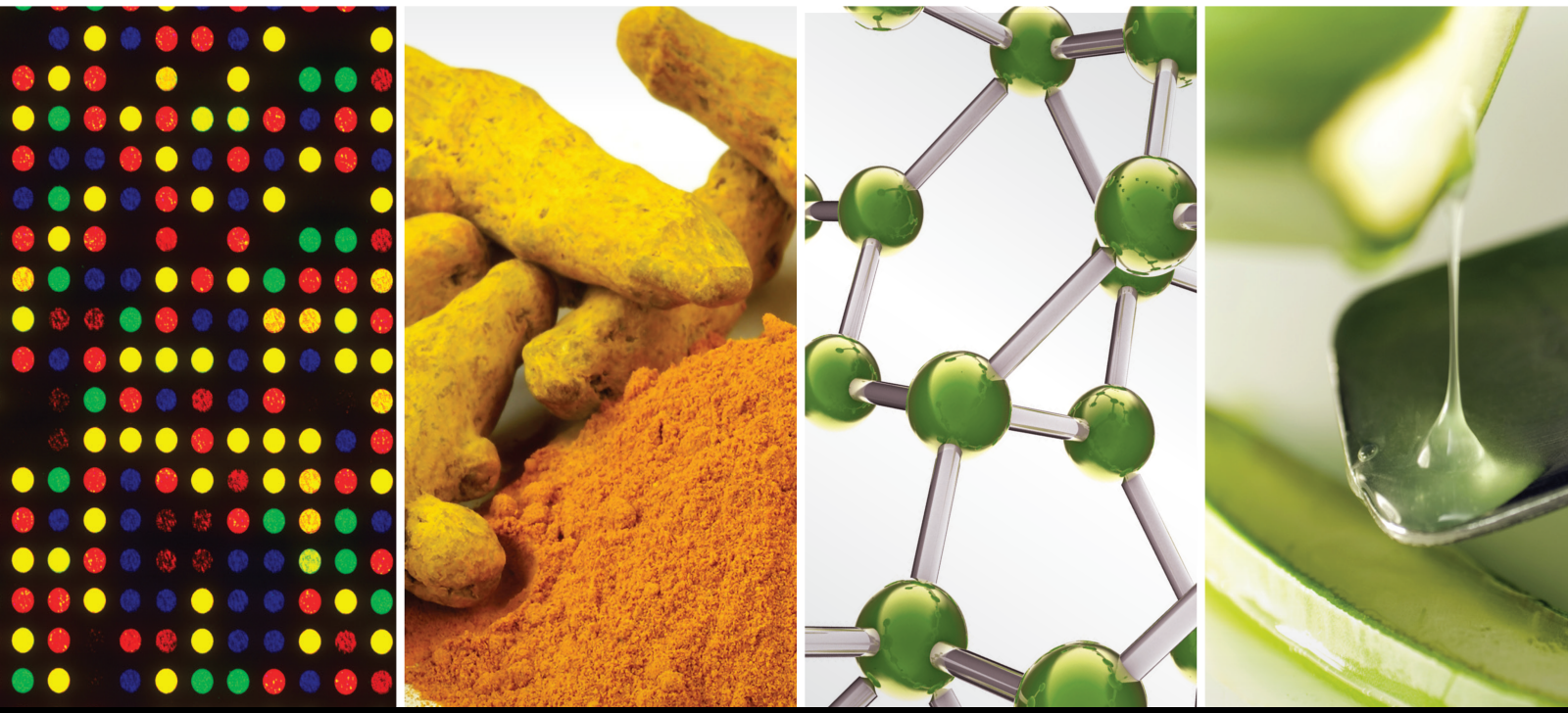
Evidence-Based Complementary and Alternative Medicine

# Complementary and Alternative Therapies for Pain Management

Lead Guest Editor: Xia Wang
Guest Editors: Shan Gao and Wei Lei



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*Research Article*

# A Randomized Clinical Hypnosis Pilot Study: Improvements in Self-Reported Pain Impact in Adults with Sickle Cell Disease

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Sickle cell disease (SCD) is characterized by recurrent painful vasoocclusive crises. Current evidence focuses on the frequency of acute pain crises resulting in emergency department use and nonplanned inpatient hospital admissions; yet few studies focus on pain sequelae outside the healthcare system or how individuals self-manage their chronic SCD-related pain. This study investigated the feasibility of a biobehavioral intervention as an adjunct nonpharmacological therapy to assist in the self-management of chronic pain. A randomized, controlled clinical trial of hypnosis was conducted in outpatients with SCD ($n = 31$). Patient-reported outcomes (PROs) administered at baseline, five, and twelve weeks from both groups included pain frequency, intensity, and quality (Pain Impact Scale (PIQ) and Numerical Rating Scales); anxiety (State-Trait Anxiety Inventory), coping strategies (Coping Strategies Scale), sleep (Pittsburgh Sleep Quality Index (PSQI)), and depression (Beck Depression Inventory (BDI)). The same PROs were collected at weeks seventeen and twenty-four from the control group after the crossover. No significant group by time interaction effects were found in any of the PROs based on the repeated-measures mixed models. The PIQ and PSQI scores decreased over time in both groups. Post hoc pairwise comparisons with the Bonferroni adjustment indicated that the mean PIQ score at baseline decreased significantly by week 12 ($p = 0.01$) in the hypnosis group. There were no significant changes across time before and after the crossover in any of the PROs in the control group. As suggested by these findings, pain impact and sleep in individuals with SCD may be improved through guided mind-body and self-care approaches such as hypnosis.

## 1. Introduction

Sickle cell disease (SCD) is the most common genetic hematologic disease in the United States, characterized by recurrent painful vasoocclusive crises. Sickle cell disease affects approximately 100,000 Americans and with an estimated 1 in 365 African-American newborns each year [1]. The disease is caused by a mutated form of hemoglobin that results in red blood cell (RBC) rigidity, lysis, and clustering. In addition, studies show that the hemoglobin released from intravascular hemolysis scavenges nitric oxide from blood plasma [2], leading to recurrent vasoocclusive crises that are usually accompanied by disabling pain. The severity and frequency of the crises present a significant impact on self-determination, independent living, and overall quality of life [3, 4]. Patients with SCD often report pain, disturbed sleep, reduced daytime functioning, and absence from work or school, all of which can be exacerbated during a vasoocclusive crisis [5, 6]. The standard of care for SCD patients during vasoocclusive crisis is pharmacologic analgesia, typically with opioids. While these pharmacological approaches may be effective for some individuals, many are ineffectively treated due to high dosage requirements, and these approaches do not prevent pain crises from occurring nor ameliorate the consequences of chronic pain in SCD. Evidence often focuses on

the frequency of acute pain crises resulting in the need to access emergency departments and/or the number of unplanned inpatient hospitalizations; however, few studies focus on chronic pain manifestations outside the typical healthcare delivery system or how patients self-manage their SCD-related pain. Furthermore, the percentage of patients who are able to self-manage their crises pain and symptoms at home without accessing healthcare professionals is not known [5]. There is growing evidence that the psychosocial and emotional consequences of chronic pain may be modifiable through nonopioid, guided mind-body and self-care approaches such as guided imagery, hypnosis, and yoga; however, it remains unclear whether SCD patients can benefit from these techniques [7–9].

Hypnosis is a cognitive-behavioral intervention that has been shown to have a powerful effect on pain management in a number of acute and chronic settings [10, 11]. Thus, adjunctive treatments using psychosocial methods designed to teach and encourage the use of self-hypnosis may positively impact the pain perception, sleep quality, functional outcomes, quality of life, and satisfaction of individuals with sickle cell, further reducing and/or preventing painful crises and healthcare utilization.

The efficacy of hypnosis has been established in treating numerous conditions including acute pain, chronic pain, burn injury progression, pulmonary illnesses, and hemophilia [12–16]. For those with clinically significant pain episodes, learning a cognitive-behavioral method such as self-hypnosis to manage their pain has proven helpful in reducing pain frequency, improving sleep quality, and decreasing use of opioids [17, 18]. Self-hypnosis training has also been shown to decrease the number of poor sleep nights, mainly by reducing the number of mild-pain nights [18]. Integrating hypnosis and the practice of self-hypnosis into the standard and palliative care of patients with sickle cell disease may also result in better pain management during their crises.

To date, there have been no published randomized, controlled trials evaluating the feasibility/efficacy of hypnosis for pain and symptom control in adults with SCD. This longitudinal clinical trial evaluated the effects of a bio-behavioral hypnosis intervention, while assessing the relationships between demographic and psychosocial variables of interest [6]. The primary research objectives of this pilot study were as follows:

Aim 1: to determine the feasibility of combining heterohypnosis and self-hypnosis as a pain and symptom management strategy in patients with SCD.

Aim 2: to test whether therapeutic heterohypnosis and self-hypnosis improve disease-related pain, anxiety, coping strategies, sleep, and depression, as compared to an education control intervention in patients with SCD.

## 2. Materials and Methods

*2.1. Design.* This was a randomized, controlled, single-crossover, repeated-measures pilot study trial of hypnosis for managing pain in SCD patients (Figure 1). For more details regarding the study design and hypnosis intervention, refer to Wallen et al. (2014) describing the full randomized controlled trial (RCT) protocol [6].

*2.2. Eligibility.* Participants with SCD were recruited into this study by referrals from physicians within one of the Vascular Therapeutic Section, Cardiovascular Branch (CB), National Heart Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH). Eligibility for this study was limited to hemoglobin SS patients since hemoglobin SC and S-$\beta$-plus-thalassemia patients typically have less pain. Eligible patients were 18 years of age or older and had a history of pain as a significant problem during a minimum of two days in the month prior to joining the study. Participants provided written informed consent after being provided with the details of the study during an initial face-to-face visit. Participants were excluded from the study if they were unwilling to experience hypnosis or to have heterohypnosis sessions recorded, were nonfluent in written and spoken English, had physical or other disabilities that prevented adequate participation in hypnotic susceptibility testing, did not wish to be video and audiotaped, had psychosis or psychotic depression, and/or had a history of seizures or epilepsy. All participants enrolled in the study received standard-of-care medical therapy while on study irrespective of study group assignment. This standard of care included the full complement of consultations including pain and palliative care services, nutrition services, social workers, spiritual ministry, rehabilitation medicine, and clinical psychiatry.

*2.3. Instruments.* Primary outcome measures included patient assessments of pain frequency, intensity, and quality as measured by the pain numerical rating scale (NRS) on an 11-point scale from 0 to 10, with 0 representing no pain and 10 equaling the worst possible pain [19]. Secondary outcome measures included face-to-face assessments of psychosocial variables including anxiety (Speilberger's State-Trait Anxiety Inventory (STAI)), coping strategies (Coping Strategies Questionnaire (CSQ)), sleep disturbance (Pittsburgh Sleep Quality Index (PSQI)), depression (Beck's Depression Inventory (BDI)), pain impact (The Pain Impact Questionnaire (PIQ-6), and healthcare utilization assessed by unplanned/emergency visits to a hospital, emergency room, or physician's office for crisis pain in the last 24 hours, as reported by patients in their daily diaries. In addition to these patient-reported outcomes (PROs), functional outcomes including ability to work and/or go to school and leave home were also analyzed as part of the daily diaries. Participants were instructed on daily documentation through a Sickle Cell Pain Diary [5, 18, 20] of pain incidence, pain severity, sleep quality, medications taken, and visits to a hospital, emergency room, or physician's office, and absence from school or work. Secondary outcome measures were collected prior to randomization, at the end of the 4-week education or hypnosis interventions, and at two-week intervals until the end of the 6-week self-hypnosis (Group A intervention) or education (Group B control) phases. Detailed description of all study measures is presented in Table 1.

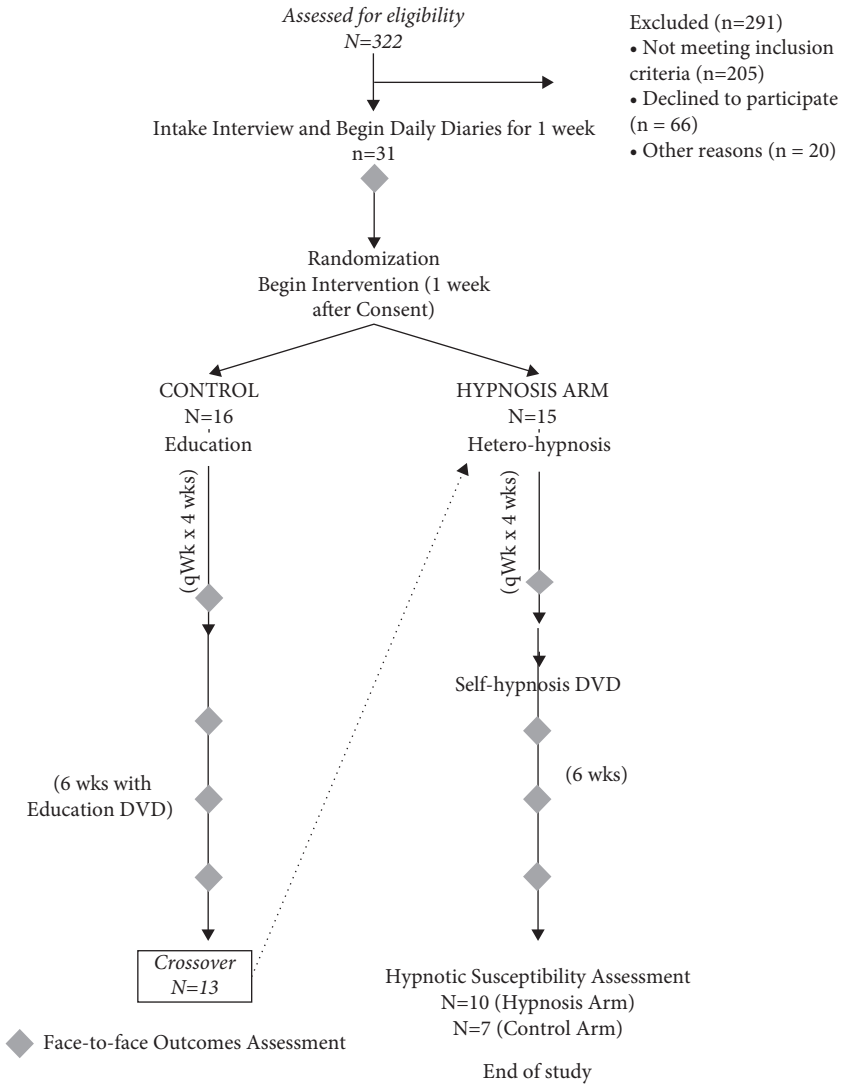


Figure 1: Hypnosis pilot: experimental versus control intervention with SCD patients. Of the 15 participants in Group A (hypnosis arm), 10 participants were considered completers and their data was used in the final analysis. Three participants withdrew (after weeks 0, 1, and 8) and two participants were lost to follow-up (one after week 0 and another after week 5). Of the 16 participants in Group B (control arm), seven completed the study. Six participants withdrew from the study at different time points (weeks 1, 4, 12, 14, and 22), whereas three were lost to follow-up: after week 5, week 14, and week 15.

*2.4. Procedures.* Upon consent and enrollment, participants completed a face-to-face intake assessment. After completing the intake questionnaires, each participant was provided with a daily pain diary and instructions on how to complete it for one week. One week following enrollment, participants returned to the outpatient clinic and were randomized to the initial hypnosis intervention group (Group A) or the education control group (Group B) of the study. For more details regarding study enrollment and randomization processes, refer to Wallen et al. describing the full randomized controlled trial (RCT) protocol [6].

Participants in Group A received hypnosis (experimental intervention) during 4 weeks of face-to-face encounters with a physician certified in hypnosis (heterohypnosis). Heterohypnosis sessions consisted of a hypnotic induction followed by individualized suggestions for analgesia, reducing anxiety, improving sleep hygiene, promoting ego-strengthening (self-efficacy), and enhancing health and well-being. Where appropriate, participants also received therapeutic suggestions specific to other symptoms. Sessions lasted about 1-1.5 hours and were typically conducted in a clinic room or other suitable setting. Hypnosis sessions were video and audiotaped for documentation purposes. Following these heterohypnosis sessions in the clinic, participants entered a self-hypnosis phase in which they were trained to perform self-hypnosis. Participants were provided with a DVD for self-hypnosis and a DVD player. For 6 weeks following the instruction period, the participants were instructed to perform self-hypnosis using customizable digital media with a recommended minimum range of three to seven times per week. Participants in the control arm (Group B) of the study received face-to-face education regarding sickle cell disease for the same length and frequency as the treatment group encounters before crossing over to

Table 1: Description of study measures.

| Study measures | Description |
|---|---|
| Pain Numerical Rating Scale (NRS) [19, 21, 22] | NRS is a numeric version of the visual analog scale in which the participants rate their pain on an 11-point scale. This verbally administered or written scale values range from 0 to 10, with 0 representing no pain and 10 equaling the worst possible pain. The scale has well-established validity. |
| State-Trait Anxiety Inventory [23–25] | This 40-item inventory assesses two distinct self-report anxiety concepts: state (transitory emotional state) and trait (habitual predisposition to anxiety). Internal consistency coefficients for this scale range from 0.86 to 0.95, whereas reliability and validity have been supported in studies of both patients and normal volunteers. |
| Coping Strategy Questionnaire (CSQ) [26, 27] | CSQ assesses participant's coping strategies for pain. Individuals rate how often they use each strategy on a 7-point scale for 6 different cognitive/behavioral coping strategies. The scale has acceptable internal reliability |
| Pittsburgh Sleep Quality Index (PSQI) [28] | PSQI assesses sleep quality and disturbance over a 30-day time interval. 19 individual items generate seven "component" scores and a global score, where a score of 5 or higher indicates poor sleep quality. This scale has been validated in populations with insomnia and other sleep disorders, psychiatric patients, and normal populations. Internal consistency and reliability coefficient range from 0.80 to 0.83 for its seven components. |
| Beck Depression Inventory (BDI) [29–31] | This 21-item inventory screens for presence and severity of depression in adults. Each item, on a 4-point scale, assesses a particular aspect of depression where higher scores are indicative of more depression. The measure is reliable and valid with adults, including the elderly. |
| Pain Impact Questionnaire (PIQ) [32] | This 6-item, patient-reported outcome measure assesses pain severity and the impact of pain on an individual's health-related quality of life (HRQOL) over the past four weeks. |
| Sickle Cell Pain Diary [5] | This pain diary examines painful crises and healthcare utilization events for each participant, noted daily during the study. The sickle cell pain diary included the entire Dinges et al.'s diary with the addition of healthcare utilization items proposed by Smith et al. |

the experimental intervention arm of the study. After completion of the self-hypnosis, an assessment was conducted to measure hypnotic ability, using the *Stanford Hypnotic Susceptibility Clinical Scale for Adults* (SHSS) [33]. This hypnosis susceptibility rating was for documentation purposes and as a potential variable that may be associated with the outcomes of the treatment.

*2.5. Statistical Analysis.* Descriptive statistics (mean and standard deviation for normally distributed continuous data, median for ordinal and nonnormally distributed continuous data, and frequencies and percentages for nominal data) were used to describe the characteristics of the study population and the outcomes (pain, anxiety, coping strategies, sleep, and depression). Correlation matrices and parametric (*t*-test and ANOVA) and nonparametric (Wilcoxon rank-sum and Kruskal–Wallis) tests were used to examine the relationships between the demographic variables and study outcomes at baseline. Overall pain diary measurements, such as percentage of days with SCD pain and other pain, average SCD pain intensity, percentage of days using pain medications during SCD pain days and non-SCD pain days, percentage of bad sleep nights during SCD pain days and non-SCD pain days, and percentage of pain-free days, were computed and compared between two groups using Wilcoxon rank-sum tests and within Group B using Friedman tests. Linear mixed model and Generalized Estimating Equations (GEE) were used to examine the daily SCD pain intensity and bad sleep night's changes over time between two groups.

Linear mixed models for repeated measures were used to examine the changes of the other nondiary outcomes over time before the crossover between the two groups. First-order autoregressive (AR1) and unstructured covariance structures were compared, and model selection was based on Bayesian Information Criterion (BIC). Friedman tests were conducted comparing the differences within Group B across all time points before and after the crossover. Wilcoxon signed-rank tests with a Bonferroni adjustment were conducted to determine pairwise differences. The level of statistical significance was set at 0.05. The data were analyzed using IBM SPSS Statistics and SAS 9.4.

## 3. Results

Out of 117 eligible patients, 31 were enrolled into the study. Table 2 describes the detailed reasons for exclusion. Of the 31 enrolled, sixteen participants were randomly assigned to the control group and fifteen were assigned to the experimental group. The study participants were 51.6% male with a mean age of 36.2 years; the majority of participants identified themselves as Black/African-American (80.6%) and considered themselves non-Hispanic (93.5%). Majority of the participants (54.8%) completed 75% or more of the pain diaries whereas a little less than half of the participants attended all four heterohypnosis sessions (Table 3). As presented in Tables 4–6 , our data showed no statistically significant differences in selected demographic, baseline clinical characteristics, and clinically relevant lab values and outcome measures between the two groups at baseline.

Using Spearman's rho nonparametric tests for baseline measures, significant correlations were found between NRS and PIQ ($rs = 0.412$, $p = 0.021$), PIQ and BDI ($rs = 0.378$, $p = 0.039$), PSQI and BDI ($rs = 0.430$, $p = 0.018$), BDI and

Table 2: Description of eligible and excluded participants with reasons.

| Recruitment status | Total |
|---|---|
| Eligible | 117 |
| Enrolled | 31 |
| *Reasons for not enrolling* | |
| Time constraints | 43 |
| Not interested/declined | 18 |
| Religion/family obligations | 5 |
| Unable to contact | 7 |
| Other reasons | 13 |
| Not eligible | 205 |
| *Reasons for noneligibility* | |
| Not SS | 55 |
| No/limited pain | 26 |
| Age, language and comorbidities exclusion | 11 |
| Unable to contact | 53 |
| Other nonspecific reasons | 60 |

Table 3: Participant adherence to study procedures ($n = 31$).

| Adherence status | Total $n$ (%) | Group A $n$ (%) | Group B $n$ (%) |
|---|---|---|---|
| *Diary completion* | | | |
| 25% or less | 8 (25.8) | 3 (20.0) | 5 (31.3) |
| 25%–49% | 2 (6.5) | 0 | 2 (12.5) |
| 50%–74% | 4 (12.9) | 2 (13.3) | 2 (12.5) |
| 75% or more | 17 (54.8) | 10 (66.7 | 7 (43.8) |
| *Hypnosis sessions attended* | | | |
| None | 9 (29.0) | 3 (20.0) | 6 (37.5) |
| Two | 5 (16.1) | 1 (6.7) | 4 (25.0) |
| Three | 4 (12.9) | 2 (13.3) | 2 (12.5) |
| Four | 13 (41.9) | 9 (60.0) | 4 (25.0) |
| *Self-hypnosis sessions* | | | |
| Minimum and maximum | 0–74 | 7–74 | 0–56 |
| Median | | 29 | 28 |

Table 4: Demographic characteristics of intervention and control group at baseline ($n = 31$).

| Characteristics | Total | Group A | Group B |
|---|---|---|---|
| Age | | | |
| Mean ± SD | 36.2 ± 11.8 | 37.7 ± 13.6 | 34.7 ± 10.0 |
| | $n$ (%) | $n$ (%) | $n$ (%) |
| 19–36 years | 19 (61.3) | 8 (53.3) | 11 (68.8) |
| 37–57 years | 12 (38.7) | 7 (46.7) | 5 (31.3) |
| Gender | | | |
| Male | 16 (51.6) | 6 (40.0) | 10 (62.5) |
| Female | 15 (48.4) | 9 (60.0) | 6 (37.5) |
| Ethnicity | | | |
| Hispanic | 2 (6.5) | 1 (6.7) | 1 (6.3) |
| Non-Hispanic | 29 (93.5) | 14 (93.3) | 15 (93.8) |
| Race | | | |
| Black/African-American | 25 (80.6) | 12 (80.0) | 13 (81.3) |
| American Indian/Alaskan Native | 1 (3.2) | 0 (0) | 1 (3.2) |
| Others | 5 (16.1) | 3 (20.0) | 2 (12.5) |
| Education | | | |
| High school or some college | 21 (67.7) | 10 (66.7) | 11 (68.8) |
| Graduate or postgraduate | 10 (32.3) | 5 (33.3) | 5 (31.3) |
| Marital status | | | |
| Married | 7 (22.6) | 3 (20.0) | 4 (25.0) |
| Not married | 24 (77.4) | 12 (80.0) | 12 (75.0) |

Comparisons using the independent two-sample $t$-test and Fisher's exact test showed no statistically significant differences for the measures shown.

STAI-state (rs = 0.456, $p = 0.011$), STAI-state (rs = 0.456, $p = 0.011$), BDI and STAI-trait (rs = 0.694, $p < 0.001$), STAI-trait and STAI-state (rs = 0.770, $p < 0.001$), STAI-trait and PSQI (rs = 0.361, $p = 0.046$), number of comorbid conditions and number of pain medications (rs = 0.674, $p < 0.001$), and NRS and number of pain medications taken at baseline (rs = 0.382, $p = 0.041$).

At baseline, patients reporting higher levels of pain intensity also reported a higher level of pain severity (rs = 0.412, $p = 0.021$) and greater number of pain medications (rs = 0.382, $p = 0.041$). Similarly, patients reporting higher levels of depression also reported poorer sleep quality (rs = 0.430, $p = 0.018$), higher PIQ (rs = 0.378, $p = 0.039$), higher STAI-trait (rs = 0.694, $p < 0.001$), and higher STAI-state (rs = 0.456, $p = 0.011$). Patients with a higher STAI-trait reported poorer sleep quality (rs = 0.361, $p = 0.046$) and higher STAI-state (rs = 0.770, $p < 0.001$). Patients with a greater number of comorbid conditions reported using a greater number of medications (rs = 0.674, $p < 0.001$).

*3.1. Primary Outcomes.* Linear mixed model for repeated measures for all primary outcomes with AR1 was found to have better fit based on the lower BIC values. No significant group by time interaction effects were found in any of the models (Table 7). Although no group differences were found in any of the primary outcomes, PIQ and PSQI scores decreased significantly over time. Pairwise comparisons (Table 8) using the Bonferroni adjustment indicated that the mean PIQ score at week 12 was significantly lower at week 12 ($p = 0.003$) than at baseline, indicating lower perceived impact of pain following the hypnosis intervention. There was no difference between baseline and week 5 ($p = 0.11$) or between week 5 and week 12 ($p = 0.20$). More specifically, the baseline and week 12 difference was only seen in Group A ($p = 0.01$), the hypnosis intervention group. Group B showed no differences across the three time points. Pairwise marginal means comparison using the Bonferroni adjustment showed that the overall mean PSQI score at week 5 was significantly higher than at week 12 ($p = 0.04$). However, no significant within-group differences were found in the PSQI scores.

Friedman tests examined the outcome measurements in group B over 24 weeks before and after the crossover (Table 9). Only cases that had data for all five time points were used in these analyses. No differences were found for any of these outcomes across time. The authors acknowledge that the lack of effect of the hypnosis intervention in the crossover for the control groups was likely a result of the small sample size.

Table 5: Baseline clinical data and clinically relevant lab values ($n = 31$).

| | Total | Group A ($n = 15$) | Group B ($n = 15$) |
|---|---|---|---|
| *Clinically relevant lab values** | Mean (SD)<br>min–max | Mean (SD) | Mean (SD) |
| Fetal hemoglobin | 12.1 (±7.3)<br>0.0–27.4 | 14.2 (±8.4) | 10.1 (±5.6) |
| Red blood cell count | 2.7 (±0.5)<br>1.76–3.82 | 2.7 (±0.5) | 2.6 (±0.6) |
| Hematocrit | 26.7 (±3.5)<br>19.3–34.2 | 27.2 (±2.4) | 26.1 (±4.3) |
| TR peak velocity ($n = 27$) | 2.7 (±0.4)<br>1.70–3.60 | 2.7 (±0.5) | 2.7 (±0.4) |
| | ***n* (%)** | ***n* (%)** | ***n* (%)** |
| *Narcotic analgesics prescribed (baseline)* | 26 (89.7%) | 11 (78.6%) | 15 (100%) |
| *Episodes of pain in the past 12 months* | | | |
| Min and max | | 0–25 | 0–20 |
| Median | | 9.5 | 4 |
| *Use of hydroxyurea at baseline* | 22 (75.9%) | 10 (71.4%) | 12 (80%) |
| *Patient reported comorbidities* | | | |
| Congestive heart failure | 3 (9.7%) | 2 | 1 |
| Chronic lung disease | 6 (19.4%) | 4 | 2 |
| Blindness or trouble seeing | 4* (12.9%) | 4 | 0 |
| Deafness or difficulty hearing | 3 (9.7%) | 2 | 1 |
| Sugar diabetes, mellitus | 0 | 0 | 0 |
| Asthma | 2 (6.5%) | 1 | 1 |
| Ulcer or GI bleeding | 1 (3.2%) | 0 | 1 |
| Arthritis or rheumatism | 4 (12.9%) | 2 | 2 |
| Sciatic or chronic back pain | 6 (19.4%) | 3 | 3 |
| High blood pressure (HTN) | 11 (35.5%) | 4 | 7 |
| Angina | 2 (6.5%) | 2 | 0 |
| Heart attack or MI | 3 (9.7%) | 3 | 0 |
| Stroke | 8 (25.8%) | 6 | 2 |
| Kidney disease | 3 (9.7%) | 3 | 0 |
| *Number of comorbid conditions* | 1.8 (±1.9) | 2.4 (±2.2) | 1.3 (±1.3) |
| *Number of medications at baseline* | 7.4 (±4.1) | 8.4 (±3.9) | 6.5 (±4.1) |

No statistically significant differences were found between groups based on the independent two-sample $t$-tests. *Normal ranges are fetal haemoglobin = 0.0–2.0; red blood cell count = 3.93–5.22; and haematocrit = 34.1–44.9.

*3.2. Diary Outcomes.* No significant differences were found in any overall pain diary measurements between two groups in the first ten weeks period and within Group B before and after the crossover (Tables 10 and 11 ).

Using the daily crisis pain intensity as the outcome with random intercept and slope, the linear mixed model showed no significant group, time, and group by time interaction effects.

The Generalized Estimating Equations (GEE) showed no significant group by time interaction and overall group effect in daily bad sleep status. The overall probabilities of bad sleep decreased over time for both groups ($p = 0.014$).

## 4. Discussion

This was the first randomized controlled clinical trial of hypnosis in adults with sickle cell disease that aimed to explore the trajectory of psychosocial variables (depression and anxiety), pain intensity, and pain impact changes over time. Our findings suggest that use of self-hypnosis techniques coupled with heterohypnosis resulted in significant decrease in pain impact and overall improvement in sleep quality over time.

Existing evidence related to the efficacy of hypnosis as a nonpharmacological intervention to address the pain and symptoms often associated with chronic disease management is mixed [34–36]. Heterohypnosis alone or followed by self-hypnosis treatment may benefit some individuals with chronic pain of various etiologies. Previous research has shown that training in different mind-body relaxation techniques, including self-hypnosis, resulted in decrease of emergency room visits and number of hospitalization and inpatient treatment days among patients with a history of painful episodes of sickle cell disease [37]. Dinges et al. reported that self-hypnosis was significantly effective in reducing milder episodes of pain, but not effective in severe sickle cell disease pain episodes while Wolfe and colleagues found that the effects of self-hypnosis on experimental dental pain resulted in increased pain thresholds and lower pain rating on VAS [18, 38].

Elkins et al. highlighted the significant effects of hypnosis on pain reduction in their review of the literature [9, 39–44]. Although our findings did not show a significant reduction in pain as measured by the NRS, our data reflect a significant decrease in pain impact assessed by the Pain Impact Scale

Table 6: Primary outcome measures at baseline ($n = 31$).

| Outcome measures | Range | Total mean (±SD) | Group A ($n = 15$) Mean (±SD) | Group B ($n = 16$) Mean (±SD) |
|---|---|---|---|---|
| VAS | 0–8 | 2.7 (±2.3) | 3.0 (±2.6) | 2.3 (±1.9) |
| PIQ* | 48–72 | 62 (±6.7) | 62.8 (±6.5) | 61.3 (±7.1) |
| BDI | 0–25 | 11 (±6.2) | 10.7 (±7.0) | 11.3 (±5.6) |
| STAI total | 40–117 | 68.6 (±18.9) | 68.2 (±18.8) | 69.0 (±19.6) |
| STAI-state | 20–57 | 32.5 (±9.6) | 32.3 (±10.1) | 32.8 (±9.4) |
| STAI-trait | 20–60 | 36.1 (±10.2) | 35.9 (±9.5) | 36.3 (±11.2) |
| CSQ | 53–209 | 138.8 (±41.6) | 141.1 (±43.7) | 136.6 (±40.8) |
| PSQI | 2–16 | 9.6 (±3.9) | 9.2 (±3.6) | 10.0 (±4.3) |

No statistically significant differences were found between groups based on the independent two-sample $t$-tests. VAS: Visual Analog Scale; PIQ: Pain Impact Questionnaire; BDI: Beck's Depression Inventory; STAI: Speilberger's State-Trait Anxiety Inventory; CSQ: Coping Strategies Questionnaire; PSQI: Pittsburgh Sleep Quality Index. *The US adult general population had an average PIQ score of $50 \pm 10$; the chronic pain patient sample had a mean score of $64 \pm 7$ [32].

Table 7: Summary of final repeated-measures mixed models of the effect of hypnosis.

| Variable | Group | | Time | | Group*time | |
|---|---|---|---|---|---|---|
| | $F$ test | $p$ value | $F$ test | $p$ value | $F$ test | $p$ value |
| VAS | 0.32 | 0.58 | 1.83 | 0.17 | 0.09 | 0.92 |
| PIQ | 0.17 | 0.68 | 6.00 | 0.005 | 0.68 | 0.51 |
| BDI | 0.21 | 0.65 | 2.02 | 0.14 | 0.41 | 0.67 |
| STAI total | 0.80 | 0.38 | 0.03 | 0.98 | 1.45 | 0.25 |
| STAI-state | 0.57 | 0.46 | 0.17 | 0.84 | 0.60 | 0.55 |
| STAI-trait | 0.95 | 0.34 | 0.93 | 0.4 | 2.77 | 0.07 |
| CSQ | 0.84 | 0.37 | 0.62 | 0.54 | 0.77 | 0.47 |
| PSQI | 0.05 | 0.82 | 3.47 | 0.04 | 0.56 | 0.57 |

Linear mixed models for repeated measures were used to test the hypnosis effect between the two groups over the three time points (baseline, week 5, and week 12). First-order autoregressive covariance structure was used in all models. VAS: Visual Analog Scale; PIQ: Pain Impact Questionnaire; BDI: Beck's Depression Inventory; STAI: Speilberger's State-Trait Anxiety Inventory; CSQ: Coping Strategies Questionnaire; PSQI: Pittsburgh Sleep Quality Index.

Table 8: Model estimated means of PIQ and PSQI.

| Variable | Group | Baseline | Week 5 | Week 12 | Overall |
|---|---|---|---|---|---|
| PIQ | A | 62.8 | 57.4 | 53.8 | 58.0 |
| | B | 61.3 | 59.4 | 56.2 | 59.0 |
| | Overall | 62.0* | 58.4 | 55.0* | 58.5 |
| PSQI | A | 9.2 | 9.8 | 7.7 | 8.9 |
| | B | 10.0 | 9.4 | 8.1 | 9.2 |
| | Overall | 9.6 | 9.6^ | 8.0^ | 9.0 |

Model estimated means from the linear mixed models for repeated measures with first-order autoregressive covariance structure. PIQ: Pain Impact Questionnaire; PSQI: Pittsburgh Sleep Quality Index. *Pairwise comparisons using the Bonferroni adjustment indicated mean PIQ score at baseline were significantly higher than at week 12 ($p = 0.003$). Pairwise marginal means comparison using the Bonferroni adjustment showed that the overall mean PSQI score at week 5 was significantly higher than at week 12 ($p = 0.04$).

Table 9: Group B within group comparisons over time before and after the crossover.

| Variable | $N$ | $\chi^2$ (d$f = 4$) | $p$ value* |
|---|---|---|---|
| VAS | 7 | 1.23 | 0.89 |
| PIQ | 7 | 5.35 | 0.25 |
| BDI | 6 | 6.63 | 0.16 |
| STAI total | 7 | 4.65 | 0.33 |
| STAI-state | 7 | 7.36 | 0.12 |
| STAI-trait | 7 | 4.98 | 0.29 |
| CSQ | 7 | 3.25 | 0.52 |
| PSQI | 7 | 1.63 | 0.80 |

VAS: Visual Analog Scale; PIQ: Pain Impact Questionnaire; BDI: Beck's Depression Inventory; STAI: Speilberger's State-Trait Anxiety Inventory; CSQ: Coping Strategies Questionnaire; PSQI: Pittsburgh Sleep Quality Index. *A nonparametric Friedman test was conducted comparing the differences within groups across time.

Table 10: Pain diary outcome measurements between two groups.

| Variable | Group A median | Group B median | $p$ value |
|---|---|---|---|
| Percentage of days with sickle cell disease (SCD) pain | 30.95 | 52.86 | 0.71 |
| Percentage of days with other pain | 56.04 | 38.96 | 0.52 |
| Average SCD pain intensity | 1.19 | 1.32 | 0.85 |
| Percentage of days using pain medications during SCD pain days | 99.17 | 94.12 | 0.72 |
| Percentage of days using pain medications during non-SCD pain days | 29.12 | 75.00 | 0.34 |
| Percentage of bad sleep nights (SCD pain days) | 24.17 | 28.42 | 0.83 |
| Percentage of bad sleep nights (non-SCD pain days) | 5.22 | 15.17 | 0.39 |
| Percentage of days unplanned ER or doctor visit | 3.81 | 1.45 | 0.51 |
| Percentage of pain-free days | 30.71 | 16.44 | 0.60 |

Table 11: Group B within group comparisons for pain diary outcomes over time before and after the crossover.

| Variable | Education median | Hypnosis median | $p$ value |
|---|---|---|---|
| Percentage of days with sickle cell disease (SCD) pain | 52.86 | 64.29 | 0.16 |
| Percentage of days with other pain | 38.96 | 39.93 | 0.71 |
| Average SCD pain intensity | 1.32 | 1.25 | >0.99 |
| Percentage of days using pain medications during SCD pain days | 94.12 | 98.11 | 0.71 |
| Percentage of days using pain medications during non-SCD pain days | 75.00 | 61.34 | 0.71 |
| Percentage of bad sleep nights (SCD pain days) | 28.42 | 36.31 | 0.48 |
| Percentage of bad sleep nights (non-SCD pain days) | 15.17 | 14.29 | 0.10 |
| Percentage of days unplanned ER or doctor visit | 1.45 | 1.92 | 0.74 |
| Percentage of pain-free days | 16.44 | 11.43 | >0.99 |

(PIQ-6) after a period of 12 weeks. As a validated self-reported measure, the PIQ-6 asks the individual to assess on average how much pain they have had over the past four weeks as well as how this pain has impacted their activities as well as their mood. These findings may be particularly important for SCD patients who not only suffer from episodes of acute pain crisis but also from the physical and emotional sequelae of chronic and often debilitating pain related to their disease. For some patients, reducing their reported current and/or daily perceived pain may not be a realistic goal, whereas building strategies to decrease the negative impact of chronic and crisis pain may be more plausible.

An important aspect of chronic pain is how adversely it affects the individual's overall quality of life. Individuals suffering from SCD crises do require access to medical services for pain control; yet it is important to consider adjunct nonpharmacological strategies for self-care. In the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic and the opioid crisis eras, self-care modalities that provide individuals with methods to improve sleep and decrease the negative impacts of their chronic pain may be particularly relevant. Self-hypnosis was taught to our intervention group (followed by the education-only group after crossover) in an effort to build a feeling of confidence and self-control over the intensity and frequency of pain. Our results show significant improvement in sleep quality over time, which is consistent with the previous findings of Haanen et al., who also reported improved sleep quality with hypnosis sessions in refractory fibromyalgic pain [9, 40]. Although at baseline, depression and poor sleep quality were significantly correlated in both the intervention and control groups in the current study, and we saw an improvement in sleep quality, our intervention did not result in a significant reduction in depression scores over time. This is in contrast to previous studies that report reductions in fatigue, anxiety, worry, nervousness, and distress with use of hypnotherapy [40, 45, 46]. Future research with a larger sample size is needed to further delineate the effects of hypnotherapy on psychosocial factors in patients with sickle cell disease.

The strength of our study lies in that it addresses the concerns raised by previous hypnosis intervention studies addressing chronic pain and symptom management, namely, the lack of standardization of the hypnotic interventions in clinical trials [9]. Our study is not without limitations in that the results may not be representative of the larger population due to the small sample size and high dropout rates, partially because of the long study duration. Furthermore, future studies may need to consider including symptomatic individuals with hemoglobin SC of Sb + thalassemia in future clinical trials to further evaluate these additional genotypes in adult patients with SCD.

## 5. Conclusions

As suggested by these findings, hypnosis may be a promising tool as an adjunct intervention to reduce pain severity and the impact of pain on an individual's health-related quality of life (HRQOL) as measured by the validated PIQ-6 patient-reported outcome measure which had not been previously used in trials evaluating the use of hypnosis to manage chronic pain in patients with sickle cell disease. Additional randomized trials with larger sample sizes and standardized hypnotic interventions are warranted.

## Data Availability

Data are available upon request. Gwenyth Wallen, the Principal Investigator, should be contacted to request the data at gwallen@cc.nih.gov.

## Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this study.

## Authors' Contributions

Dr. Alyssa T. Brooks conducted this collaborative research while at the National Institutes of Health Clinical Center. Conceptualization was done by GRW; formal analysis was conducted by GRW and LY; resources were obtained by GRW; original draft was written by GRW, NK, KRM, LY, and ATB; review and editing were done by GRW, KRM, NK, LY, and ATB.

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*Research Article*

# Herbal Prescription SH003 Alleviates Docetaxel-Induced Neuropathic Pain in C57BL/6 Mice

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Docetaxel-based therapy has been applied to kill cancers including lung and breast cancers but frequently causes peripheral neuropathy such as mechanical allodynia. Lack of effective drugs for chemotherapy-induced peripheral neuropathy (CIPN) treatment leads us to find novel drugs. Here, we investigated whether and how novel anticancer herbal prescription SH003 alleviates mechanical allodynia in mouse model of docetaxel-induced neuropathic pain. Docetaxel-induced mechanical allodynia was evaluated using von Frey filaments. Nerve damage and degeneration in paw skin of mice were investigated by immunofluorescence staining. Neuroinflammation markers in bloodstream, lumbar (L4-L6) spinal cord, and sciatic nerves were examined by ELISA or western blot analysis. Docetaxel (15.277 mg/kg) was intravenously injected into the tail vein of C57BL/6 mice, and mechanical allodynia was followed up. SH003 (557.569 mg/kg) was orally administered at least 60 min before the mechanical allodynia test, and von Frey test was performed twice. Docetaxel injection induced mechanical allodynia, and SH003 administration restored withdrawal threshold. Meanwhile, degeneration of intraepidermal nerve fibers (IENF) was observed in docetaxel-treated mice, but SH003 treatment suppressed it. Moreover, docetaxel injection increased levels of TNF-$\alpha$ and IL-6 in plasma and expressions of phospho-NF-$\kappa$B and phospho-STAT3 in both of lumbar spinal cord and sciatic nerves, while SH003 treatment inhibited those changes. Taken together, it is worth noting that TNF-$\alpha$ and IL-6 in plasma and phospho-NF-$\kappa$B and phospho-STAT3 in spinal cord and sciatic nerves are putative biomarkers of docetaxel-induced peripheral neuropathy (DIPN) in mouse models. In addition, we suggest that SH003 would be beneficial for alleviation of docetaxel-induced neuropathic pain.

## 1. Introduction

Cancer patients receive chemotherapy to kill malignant tumors and improve survival rate whereas it unfortunately causes severe side effects [1, 2]. CIPN is one of the painful side effects occurring in approximately 30–70% of patients and characterized by damaged peripheral neurons [3–6]. The main symptoms include pain, muscle weakness, and muscle spasms, resulting in a decrease in the quality of life [7]. Taxanes (paclitaxel and docetaxel) are useful anticancer drugs but cause CIPN [8]. Docetaxel is one of the cytotoxic anticancer drugs and exhibits an anticancer effect by binding to tubulin, resulting in impairment of microtubule homeostasis and mitotic arrest [9]. US Food and Drug Administration (FDA) approved docetaxel as an anticancer drug against multiple types of cancers including non-small-cell lung and breast cancers [10, 11]. Of note, docetaxel is associated with acute pain syndrome in several cancer patients [12–17]. Development of painful symptoms by DIPN may lead to discontinuation of cancer treatment regardless of therapeutic benefit of chemotherapy [18–20]. Until now, there have been no effective therapeutic options for DIPN in cancer patients [21]. Thus, this drives us to find novel medicines for treatment of DIPN.

Cancer chemotherapy typically damages the distal sensory neurons of hands or feet with severe pain [22].

Nonclinical studies have demonstrated that peripheral nervous system tissues, which are mainly damaged in CIPN model, include sciatic nerves, lumbar spinal cord, and dorsal root ganglion (DRG) [23, 24]. While the mechanism underlying CIPN is still unclear, several CIPN studies have demonstrated that NF-$\kappa$B has been suggested to be one of the readouts for neuropathic pain [8, 25]. It was reported that activation of NF-$\kappa$B is associated with spinal cord and sciatic nerve injury in rodent model [26]. Moreover, inhibition of NF-$\kappa$B pathway can ameliorate chronic pain in neuropathic pain rodent model. Besides NF-$\kappa$B, STAT3 pathway has also been suggested to be involved in neuropathic pain. It has been reported that paclitaxel treatment induces neuropathic pain with increased expression of phosphorylated JAK2 and STAT3 [27]. Furthermore, another study demonstrated that chemotherapeutic agent bortezomib-induced mechanical allodynia is associated with STAT3 activation in DRG [28]. Moreover, TNF-$\alpha$-mediated activation of STAT3 plays a critical role in CIPN [29]. Therefore, activation of NF-$\kappa$B and STAT3 can be regarded as a biomarker for CIPN. However, there is no evidence to support the fact that those biomarkers are associated with DIPN.

Novel herbal prescription SH003 is the traditional Chinese medicine (TCM) theory-based anticancer drugs composed of *Astragalus membranaceus*, *Angelica gigas*, and *Trichosanthes kirilowii* Maximowicz. The anticancer effect of SH003 against breast, lung, and prostate cancer has been demonstrated through several *in vitro* and *in vivo* studies [30–38]. The safety of SH003 was proved by phase I clinical study for solid tumors, and phase II clinical study for wild-type EGFR lung cancer patients is in progress [39–41]. Meanwhile, phase I/II clinical study of SH003 combined with docetaxel for breast and lung cancer patients is also in progress (NCT01755923, https://clinicaltrials.gov). While it is a crucial point that TCM-based herbal medicines and supplements are the representative alternative treatment for improving health problems including cancer-related side effects [42–46], we needed to investigate whether novel herbal medicine SH003 alleviates docetaxel-mediated adverse effects in both nonclinical and clinical studies.

The purpose of the present study was to evaluate the mitigative effect of SH003 on DIPN in C57BL/6 mice. Intravenous injection of docetaxel decreased the withdrawal threshold of von Frey filament compared to Control group whereas oral administration of SH003 relieved this symptom. ELISA analysis showed that levels of TNF-$\alpha$ and IL-6 in plasma were upregulated by docetaxel injection while SH003 treatment revered it. Docetaxel-induced upregulation of phospho-NF-$\kappa$B and phospho-STAT3 expression in sciatic nerve and spinal cord was inhibited by SH003 treatment. Histological analysis of mouse foot pad skin indicated that administration of SH003 relieved docetaxel-induced damage on peripheral nerve fibers. Therefore, the present study suggests that SH003 is applicable to alleviate DIPN.

## 2. Materials and Methods

*2.1. SH003 and Docetaxel.* SH003 powder was prepared as described previously. In brief, *Astragalus membranaceus* (333 g), *Angelica gigas* (333 g), and *Trichosanthes kirilowii* Maximowicz (333 g) were mixed and then extracted with 10 times volume of 30% ethanol at 100°C for 3 h. This process was performed 2 times. The extract was dried at reduced pressure (40 Torr) at 60°C for 18 h. Dried SH003 was stored at −20°C. Docetaxel was purchased from Sigma-Aldrich (#01885–25MG-F, St. Louis, MO, USA). Docetaxel was dissolved in DMSO and stored at −20°C.

*2.2. Determination of Drug Dose.* No-observed-adverse-effect level (NOAEL) of SH003 determined by phase I clinical trials was 4,800 mg/day [41]. Maximum tolerable dose (MTD) of docetaxel in cancer patients was 75 mg/m$^2$ [40]. Animal equivalent dose was calculated by the following equation [47]:

$$\text{human equivalent dose}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{animal dose}\left(\frac{\text{mg}}{\text{kg}}\right) \times \left(\frac{\text{weight animal (kg)}}{\text{weight human (kg)}}\right)(1 - 0.75). \quad (1)$$

Human and mice weights are 65 kg and 0.02 kg, respectively. Since there is an individual difference in metabolic rate, exponent for body surface area is 0.75. Thus, animal equivalent dose of SH003 and docetaxel was determined as 557.569 mg/kg and 15.277 mg/kg, respectively.

*2.3. Docetaxel-Induced Neuropathy Mouse Model and Experiment Schedule.* All procedures in animal experiments were approved by Kyung Hee University Institutional Animal Care and Use Committee (KHU-IACUC; KHSASP-19-322). C57BL/6N mice (5 weeks old) were purchased from Nara Biotech (Seoul, South Korea). Mice were housed under constant temperature (24 ± 2°C) with light-dark cycle, and food and water were freely available. Two experiments were performed. The timelines of experiment "1" and experiment "2" are shown in Figure 1.

In experiment "1," mice were randomly divided into different treatment groups as follows: Control ($n = 6$) and Docetaxel ($n = 6$). Before injection of docetaxel on the 1$^{st}$ day, baseline withdrawal threshold in each mouse was determined. Mice were treated with a single intravenous injection of docetaxel to model DIPN, and neuropathic symptom was followed up.

In experiment "2," mice were randomly divided into different groups as follows: "Control" ($n = 5$), "Docetaxel" ($n = 5$), "Docetaxel + SH003" ($n = 5$). After determination of baseline withdrawal threshold by von Frey test on the 1$^{st}$ day,

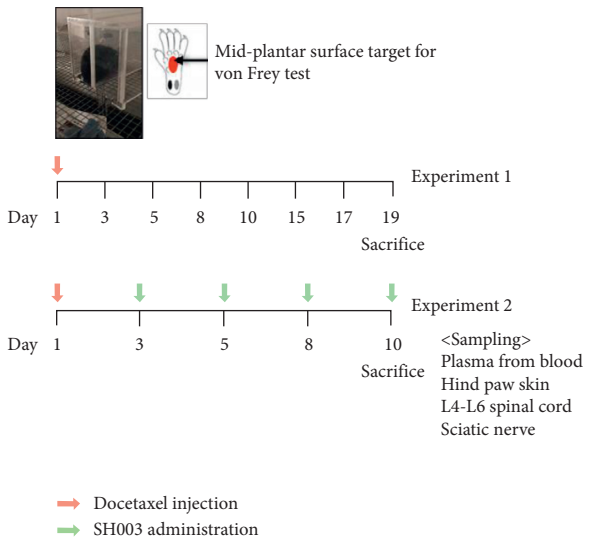



Figure 1: Timelines of the *in vivo* experimental design.

mice in "Docetaxel" and "Docetaxel + SH003" were intravenously injected with 50 $\mu$L docetaxel while mice in "Control" were injected with the same volume of DMSO, which did not exceed 5 mL/kg according to guidelines [48]. SH003 was orally administered at least 60 min before the performance of mechanical allodynia test. SH003 administration and von Frey filament test were performed 2-3 times a week. At the end of the experiment, mice were euthanized by 100% carbon dioxide in chamber by 30–70%/min filling rate, followed by cervical dislocation. The hind paw skin of mice was collected for staining of IENF. For evaluation of the inflammatory damage on peripheral neurons, lumbar (L4-L6) spinal cord and both left and right sciatic nerves were collected.

*2.4. Measurement of Mechanical Allodynia.* Mechanical allodynia was evaluated using von Frey filaments (JD-SI-11F, Jeungdo B and P, Seoul, South Korea) according to standard operating procedure from the Jackson Laboratory mouse neurobehavioral phenotyping facility. In brief, the room atmosphere was maintained with constant temperature (24 ± 2°C), humidity (50 ± 20%), and lighting (400–500 lux) without noise. Prior to the start of von Frey test, mice were individually placed on stainless steel mesh floor with acrylic cover (L*W*H= 10*8*7 cm) and left without disturbance for a minimum of 30 min to acclimate. The midplantar surface of left hind paw of mice was poked with filament (0.4 g) for 3 seconds until it bends, and each application was presented at least 3 times. If there was no response with the starting filament (0.4 g), it was changed to the next highest filament (0.6 g) in the series and continuously moved through the series in order until a withdrawal response was observed or the 2 g filament was presented. If there was response with the starting filament, it was moved to the next lowest filament in the series until a withdrawal response was not observed or the 0.02 g filament was presented. Licking and shaking of the affected paw were determined as the withdrawal responses. The whole procedure from acclimation to determination of withdrawal responses was repeated once again. Mechanical allodynia threshold values from two independent experiments were recorded and averaged in each group.

*2.5. Immunofluorescence Staining of IENF.* The hind paw skin was isolated from all mice and immediately fixed with 4% paraformaldehyde for 6 h. Fixed skin samples were dehydrated using 30% sucrose solution for 16 h. For the cryosection, dehydrated tissues were embedded in OCT compound and frozen at liquid nitrogen. Frozen sections of 20 $\mu$m were stained with anti-PGP9.5 antibody (1 : 800, ab8189, Abcam, Cambridge, UK) and IgG secondary antibody conjugated with Alexa Fluor 488 (1 : 2,000, A28175, Invitrogen Co., Carlsbad, CA, USA). The images were acquired using Zeiss LSM 5 PASCAL confocal laser scanning microscope system (Carl Zeiss AG, Oberkochen, Germany) at a magnification of 10x.

*2.6. Enzyme-Linked Immunosorbent Assay (ELISA).* Levels of inflammatory cytokines TNF-α and IL-6 were assessed using a DuoSet ELISA kit (555268 and 555240, respectively, BD Biosciences, San Diego, USA) according to the manufacturer's instructions. In brief, the whole blood was collected from left ventricle and transferred to EDTA tube (367835, BD Biosciences, San Diego, USA). After centrifugation at 2,000 rpm for 10 min, plasma from blood was prepared and stored in a deep freezer at −80°C until use. 96-well plates were coated with capture antibody in ELISA coating buffer and incubated overnight at 4°C. The plates were then washed with wash buffer and subsequently blocked with assay diluent at RT for 1 h. Diluted standard and plasma samples were added to plates and incubated at RT for 2 h. Detection antibody and streptavidin-conjugated horseradish peroxidase were added to the plates, followed by incubation at RT for 1 h. Tetramethylbenzidine substrate was added and incubated at RT for 0.5 h. The reaction was ended by adding stop buffer. The optical density was measured at 450 nm on an automated ELISA reader (Versa Max, Molecular Devices, CA, USA).

*2.7. Western Blot Analysis.* Total protein from lumbar (L4-L6) spinal cord and sciatic nerves were extracted using RIPA buffer (R2002, Biosesang, Gyeonggi-do, South Korea) with protease and phosphatase inhibitors. The same amount of proteins was quantified by Bradford protein assay (Bio-Rad, Hercules, CA, USA). The proteins were separated by 8 or 15% SDS-PAGE, followed by transfer to nitrocellulose membranes. After blocking with blocking buffer including 1% (w/v) skim milk and 1% (w/v) BSA at RT for 1 hour, the membrane was incubated with anti-NF-κB (#8242), anti-phospho-NF-κB (#3033), anti-STAT3 (#4904), anti-phospho-STAT3 (#9145), and anti-GAPDH (#5174) at 4°C for 16–24 h. All antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Horseradish peroxidase-(HRP-) conjugated secondary IgG antibodies (Calbiochem, San Diego, CA, USA) were further incubated with the

membrane at RT for 1 h. An enhanced chemiluminescence kit (DG-WP100, DoGen, Seoul, South Korea) was used for detection of HRP signal.

*2.8. Statistical Analysis.* Statistical analysis was performed using Prism (GraphPad, San Diego, CA, USA). The differences of means between the groups were analyzed by one-way or two-way ANOVA using Tukey's or Sidak's multiple comparisons test, respectively. $P$ value $< 0.05$ means statistically significant difference. Results were represented as mean ± standard deviation (SD) or standard error of the mean (SEM).

## 3. Results and Discussion

*3.1. Intravenous Injection of DIPN in C57BL/6 Mice.* Docetaxel is a well-known anticancer drug with severe side effects including CIPN with pain in the hands and feet [49,50]. The present study was performed to model DIPN. Docetaxel was intravenously injected into mice tail vein, and mechanical allodynia threshold by von Frey test was followed up. On the 5$^{th}$ day, mechanical withdrawal threshold of Docetaxel group began to be lower than that of Control group (Figure 2(a)). On the 10$^{th}$ day, the biggest difference of mechanical allodynia threshold between Control group and Docetaxel group occurred, while there were no differences after the 15$^{th}$ day (Figure 2(a)). Drug treatment did not affect body weights of mice until 19 days (Figure 2(b)). Therefore, we determined to perform further efficacy study until the 10$^{th}$ day. It is common for taxane-based adjuvant chemotherapy to induce acute pain syndrome [51]. In fact, the incidence of taxane acute pain syndrome is more common with docetaxel than paclitaxel whereas docetaxel triggers less peripheral neuropathy than paclitaxel [16, 52]. Docetaxel-mediated pain flare occurs within 4–5 days after docetaxel chemotherapy and lasts about 4 days [52]. Consistent with clinical findings, *in vivo* model for docetaxel-related acute pain syndrome was successfully developed by a single intravenous injection of docetaxel at MTD.

*3.2. Oral Administration of SH003 Mitigated Docetaxel-Induced Mechanical Allodynia at Hind Paws of C57BL/6 Mice.* Next, we further examined whether SH003 mitigates docetaxel-induced mechanical allodynia. Although mechanical threshold of "Docetaxel" was higher than that of "Docetaxel + SH003" on the 3$^{rd}$ day, the threshold value of two groups was not statistically different with "Control." While docetaxel treatment induced mechanical allodynia, SH003 treatment relieved it on the 8$^{th}$ and 10$^{th}$ day (Figure 3(a)). Drug treatment did not affect body weights of mice until 10 days (Figure 3(b)). The data showed that SH003 administration mitigated DIPN. SH003 is a TCM-based novel herbal mixture for treating several malignant cancers including breast, prostate, and lung cancers. Anticancer effect and mode of action have been clearly demonstrated by numerous nonclinical studies [30–38]. In fact, conventional chemodrugs have adverse side effects because of their cytotoxicity to healthy cells as well as cancer cells regardless of therapeutic benefit. Our previous study reported that SH003 is safe in rats according to toxicity studies with Good Laboratory Practice (GLP) regulations [36]. Oral administration of SH003 at various doses (500; 1,000; 2,000 mg/kg) did not show any problem in mortality, food intake, haematological value, organ weight, histopathology, etc. In sum, we suggest that SH003 is safe and effective herbal medicine for treatment of DIPN as well as cancer.

*3.3. SH003 Administration Inhibited Docetaxel-Induced Reduction of IENF at Hind Paws of C57BL/6 Mice.* IENF are bare nerve endings within junction between dermis and epidermis and play a role in transmission of peripheral pain [53, 54]. Clinically, chemotherapy damages distal IENF, resulting in loss of IENF in hands and feet in patients with CIPN. [55–59]. In the rodent CIPN model, degeneration of IENF in paw skin is detected although the contribution of IENF loss to the pathobiology of CIPN is not fully elucidated [60–62]. Of note, chemotherapeutic drugs including paclitaxel and oxaliplatin induce loss of IENF with severe pain in rodent model while prevention of IENF loss inhibits neuropathic pain [53, 55, 63]. Thus, the reduction of IENF loss could be regarded as one of therapeutic markers in CIPN model. We further investigated the therapeutic effect of SH003 on IENF degeneration in mice paw skin. Compared to Control, IENF seem to be fragmented and degenerated (white arrow) in docetaxel-treated mice, whose morphological changes indicate axonal degeneration (Figures 4(a) and 4(b)). SH003 treatment decreased docetaxel-induced histopathological changes whereas a layer (yellow arrow) was thickened (Figure 4(c)). This layer located in the mid-epidermis is stratum lucidum containing dead keratinocytes and is present in the palms and soles [64]. The thickness of this layer is regulated by the rate of mitosis of the epidermal cells [65]. Stratum lucidum layer contains a large amount of eleidin which is known to protect skins by blocking infiltration of water [66]. However, the role of stratum lucidum in neuropathic pain is unknown yet. Although further studies are still needed to investigate the possible role of components in stratum lucidum in DIPN model, our data suggest that histological change in stratum lucidum is one of the therapeutic markers for SH003 on DIPN.

*3.4. Oral Administration of SH003 Inhibited Docetaxel-Induced Production of Proinflammatory Cytokines in Bloodstream of C57BL/6 Mice.* In CIPN model, pain mediators including inflammatory cytokines are increased in peripheral nerve spinal cord and sciatic nerves [67]. *In vivo* studies demonstrated that levels of TNF-$\alpha$ and IL-6 are elevated in CIPN animal model [68, 69]. It was also demonstrated that neutralizing antibodies against TNF-$\alpha$ or IL-6 reduce chemotherapy-induced allodynia [69, 70]. The present study investigated whether SH003 reduces TNF-$\alpha$ and IL-6 in DIPN model. Docetaxel injection increased levels of TNF-$\alpha$ and IL-6 in plasma sample (Figures 5(a) and 5(b)). The present data showed that DIPN is associated with inflammatory neuropathy [8]. In SH003 group, the inflammatory

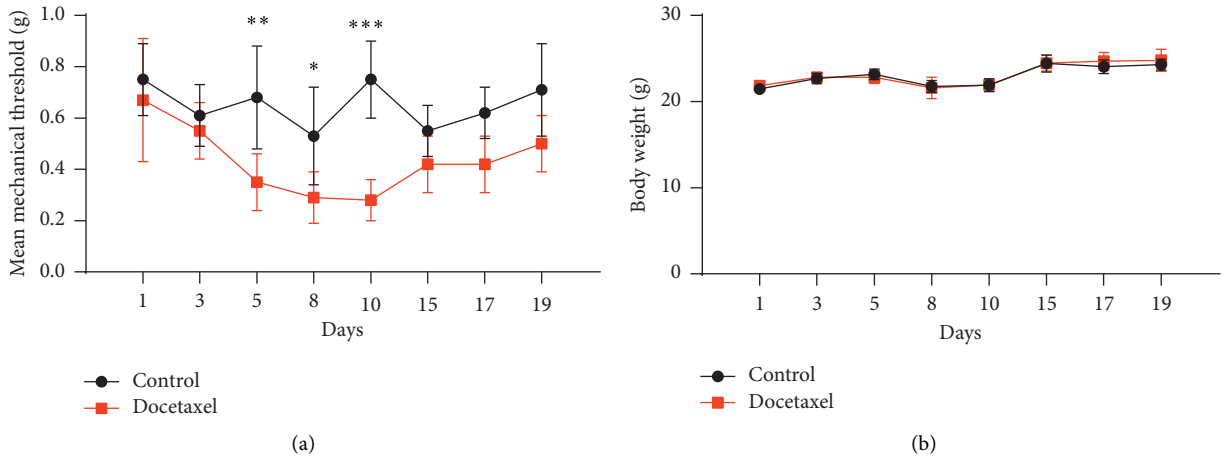



Figure 2: Docetaxel injection induced mechanical allodynia in C57BL/6 mice. The mice were divided into "Control" ($n = 6$) and "Docetaxel" ($n = 6$). Docetaxel was intravenously injected via tail vein of C57BL/6 mice on the 1st day, and mechanical allodynia threshold by von Frey filament was followed up. Mean mechanical threshold (a) and body weight (b). The differences of means between the groups were analyzed by two-way ANOVA using Sidak's multiple comparisons test ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$; Docetaxel *vs.* Control).

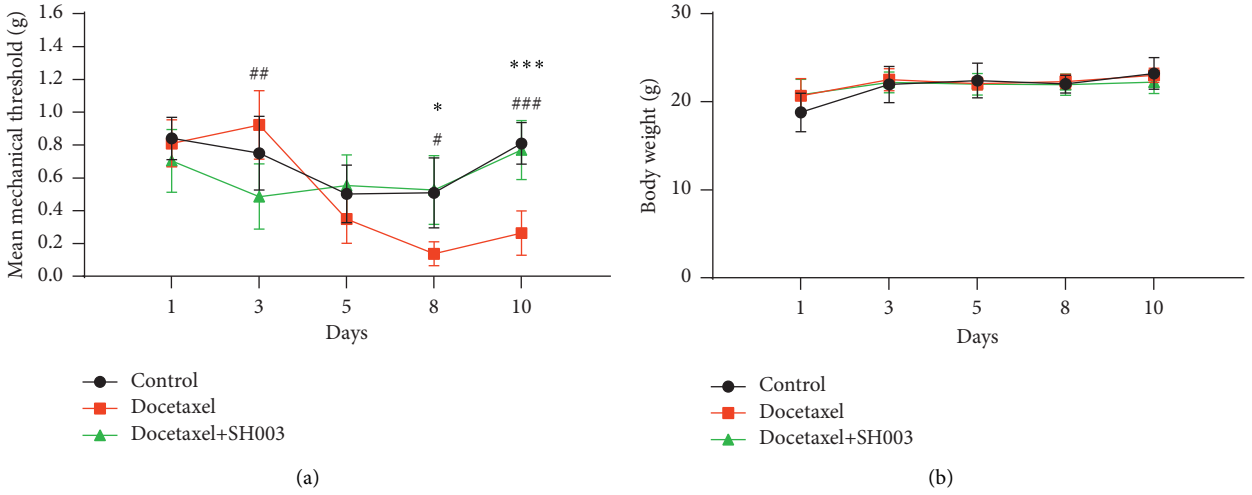
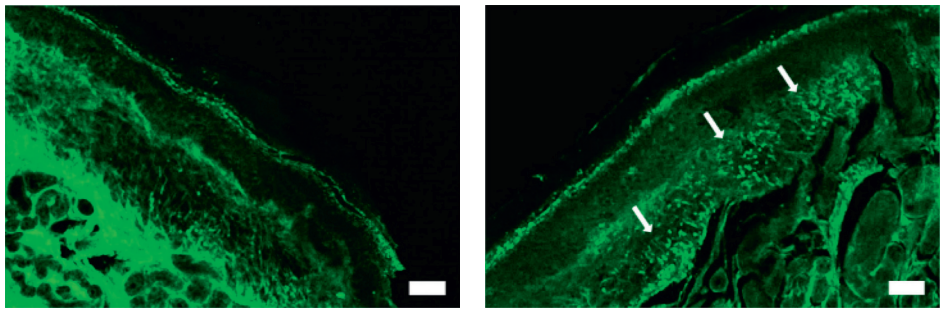
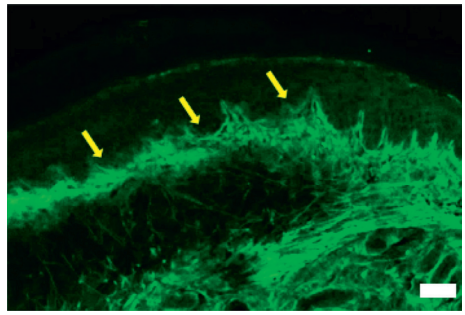



Figure 3: Effect of SH003 administration on docetaxel-induced mechanical allodynia in C57BL/6 mice. The mice were divided into Control ($n = 5$), Docetaxel ($n = 5$), and Docetaxel + SH003 ($n = 5$) groups. Docetaxel was intravenously injected via tail vein of C57BL/6 mice on the 1st day, and mechanical allodynia threshold by von Frey filament was followed up. From 3rd day, SH003 was orally administered at least 60 min before the performance of mechanical allodynia test. Mean mechanical threshold (a) and body weight (b). Data are presented as mean ± SEM. The differences of means between the groups were analyzed by two-way ANOVA using Sidak's multiple comparisons test ($^{*}P < 0.05$, Docetaxel *vs.* Control; $^{***}P < 0.001$, Docetaxel *vs.* Control; $^{\#}P < 0.05$, Docetaxel + SH003 *vs.* Docetaxel; $^{\#\#\#}P < 0.001$, Docetaxel + SH003 vs. Docetaxel).



(a) (b)

Figure 4: Continued.



(c)

Figure 4: Effect of SH003 administration on degeneration of IENF induced by docetaxel. The hind paw skins were isolated and prepared for immunofluorescence staining of IENF in Control (a), Docetaxel (b), and Docetaxel + SH003 (c) groups. The representative images of PGP 9.5-labeled IENF in paw skins were acquired using Zeiss LSM5 PASCAL confocal laser scanning microscope system. White scale bar indicates 50 $\mu$m (magnification: 10x). White and yellow arrows indicate the degenerated IENF and stratum lucidum layer, respectively.

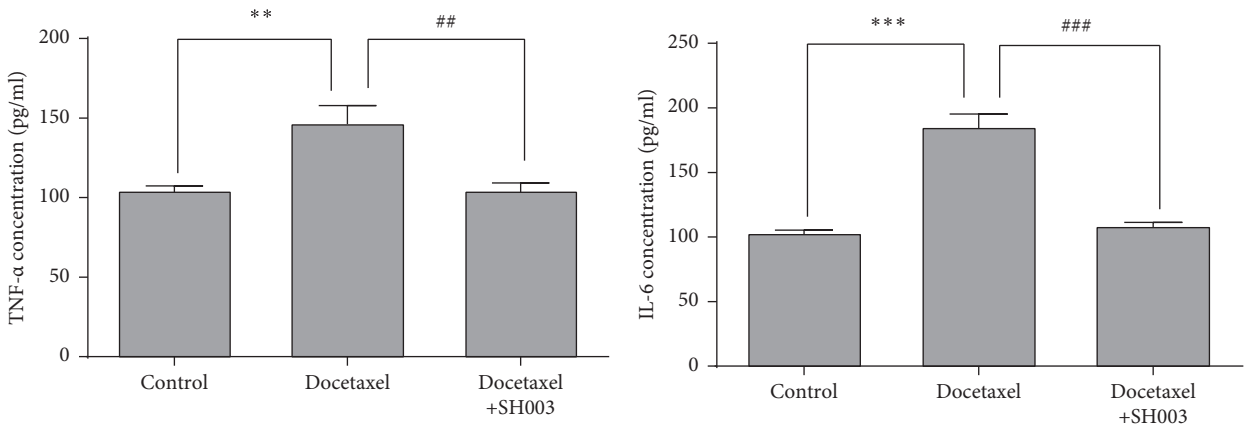



Figure 5: Effect of SH003 administration on the expression of TNF-$\alpha$ and IL-6 in plasma of DIPN mice. Plasma sample was prepared, and levels of inflammatory cytokines TNF-$\alpha$ (a) and IL-6 (b) were assessed using a DuoSet ELISA kit according to the manufacturer's instructions. Data are presented as mean ± SD of the mean ($n = 5$ for each group). The differences of means between the groups were analyzed by one-way ANOVA using Tukey's post hoc test ($^{**}P < 0.001$, Docetaxel *vs.* Control; $^{***}P < 0.001$, Docetaxel *vs.* Control; $^{\#\#}P < 0.01$, Docetaxel + SH003 *vs.* Docetaxel; $^{\#\#\#}P < 0.001$, Docetaxel + SH003 *vs.* Docetaxel).

level increased by docetaxel was reduced as much as Control group (Figures 5(a) and 5(b)). We also demonstrated that SH003 inhibition of DIPN is mediated by relieving inflammation.

*3.5. Oral Administration of SH003 Inhibited Docetaxel-Induced Phosphorylation of NF-κB and STAT3 in Both L4-L6 Spinal Cord and Sciatic Nerve of C57BL/6 Mice.* NF-κB and STAT3 would be one of the readouts for CIPN [8, 25–29]. Moreover, TNF-$\alpha$ and IL-6 are involved in the activation of NF-κB and STAT3 in neuroinflammation [71–73]. Thus, we further examined whether SH003 treatment inhibits NF-κB and STAT3 in both of lumbar spinal cord and sciatic nerves. Docetaxel treatment increased the expression of phospho-NF-κB and phospho-STAT3 in both L4-L6 spinal cords and sciatic nerves whereas SH003 treatment reversed it (Figures 6(a) and 6(b)). The present study suggested that activation of NF-κB and STAT3 is a putative biomarker of DIPN while further studies are necessary to decipher how they are involved in the development and progression of DIPN. Moreover, SH003 inhibition of NF-κB and STAT3 shown in our data would be a hint for SH003 application to other peripheral neuropathic diseases. Deregulation of NF-κB and STAT3 appears to be involved in the progression of neuropathic pain in diabetes [74–77]. Alcoholic neuropathy is tightly linked to NF-κB deregulation [78, 79]. NF-κB deregulation is likely to be related to dysimmune neuropathies such as acute Guillain–Barré syndrome [80]. Therefore, SH003 may be applicable for treating other neuropathic diseases related to the deregulation of NF-κB and STAT3.

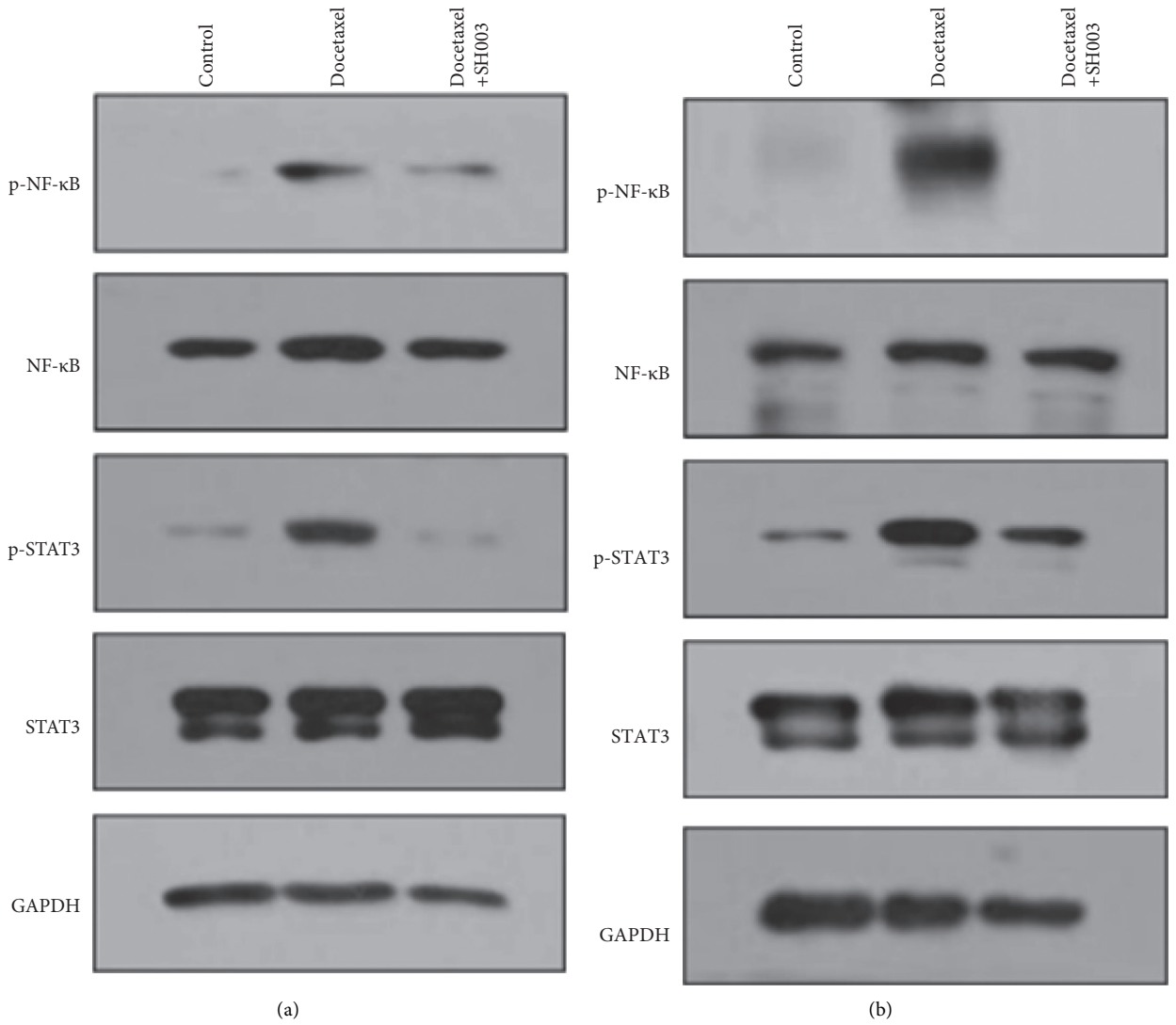



Figure 6: Effect of SH003 administration on the expression of NF-$\kappa$B and STAT3 in lumbar (L4-L6) spinal cord and sciatic nerves of DIPN mice. At the end of the experiment, lumbar (L4-L6) spinal cord and sciatic nerves were isolated. Total proteins were extracted and separated by SDS-PAGE. Expression of NF-$\kappa$B and STAT3 from spinal cord (a) and sciatic nerves (b) was analyzed by western blotting. GAPDH was used as internal marker.

## 4. Conclusions

Single injection of docetaxel at MTD can develop acute pain syndrome model in C57BL/6 mice. Moreover, activation of NF-$\kappa$B and STAT3 in sciatic nerves and L4-L6 spinal cords and upregulation of TNF-$\alpha$ or IL-6 in bloodstream may be biomarkers of DIPN in mouse model. However, further studies are needed to investigate the role of those putative biomarkers in the development and progression of DIPN. Furthermore, we suggest that SH003 administration is able to relieve docetaxel-induced neuropathic pain and this efficacy needs to be monitored in current clinical studies.

## Data Availability

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

## Acknowledgments

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*Research Article*

# Comparative Effectiveness of Collaborative Treatment with Korean and Western Medicine for Low Back Pain: A Prospective Cohort Study

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In Korea, low back pain is the ailment that is most frequently treated using collaborative care regimens that include aspects of Western and traditional Korean medicine. As part of a national pilot project on the collaboration between Western and Korean medicine, we aimed to investigate the clinical effectiveness of collaborative treatment and compare it with treatment methods that involved only Korean or Western Medicine practices for patients with low back pain. This nationwide, multicenter, prospective, observational, and comparative study spanned 8 weeks, during which patients with low back pain were evaluated at three time points (at baseline, 4 weeks, and 8 weeks). The primary outcome was low back pain-related disability measured by the Oswestry Disability Index, while the secondary outcomes included severity of low back pain (as on a numeric rating scale) and quality of life (as per a 5-level EuroQol-5 dimensions questionnaire). We analyzed 150 patients (including 129 per-protocol cases) and found that the Oswestry Disability Index and 5-level EuroQol-5 dimensions showed statistically significant differences over time between the collaborative treatment group and the sole treatment group after adjusting for sex, income level, and age. Conversely, the numeric rating and EuroQol-visual analog scales showed no significant between-group differences over time. Based on our findings, we believe that collaborative treatment that includes parallelly administered aspects of Western and Korean medicine can benefit patients with low back pain by facilitating functional improvements and lead to a better quality of life.

## 1. Introduction

Low back pain (LBP) affects 540 million people across the world [1] and the loss of working hours due to LBP increased by 54% in 2015 compared to 1990 [2]. A multidisciplinary approach is imperative for treating LBP effectively [3], which should ideally include a combination of pharmacological and nonpharmacological treatments that result in functional recovery of muscles and tendons [4, 5].

In South Korea, LBP is one of the diseases most frequently treated using traditional Korean Medicine (KM), which is often administered parallelly with Western Medicine (WM) or as a gradual successive step after a WM regimen [6]. Korea has a dual medical system wherein WM doctors (MDs) and KM doctors (KMDs) operate cohesively. This dual system is advantageous because it increases patient satisfaction and expands the range of treatment options [7, 8]; however, its pitfalls are that the medical costs increase due to redundant treatments from both systems and the treatment modality becomes dependent on the patients' choices, which increases the conflict between the KM and WM systems [9, 10].

To address these concerns, the Korean Ministry of Health and Welfare launched the "WM-KM collaborative treatment (CT) pilot project" to investigate the utility of collaborative treatment (CT), develop an ideal CT model for each disease, and evaluate the clinical efficacy and cost-effectiveness of CT. Initiated in November 2017, this second-stage project aims to

assess the feasibility of providing a "collaboration fee" to the participating institutions from the National Health Insurance System as an additional reimbursement. This fee seeks to facilitate collaboration between MDs and KMDs by applying insurance coverage to both WM and KM treatments when a patient is treated by both an MD and KMD for the same disease on the same day.

In conjunction with this pilot project, we aimed to conduct a prospective observational analysis of the Registry for Korean Medicine and Western Medicine Collaborative Treatment (REKOMENT) to compare the clinical effectiveness of CT to that of sole treatment (ST) with WM or KM for patients with LBP––an ailment that is largely treated using CT in Korea.

## 2. Methods

*2.1. Study Design and Setting.* This multicenter, prospective, observational study targeted all LBP patients who visited any of the hospitals participating in the WM-KM collaborative treatment pilot project from 7 November 2017 to 14 October 2019. Four university-affiliated hospitals and three KM hospitals participated in this research project and each institution's IRB approved the study: Korean Medicine Hospitals of Dongguk University (DUBOH-IRB 2018-0002), Daejeon University (DJDSKH-18-BM-06), Dongshin University (DSMPOS18-2), and Gachon University (18-104) and Design Hospital (P01-201806-21-001), Samse Hospital (P01-201807-21-004), and the Mokhuri Hospital (P01-201807-21-011). The study design was registered at "Clinical Research Information Service" (CRIS) as a prospective study, and the details can be found in the published study protocol [11]. The entire research process was conducted in accordance with the Declaration of Helsinki and the Good Research Practices recommended by the International Society for Pharmacoeconomics and Outcomes Research (ISPOR).

*2.2. Participants.* This study included LBP patients over the age of 19 who visited any of the aforementioned clinics for the first time and voluntarily provided their written informed consent to participate. The patients who were currently participating in another clinical trial, or found it difficult to comply with the study schedule, or comprehend and respond to study questionnaires were excluded.

Announcements regarding the study were displayed publicly at the institutions to facilitate equal-opportunity participation.

*2.3. Patient Groups.* As this was an observational study, participants were treated using individualized treatment plans that were designed according to their specific disease condition, the duration and severity of LBP, and underlying diseases, if any. The Korean Medicine clinical practice guideline outlines the general treatment principles for chronic LBP [12], and in our study, the attending MD or KM used their expertise to decide whether a particular participant should receive CT or ST (with either WM or KM). More details regarding the treatment methods are outlined in "clinical pathways for collaborative treatment of low back pain" provided as a supplementary file. ST treatment methods are also included in the CT pathways. Participants who received CT were assigned to the CT group and those who received ST were assigned to the ST group. We assessed differences between the CT and ST groups by comparing their baseline characteristics.

The participants received routine care and were neither subjected to nor denied any specific treatments by partaking in this study. Afterwards, those patients who had incurred a collaboration fee even once over the course of the study were categorized and analyzed as part of the CT group. A collaboration fee required a written proof of collaborative care provided by both the MD and the KMD who treated the patient for the same disease on the same day.

*2.4. Study Blinding.* The subjects and practitioners (KMs and MDs) could not be blinded because of the observational design of the study. However, the evaluators who conducted the assessment and performed the statistical analysis were blinded.

*2.5. Outcome Measurements.* The participants undertook surveys that were conducted at three time points: immediately after initial treatment (baseline), after 4 weeks, and after 8 weeks. The baseline characteristics were collected during the first survey and clinical indicators were evaluated in every survey. The baseline surveys were conducted by researchers from each institution via face-to-face interviews, while the second and third surveys were conducted by one researcher via a phone interview. All the surveys were conducted using a well-defined questionnaire and lasted 5–10 minutes each.

*2.6. Primary Outcome.* The primary endpoint in this study was the Oswestry Disability Index (ODI) score, which captures the intensity of LBP and the degree of LBP-related disability caused in daily life [13]. The ODI measures 10 parameters, namely, pain intensity, personal care, lifting, walking, sitting, standing, sleeping, sex life, social life, and traveling [14]. The Minimal Important Difference (MID) refers to the minimum clinically important difference that measures changes in the patient's condition [15]; for the ODI, the MID was set at 10 points [16]. In this study, we used the Korean version of the ODI proposed by Kim et al. [17].

*2.7. Secondary Outcomes.* The secondary endpoints in this study were the numeric rating scale (NRS), 5-level EuroQol-5 dimensions (EQ-5D-5L), and EuroQol-visual analog scale (EQ-VAS) measurements.

NRS is an index that converts the LBP-related pain intensity into numerical values using the patient's verbal rating of their LBP. It is scored between 0 (no pain) and 10 (worst pain imaginable) [18]. The MID of NRS for LBP was established at 2 points [16, 19].

EQ-5D-5L measures the patient's health-related quality of life (HRQOL) [20], which is rated according to five response levels to questions in five dimensions, namely, mobility, self-care, usual activity, pain/discomfort, and anxiety/depression. We used the officially verified Korean version of the EQ-5D-5L [21].

EQ-VAS calculates the overall health level of the patient using a vertical scale that ranges from 0 to 100 points [22]. We calculated the utility value of HRQOL and analyzed it using the recently reported national tariff, which was developed and published to translate the five domain values of EQ-5D into utility values that could be scored between 0 (dead) and 1 (perfect health) [23].

The higher the value calculated using EQ-5D-5L, the higher was the HRQOL. The MID of EQ-5D and EQ-VAS for chronic LBP were set at 0.08 and 10.5, respectively [19, 24].

*2.8. Statistical Analysis.* Continuous variables were analyzed after being tested for normality using the Shapiro–Wilk test. Differences in categorical variables between groups were analyzed using the Chi-square and Fisher's exact tests. Between-group differences in the means of continuous variables were analyzed using the Student's *t*-test and the Mann–Whitney *U* test. Differences in more than three groups were analyzed using analysis of variance (ANOVA) and the Kruskal–Wallis test.

Observations for the primary endpoint were presented as per-protocol (PP) data, and the analysis of the difference in mean variations between the two groups during the observation period was presented by performing repeated-measures ANOVA. The clinical effectiveness was analyzed using intention-to-treat (ITT) data. The amount and mechanisms of missing data were verified after processing the missing items using multiple imputation (MI). The final results were generated following analysis with the Generalized Linear Mixed Effect Model (GLMM), a random-effects model used to analyze longitudinal data.

All data analyses were performed using the Stata MP version 14.2 software (StataCorp, Texas, USA). The statistical significance was considered at a $P$ value of $<0.05$.

## 3. Results

*3.1. Participants.* We screened and registered 163 patients, of which 13 were excluded because they visited the hospital only once. Then, we analyzed 150 patients as ITT subjects, but only 129 had complete data with no missing items. These 129 patients became the PP subjects for our clinical effectiveness analysis. Of the 150 patients included in the ITT analysis, 74 patients had received both WM and KM treatments so they were labelled as the CT group, and the rest of the 76 patients who received only usual care were labelled as the ST group. Four patients in the CT group and 17 patients in the ST group were excluded from the PP analysis because of missing data (Figure 1). Of the 21 patients with missing data, 1 was missing an evaluation variable in the baseline survey, 18 were unable to complete the second and third surveys, and 2 were lacking in their hospital medical records and administrative data.

*3.2. Baseline Characteristics of Patients.* There were no significant differences between the two groups according to age, diagnosis, NRS, and EQ-VAS values; however, the baseline ITT analysis showed significant between-group differences according to sex, income level, and ODI and EQ-5D-5L measurements. We used these baseline differences to develop an imputation model and analyze the effectiveness of the GLMM (Table 1).

*3.3. Missing Patient Data.* The final proportion of patients with missing data in terms of the main variables of primary and secondary endpoints was 14.7%, with the missing data representing 8.0% of the total data. We performed Little's test to check missing mechanisms. The results showed a missing completely at random (MCAR) mechanism (Chi-square value = 48.2677, $P$ value = 0.3813). On verifying the covariate association of the base variables (sex, income level, and age), we observed an estimated covariance-dependent missing completely at random (CD-MCAR) mechanism. Thus, after processing the missing data by MI, which sets the base variables as explanatory variables, we used ITT data for our analysis (Table 2).

*3.4. Effectiveness Outcomes*

*3.4.1. Mean Changes in Outcomes during the Study Period (PP).* Primary outcome: the measured ODI was $27.01 \pm 15.28$ in the ST group and $35.49 \pm 14.98$ in the CT group immediately after initial treatment. After 4 weeks, the ODI was $22.37 \pm 15.30$ in the ST group and $21.29 \pm 12.37$ in the CT group. After 8 weeks, the ODI was $19.81 \pm 15.08$ in the ST group and $17.52 \pm 15.68$ in the CT group.

These results indicated that both groups experienced a statistically significant reduction in ODI over time (within-group effects). This reduction also showed statistically significant between-group differences, which suggested that the degree of disability caused by LBP reduced markedly in the CT group compared to the ST group ($P < 0.001$) (Table 3, Figure 2).

Secondary outcomes: the measured NRS was $5.15 \pm 1.89$ in the ST group and $5.41 \pm 1.82$ in the CT group immediately after initial treatment. After 4 weeks, the NRS was $3.29 \pm 1.88$ in the ST group and $2.93 \pm 1.63$ in the CT group. After 8 weeks, the NRS was $2.64 \pm 1.99$ in the ST group and $2.51 \pm 2.10$ in the CT group. These results showed that both groups experienced a statistically significant reduction in NRS over time (within-group effects). However, there were no significant between-group differences over time.

The measured EQ-5D-5L was $0.74 \pm 0.13$ in the ST group and $0.68 \pm 0.15$ in the CT group immediately after initial treatment. After 4 weeks, EQ-5D-5L was $0.83 \pm 0.11$ in the ST group and $0.84 \pm 0.10$ in the CT group. After 8 weeks, EQ-5D-5L was $0.87 \pm 0.14$ in the ST group and $0.89 \pm 0.12$ in the CT group. The results indicate that both groups experienced a statistically significant increase in EQ-5D-5L over time,

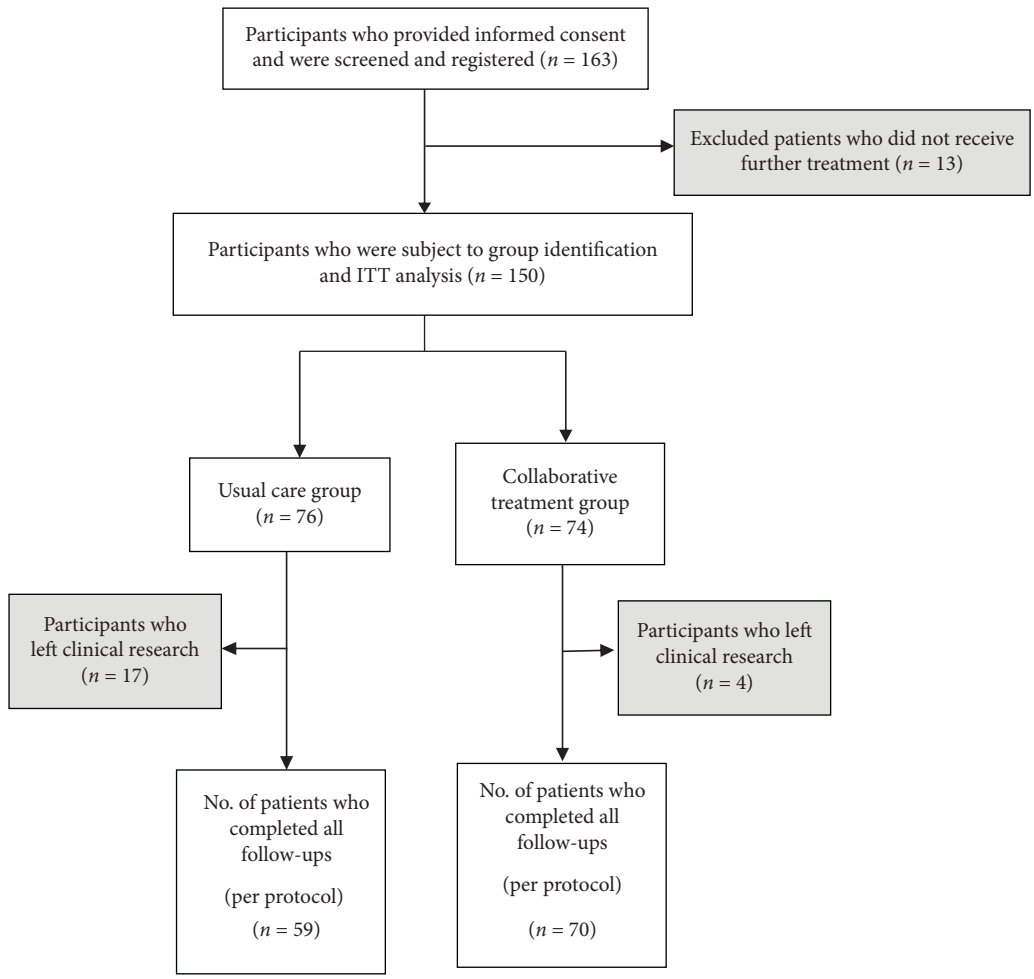


Figure 1: Flow diagram of patient selection.

Table 1: Baseline characteristics of patients.

| Variables | | Usual care $N = 76$ | | Collaboration $N = 74$ | | $P$ value |
|---|---|---|---|---|---|---|
| Sex | Male | 24 | 31.58% | 39 | 52.7% | 0.009** |
| | Female | 52 | 68.42% | 35 | 47.3% | |
| | Unknown | 54 | 71.05% | 41 | 55.41% | 0.003** |
| Income (monthly) (won) | Under 2 million | 5 | 6.58% | 4 | 5.41% | |
| | 2–5 million | 8 | 10.53% | 26 | 35.14% | |
| | 5–10 million | 7 | 9.21% | 2 | 2.7% | |
| | Over 10 million | 2 | 2.63% | 1 | 1.35% | |
| Age (years old) | | 44.39 | 17.33 | 48.78 | 13.9 | 0.0896 |
| Diagnosis | Dorsalgia | 37 | 48.68% | 33 | 44.59% | 0.825 |
| | Sprain | 29 | 38.16% | 29 | 39.19% | |
| | HIVD and others | 10 | 13.16% | 12 | 14.67% | |
| ODI | | 26.75 | 15.32 | 35.44 | 14.98 | 0.0006** |
| NRS | | 5.066 | 2.016 | 5.466 | 1.80 | 0.2036 |
| EQ-5D-5L | | 0.7344 | 0.1444 | 0.6786 | 0.1522 | 0.0226** |
| EQ-VAS (mm) | | 54.46 | 16.65 | 55.03 | 20.23 | 0.8527 |
| Duration (day) | | 198.8 | 820.90 | 85.53 | 465.40 | 0.3019 |

*Frequency, mean, and percent or standard deviation; **$P$ value < 0.05; HIVD: herniated intervertebral disc; ODI : Oswestry Disability Index; NRS: numeric rating scale; EQ-5D-5L: 5-level EuroQol-5 dimensions; EQ-VAS : EuroQol-visual analog scale.

both within each group and between the two groups. This suggests that the HRQOL increased markedly in the CT group compared to the ST group (between-group effects) ($P = 0.001$).

The measured EQ-VAS was $55.69 \pm 16.4$ in the ST group and $55.84 \pm 19.79$ in the CT group immediately after initial treatment. After 4 weeks, the EQ-VAS score was $68.14 \pm 16.40$ in the ST group and $71.20 \pm 16.32$ in the CT

Table 2: Summary of censored data for outcome variables.

| Variables | Never censored | Ever censored | Total |
|---|---|---|---|
| Participant (N) | | | |
| Control group | 59 | 17 | 76 |
| Case group | 69 | 5 | 74 |
| Total | 128 | 22 | 150 |
| Participant (%) | | | |
| Control group | 77.63 | 22.37 | 100.00 |
| Case group | 93.24 | 6.76 | 100.00 |
| Total | 85.33 | 14.67 | 100.00 |
| Potential periods of observation (day) | | | |
| Control group | 708 | 204 | 912 |
| Case group | 828 | 60 | 888 |
| Total | 1536 | 264 | 1800 |
| Periods with data available (day) | | | |
| Control group | 708 | 85 | 793 |
| Case group | 828 | 35 | 863 |
| Total | 1536 | 120 | 1656 |
| Periods with data available (%) | | | |
| Control group | 100.00 | 41.67 | 86.95 |
| Case group | 100.00 | 58.33 | 97.18 |
| Total | 100.00 | 45.45 | 92.0 |
| Total percent of the periods with censored data (%) | 0 | 54.55 | 8.00 |
| Total percent of patient numbers censored | | | 14.67% |
| | | Chi-square distance | $P$ value |
| MCAR test result | | 48.26 | 0.38 |
| MCAR (CDM) test result | | 102.77 | 1.00 |

MCAR: missing completely at random; CDM: covariate dependent missingness.

Table 3: Mean changes in the outcomes of effectiveness per group over time and the results of repeated-measures Analysis of Variance (per-protocol).

| Outcome measure | Treatment group | Baseline | | 4 weeks | | 8 weeks | | RM ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | Group | Time | Group × time |
| ODI | Usual care | 27.01 | 15.28 | 22.37 | 15.30 | 19.81 | 15.08 | 0.438 | <0.001* | <0.001* |
| | Collaboration | 35.49 | 14.98 | 21.29 | 12.37 | 17.52 | 15.68 | | | |
| NRS | Usual care | 5.15 | 1.89 | 3.29 | 1.88 | 2.64 | 1.99 | 0.763 | <0.001* | 0.200 |
| | Collaboration | 5.41 | 1.82 | 2.93 | 1.63 | 2.51 | 2.10 | | | |
| EQ-5D-5L | Usual care | 0.74 | 0.13 | 0.83 | 0.11 | 0.87 | 0.14 | 0.555 | <0.001* | 0.001* |
| | Collaboration | 0.68 | 0.15 | 0.84 | 0.10 | 0.89 | 0.12 | | | |
| EQ-VAS | Usual care | 55.69 | 16.45 | 68.14 | 16.40 | 69.81 | 16.72 | 0.302 | <0.001* | 0.482 |
| | Collaboration | 55.84 | 19.79 | 71.20 | 16.32 | 73.91 | 19.27 | | | |

*$P$ value < 0.05; SD: standard deviation; ODI: Oswestry Disability Index; NRS: numeric rating scale; EQ-5D-5L: 5-level EuroQol-5 dimensions; EQ-VAS: EuroQol-visual analog scale. For ODI and NRS, the decreased amount indicates the degree of improvement of the symptoms (and how effective the treatment was). For EQ-5D and EQ-VAS, the increased amount indicates the same as above.

group. After 8 weeks, the EQ-VAS score was 69.81 ± 16.72 in the ST group and 73.91 ± 19.27 in the CT group. The results showed that both groups experienced a statistically significant increase in EQ-VAS over time (within-group effects), but there were no significant between-group differences (Table 3, Figures 3–5).

*3.4.2. Effectiveness Outcomes When Adjusting Covariates (ITT).* After verifying and performing MI on the missing data, we observed that the clinical effectiveness of both types of treatments had changed over time. We analyzed the between-group differences in this change using GLMM. When we adjusted the variables of sex, income level, age, and diagnosis group, ODI and EQ-5D-5L showed statistically significant differences between the two groups over time. Conversely, NRS and EQ-VAS scores showed no between-group differences over time (Table 4).

## 4. Discussion

A recent study used national health insurance data in Korea to analyze the medical costs of WM and KM for patients with joint diseases and revealed that the largest portion of WM costs were attributed to physiotherapy and more than 70% of KM costs referred to medical procedures, such as acupuncture, moxibustion, and cupping [25]. Even though WM and KM apply different treatments

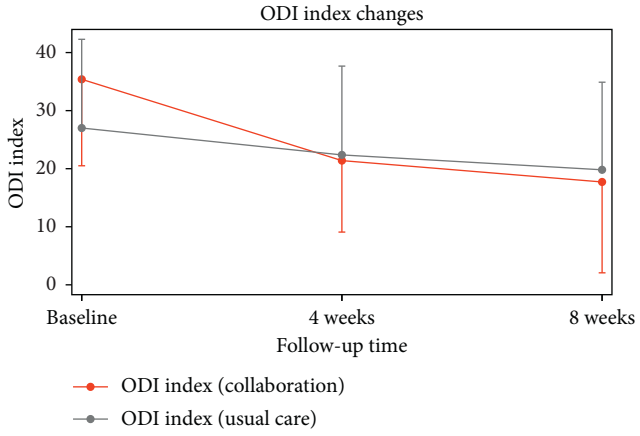


Figure 2: Mean changes in ODI during the follow-up period. ODI: Oswestry Disability Index.

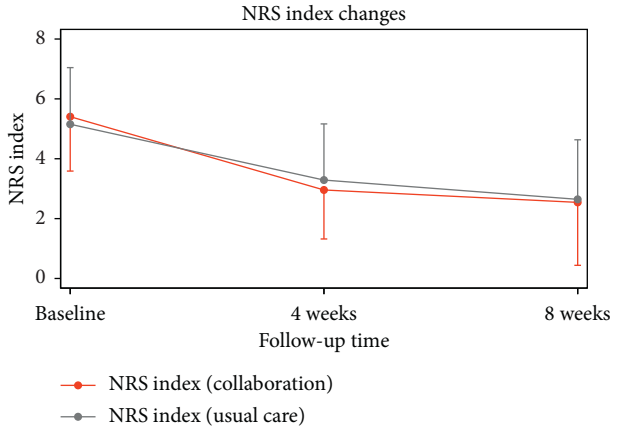


Figure 3: Mean changes in NRS during the follow-up period. NRS: numeric rating scale.

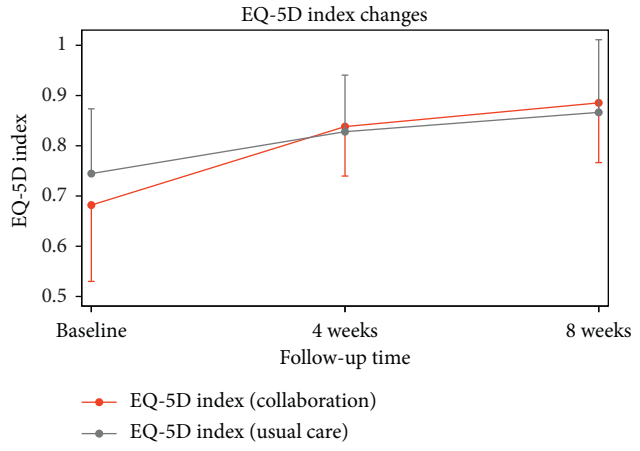


Figure 4: Mean changes in EQ-5D-5L during the follow-up period. EQ-5D-5L: 5-level EuroQol-5 dimensions.

for the same disease, the study found that patients who received both WM and KM treatments for the same disease on the same day could not receive insurance coverage for these procedures, which burdened them with

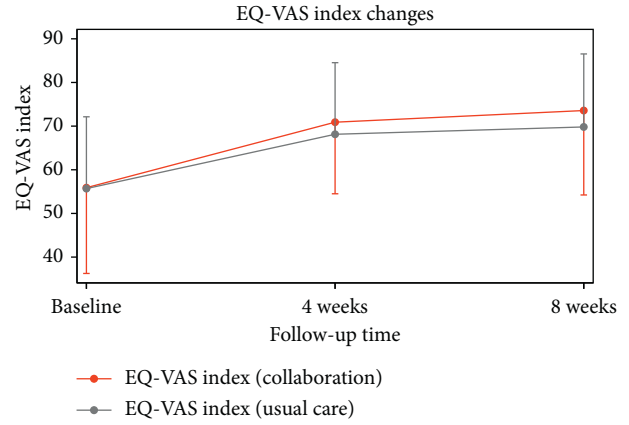


Figure 5: Mean changes in EQ-VAS during the follow-up period. EQ-VAS: EuroQol-visual analog scale.

medical expenses. However, this should not be the norm because medical institutions in Korea have been legally allowed to perform CT since 2010 and the use of CT has been growing [26].

In this study, we defined CT as a medical treatment that combines aspects of WM and KM and is administered in a hospital setting by both WM and KM doctors who share a patient's medical information and consult each other to plan the treatment [27]. For example, if an MD decides that a patient requires KM treatment in addition to analgesic medicine and physical therapy, the MD can consult a KMD and collaboratively design a treatment plan that includes some KM techniques to enhance the effectiveness of the overall treatment and avoid duplication.

The first-stage trial project of REKOMENT that started in July 2016 revealed that, currently, LBP is the ailment that is most frequently treated using CT in Korea [7]. In this study, we enrolled a wide pool of patients who are participating in the ongoing "WM-KM CT pilot project" in Korea and analyzed patient data from REKOMENT to assess the clinical effectiveness of CT on LBP. Therefore, the participants' disease duration, severity, cause, and accompanying symptoms varied greatly, and we set the observation period to 8 weeks to evaluate the short-term effectiveness of CT in its early phase of administration.

We also examined the factors that influenced each patient's decision to undergo CT. The participants' baseline characteristics showed that those with a relatively severe LBP-related disability and a low quality of life tended to choose CT. This finding is consistent with those of previous studies, which showed that patients with spinal cord disease (who usually experience severe LBP) tended to opt for KM treatments, such as acupuncture, moxibustion, cupping, and Chuna therapy [28]. Further, patients with limiting LBP chose acupuncture and chiropractic treatments more often than those with nonlimiting LBP [29]. However, since our study was conducted in seven CT-focused institutions, it was difficult for us to accurately convey the general situation of CT use in Korea. Therefore, comprehensive, large-scale studies that include standard medical

Table 4: Results of the generalized linear mixed model analysis of effectiveness outcomes (intention-to-treat).

| Random effects | ODI | | NRS | | EQ-5D-5L | | EQ-VAS | |
|---|---|---|---|---|---|---|---|---|
| | $\beta$ | SE | $\beta$ | SE | $\beta$ | SE | $\beta$ | SE |
| Collaboration | 11.98* | 3.37 | 0.36 | 0.44 | −0.08* | 0.03 | −2.06 | 4.27 |
| Follow-up time | −3.54* | 1.08 | −1.25* | 0.15 | 0.07* | 0.01 | 7.37* | 1.42 |
| Collaboration × follow-up time | −5.17* | 1.49 | −0.19 | 0.20 | 0.04* | 0.01** | 1.70 | 1.98 |
| Sex | 0.38 | 2.08 | 0.13 | 0.26 | −0.02 | 0.02 | −5.66* | 2.09 |
| Age | 0.12 | 0.07 | 0.02* | 0.01 | 0.00 | 0.00 | −0.04 | 0.07 |
| Diagnosis (vs. sprain) | −1.58 | 2.24 | −0.31 | 0.28 | −0.01 | 0.02 | −1.12 | 2.24 |
| (vs. HIVD) | −2.12 | 3.00 | 0.47 | 0.37 | −0.02 | 0.02 | −3.37 | 2.99 |
| Intercept | 24.59* | 3.89 | 5.08* | 0.50 | 0.72* | 0.03 | 54.62* | 4.38 |

*$P$ value < 0.05; ODI : Oswestry Disability Index; NRS: numeric rating scale; EQ-5D: 5-level EuroQol-5 dimensions; EQ-VAS : EuroQol-visual analog scale; SE: standard error; HIVD: herniated intervertebral disc. Reference group of diagnosis dummy variable is dorsalgia.

institutions should be conducted to understand the general utility of CT and the main factors that influence patient decisions.

In terms of clinical effectiveness, our study showed that patients in both the CT and ST groups experienced significant positive changes in pain intensity, daily-life disability, and HRQOL. However, we observed that CT was more effective than ST in reducing disability and improving the HRQOL.

A clinical study on chronic back pain stated that it was clinically significant if the NRS score decreased by more than 2.4 points [30] or varied by more than 20% between two time points [31, 32]. In our study, LBP significantly reduced in intensity in both the CT and ST groups. When patients self-assess chronic pain, they tend to be influenced by nonpainful factors, such as experience and emotions regarding the disease condition and treatment, and they consider pain intensity as a comparative rather than a linear index [33]. These factors were likely to have played a role in our study because it was not a double-blind study. Nonetheless, ODI, which measures the degree of disability caused by LBP, evaluates pain intensity according to a five-point scale using questions on daily life, physical activity, and social life. [34]. The ODI questions are more detailed than those of NRS and the answer choices are relatively objective (e.g., walking distance, standing time, and sleeping time); therefore, it is likely that the patients' conditions are reflected objectively and in more detail. Furthermore, patients with musculoskeletal diseases tend to restrict specific movements to avoid pain; therefore, while evaluating pain relief, it is important to consider not only pain intensity but also limitations in the patient's overall function [35].

Chronic LBP is caused by neuroplastic changes in sensorimotor control; thus, cognitive-based interventions, such as education and physical interventions, have the potential for clinical use [36]. Acupuncture, a major treatment in KM, induces peripheral sensory stimulation and is considered a bottom-up physical intervention to address neuroplastic changes. Therefore, for patients with chronic LBP, acupuncture might improve impaired somatosensory processing by improving tactile precision and reduce grey matter to facilitate greater improvement and increased fractional anisotropy in the posterior cortical somatosensory region [37]. Although there was no significant difference between the two groups in our study, findings from the cited studies warrant further research on chronic pain.

There are several limitations to this study. First, this was not a randomized controlled study and the patients were not blinded. So, we could not rule out all the factors that may influence the results, including placebo effects. Therefore, the findings of this study cannot be used to draw definite conclusions on the efficacy of ST or CT. Second, this research included all patients whose chief complaint was LBP; thus the diseases that caused the LBP may vary greatly. We used statistical analysis methods to exclude the effects of age, the severity of symptoms, and diagnosis, in order to reveal the impact of ST and CT. Since this was a multicenter study, the differences between each clinician's technique and treatment method contributed to the heterogeneity of the results. However, this study analyzed the hospitals following CT protocols as part of the "WM-KM CT pilot project." Therefore, we consider the variation in treatment methods and techniques to be minimal and unsubstantial. However, further studies focusing on a more specific causative disease are needed in the future. Third, as a multicenter, prospective cohort study, the number of patients we included was relatively small, which makes it difficult to generalize our results. To address these limitations, large-scale controlled trials are needed in the future to investigate the clinical effectiveness of CT on LBP in more detail.

Overall, in our study, patients who were treated for LBP using CT showed significant improvement in daily-life disability and HRQOL compared to those who received ST, which means that CT provides additional benefits to LBP patients and aids in faster recovery.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

Hye-Yoon Lee and Min Kyoung Cho contributed equally to this work. NamKwen Kim, Hye-Yoon Lee and Eun Hye Hyun conceived the research question and designed and carried out the study. NamKwen Kim supervised the overall research and conducted the statistical analysis. Min Kyoung Cho, Hye-Yoon Lee, and Na-Gyeong Gong drafted the manuscript. Hye-Yoon Lee, Se Yeon Lee, and NamKwen Kim provided the critical revision of the draft. All authors actively participated in the research and approved the final version of the paper.

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## Supplementary Materials

The supplementary file includes four clinical pathways depending on the disease entity associated with low back pain: (1) clinical pathway for collaborative treatment of low back pain, (2) clinical pathway for collaborative treatment of low back sprain, (3) clinical pathway for collaborative treatment of lumbar herniated intervertebral disc, and (4) clinical pathway for collaborative treatment of lumbar intervertebral disc disorder. Such variables as symptom severity and disease entity were controlled using statistical methods. (*Supplementary Materials*)

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*Research Article*

# The Efficacy of Backward Walking on Static Stability, Proprioception, Pain, and Physical Function of Patients with Knee Osteoarthritis: A Randomized Controlled Trial

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*Objective*. Impaired static stability and proprioception have been observed in individuals with knee osteoarthritis (KOA), which serves as a major factor increasing risk of fall. This study aimed to investigate the effects of backward walking (BW) on static stability, proprioception, pain, and physical function in KOA patients. *Methods*. Thirty-two subjects with knee osteoarthritis were randomly assigned to either an BW group (BG, $n = 16$) or a control group (CG, $n = 16$). The participants in the BG received combination treatment of a 4-week BW training and conventional treatments, while those in the CG was treated with conventional treatments alone. All the participants were tested for the assessment of static stability [center of pressure (COP) sway, including sway length (SL, mm) and sway area (SA, $mm^2$)] and proprioception [average trajectory error (ATE, %) and completion time (CT, second)]. Additionally, pain and knee function scores were measured by the numerical rating scale (NRS) and the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index, respectively. The assessments were conducted before and after intervention. *Results*. The COP sway (SA and SL), ATE, NRS, and WOMAC showed a significant decline at week 4 in the two groups in contrast to their baseline ($P < 0.05$). Moreover, after 4-week intervention, the SA [$(610.50 \pm 464.26)$ $mm^2$ vs. $(538.69 \pm 420.52)$ $mm^2$], NRS [$(1.56 \pm 0.63)$ vs. $(2.25 \pm 0.86)$], and WOMAC [$(11.69 \pm 2.50)$ vs. $(16.19 \pm 3.94)$] showed a significantly greater decrease in the BG compared to the CG ($P < 0.05$, respectively). However, the proprioception (ATE and CT) was closely similar between both groups at week 4 ($P > 0.05$). *Conclusion*. BW is an effective adjunct to conventional treatment in reducing pain, improving physical function and static stability for KOA patients. It should be taken into consideration when developing rehabilitation programs for people with KOA.

## 1. Introduction

Knee osteoarthritis (KOA), as a common disease, heavily compromises the health of the elderly. With the growing population of obesity and aging, the prevalence of KOA will become higher, which has been a serious global health concern [1]. Individuals with KOA always demonstrate severe symptoms including poor balance [2], stability deficits [3, 4], and impaired proprioception [5], in addition to joint swelling, pain, stiffness, muscle weakness, deformity, reduced joint motion, and disability [1]. Posture control is viewed as a key factor for the incidence of falls. Imbalance in the center of gravity of the body could reduce stability and increases the risk of falls [6], which would result in bone fractures or fatal injuries for older adults. Meanwhile, proprioception could influence the ability of limb coordination, which played a great role in postural control [7]. Therefore, proprioception impairment was harmful to the

balance of skeletal muscles around the knee joint and increased the risk of falling [3]. As was reported, pain served as an important factor for postural sway, proprioception, and quadriceps strength in subjects with KOA [8, 9]. Medical treatments could alleviate symptomatic pain and consequently contribute to be benefit for the improvements of balance, posture stability, and proprioception [10, 11]. However, medications had some limitations in the clinical practice due to the side effects [12]. Thus, it is critical and urgent to explore a safe, effective, and feasible therapy to improve balance, stability deficits, and impaired proprioception.

In recent years, with the continuous exploration of clinical practices for the treatment of KOA, many complementary and alternative medicine methods have been developed, such as Tai chi [13], herbal remedies [14–16], and Baduanjin [17]. Backward walking (BW) training is recently introduced as a physiotherapy treatment for KOA patients, and several studies [18–20] suggested that a BW program exerts an impact on pain, functional disability, quadriceps muscle strength, and performance in the patient with KOA. The latest meta-analysis [21] showed that BW, as an adjunctive therapy, with conventional treatment was effective and worth to promote in patients with KOA. Furthermore, current evidence reveals that it had been considered as a potential strategy to improve balance performance and prevent falling for health subjects [22] and the people suffering from stroke [23], or cerebral palsy [24]. It was previously proved that significant improvements on balance and gait were observed after 4-week BW training [22, 25, 26].

Until now, only one study has reported that, for KOA patients, BW has benefit for balance improvement evaluated by using a subjective scale. The effects of BW on static stability and proprioception for patients with knee osteoarthritis are still unreported. Consequently, the aim of the present study is to investigate whether the pain, physical function, postural stability, and proprioception of KOA patients could be improved following a 4-week BW intervention using a randomized controlled trial (RCT).

## 2. Methods

*2.1. Study Design.* This research was designed as a pilot RCT to explore the effect of BW on postural stability and proprioception in patients with KOA. It was carried out at the Guangdong Second Traditional Chinese Medicine Hospital from September 15, 2019, to May 15, 2020. Ethical approval was obtained from the Ethics Committee of Guangdong Second Traditional Chinese Medicine Hospital (no. E1949) and it was registered at the China Clinical Registration Center (Registration no. ChiCTR1900026400). In this study, all included participants provided written informed consent and could withdraw from the study at any time.

*2.2. Participants.* A total of 48 participants with KOA diagnosed by the American College of Rheumatology clinical criteria [27] were enrolled from outpatients of the hospital. The other inclusion criteria were (i) age from 50 to 75 years, (ii) Kellgren/Lawrence [28] (K/L) grade ≥1 in one or both knees, (iii) no balance training experience, such as Tai Chi, Baduanjin, and Yoga, prior to six months before enrollment, and (iv) an ability to stand independently on the platform for 30 seconds without any assistive device for static stability test and depict 5 circles within 120 seconds for the proprioception assessment. The exclusion criteria were (i) presence of any known inflammatory rheumatic disease/arthritis; (ii) concomitant neurologic diseases, such as stroke, Parkinson's disease, severe cardiovascular, respiratory, spinal cord injury, or other musculoskeletal diseases; (iii) presence of acute joint effusion in knees [29]; (iv) use of any medications that could affect the musculoskeletal system or postural stability; and (v) history of ankle diseases and lower extremity fracture/surgery.

The included patients in the study were randomly assigned to either a BW group (BG) or a control group (CG) in a 1:1 ratio by using a balanced randomization method in accordance with the random number table. The numbers were kept at a locked location in a sealed, opaque envelope, to be later opened on the participants' agreement to participate.

*2.3. Interventions.* The included participants received conventional treatment comprising acupotomy, medications, and routine exercise, once a week for 4 weeks. Based on the previous method [30], the subjects in both groups were treated with needle-knife (Hanzhang Acupotome; Beijing Huaxia Acupotome Medical Equipment Factory, Beijing, China) therapy at the dominant inserted points of Neixiyan (Ex-LE4) and Waixiyan (Ex-LE5), as well as the conjugate points Dubi (ST35) and Xuehai (SP10). The prescribed acupotomy treatment was performed by an experienced therapist (XM Xu, a Chief Physician with 30-year clinical experience) for the participates, once a week for 4 weeks. All of the patients were prescribed with an oral medication, Celebrex capsules (Pfizer, H20140106, 0.2 g/d, once a day), for the first 6 days, while no extra painkillers were used in the next 3 weeks. Additionally, straight leg raising, as a routine exercise, was prescribed to practice at home for both legs, 1 set of 10 repetitions, twice a day, and gradually increase exercise time to 3 sets over the 4-week period, according to their pain intensity (pain score $<3$) evaluated by using numerical rating scale (NRS) [31].

Participants allocated to the CG received the acupotomy therapy and completed the routine exercise as mentioned above. Participates in the CG were asked to maintain their daily habits and were discouraged from taking any other exercise. Patients allocated to BG were required to take part in BW training, in addition to the conventional treatment as the same treatment as the patients in the CG. According to the previous training program [18], BW program consisted of 10 min of BW training with 5-min warm-up and cool-down sessions 3 days a week for 4 weeks at their comfortable walking speed. Participates took the BW training session in the hospital for the first time under the supervision of another therapist (ZH Chen). After the initial training in hospital, the participants were instructed to continue to practice at home for the remaining

time (till week 4) and gradually increase their walking time up to 30 min over the 4-week period, if they did not obtain an increasing pain score (NRS < 3). All participants were reminded and checked up via telephone.

*2.4. Outcomes.* The demographic characteristics were collected at baseline. Static stability, proprioception, NRS, and the Western Ontario and McMaster Universities Osteoarthritis (WOAMC) Index [32] were determined by a trained therapist (WJ Chen) who was blinded to the group allocation during evaluations at two time points: baseline (week 0) and week 4.

*2.4.1. Assessment of Static Stability and Proprioception.* The parameters of center of pressure (COP) were always measured to assess postural stability, which served as an assessment for postural stability [4]. During the measurement, the participate was required to stand statically with both legs on the Dynamic and Static Balancing Instrument (Pro-kin 254P, TecnoBody Company, Italy) for 30 seconds, and COP sways including COP sway length (SL, mm) and sway area (SA, $mm^2$) were documented automatically. The participants were tested with open eyes and their upper limbs placed on the side of body. As was reported, the smaller value of COP sways (SL and SA) revealed the better postural stability [5].

Proprioception measurement was conducted on the same machine. The participates were required to depict 5 circles (the left foot in a counterclockwise direction and the right foot in a clockwise direction) along the trajectory specified in the prescribed time (120 seconds), as was prompted by the system. Additionally, the subjects were administrated to complete the task with the fastest speed and the best accuracy. During proprioception testing, the participants' upper limbs were placed on the handrail of the machine. The average trajectory error (ATE, %) and completion time (CT, second) was recorded for the measurement of proprioception [33]. The smaller ATE meant more accurate proprioception; and shorter CT represented better proprioception.

Prior to testing, participants were asked to familiarize themselves with the testing process and conduct two simulation tests. Sufficient rest periods were given between trials. All participants were tested by the same researcher (WJ Chen) in the same way, requiring the test environment to be quiet and the body to maintain a standard position.

*2.4.2. Assessment of NRS and WOMAC Score.* Pain and knee function score measured for the participates by using the NRS and the WOMAC, respectively, were assessed at baseline and week 4. NRS, a self-rated scale, indicates the level of pain (0 = no symptoms; 10 = extreme symptoms). WOMAC index comprises 3 components (24 items in total), pain (5 items), stiffness (2 items), and function (17 items). Each item graded in a numerical rating scale ranges from 0 ("none") to 4 ("extreme"), and the total score of the 24 items is 96 (pain: 20; stiffness: 8; function: 68).

*2.4.3. Safety Record.* Any occurrences of adverse events during the study would be recorded, and the affected participate would be instructed to discontinue the treatment. Meanwhile, necessary measures would be taken to deal with the adverse events.

*2.5. Statistical Analysis.* The required sample size was determined taking as a reference the data (effect size = 0.59, 1-$\beta$ = 0.80, $\alpha$ = 0.10) described by Burcal et al. [34]. We performed the statistical analyses by using SPSS 25.0 (IBM Corp., NY, USA) software. Shapiro-Wilk test was used to assess normality for continuous characteristics. Based on the result of normality assessment, *T* test or nonparametric test (Mann-Whitney) was preformed to assess the differences between two groups. The categorical variables were assessed by chi-square test for between-group comparison. Comparing the proprioception and COP sway parameters before versus after intervention between intragroup, the paired Student's t-test was used for normal distribution; otherwise Wilcoxon Signed-rank test was used. Two-way repeated measures analysis of variance (RM ANOVA) (group × time) was employed to examine the interaction effect between group and time. If a significant interaction was detected, Student's t test for unpaired or paired data was employed. All continuous variables were presented as mean ± standard deviations. Statistical significance was accepted at $P < 0.05$.

## 3. Results

*3.1. Participants Characteristics.* A total of 32 patients were included in this study after being screened against the selection criteria. Finally, thirty-two included participates were randomly assigned to the BG (3 males and 13 females) or the CG (3 males and 13 females). The flow chart of the participants of this RCT was illustrated in Figure 1. The age, gender, weight, height, body mass index (BMI), and K/L of the two groups were closely similar. Baseline demographics of both groups are presented in Table 1.

*3.2. Static Stability.* Table 2 displays pre- and post-intervention values regarding SL and SA. At baseline, no significant difference was found between the two groups. The results of the present study showed that no significant group × time interaction effects in SL ($F = 2.063$, $P = 0.156$, $\eta$ [2] = 0.033) and SA ($F = 1.075$, $P = 0.304$, $\eta$ [2] = 0.018) were found. Significant decline was observed in SA and SL between the pre- and posttreatment measurements of the BG, $P < 0.01$ and $P < 0.01$, respectively. Moreover, BG had a significantly greater reduction than CG in SA (mean changes, 339.6 versus 90.31; $P = 0.013$). The example of COP sway before and after intervention was illustrated in Figures 2(a) and 2(d).

*3.3. Proprioception.* At baseline, ATE and CT were closely similar between BG and CG. As was shown in Table 3, no group × time interaction effect was found in ATE and CT in both legs of the two groups. After 4-week intervention, BG

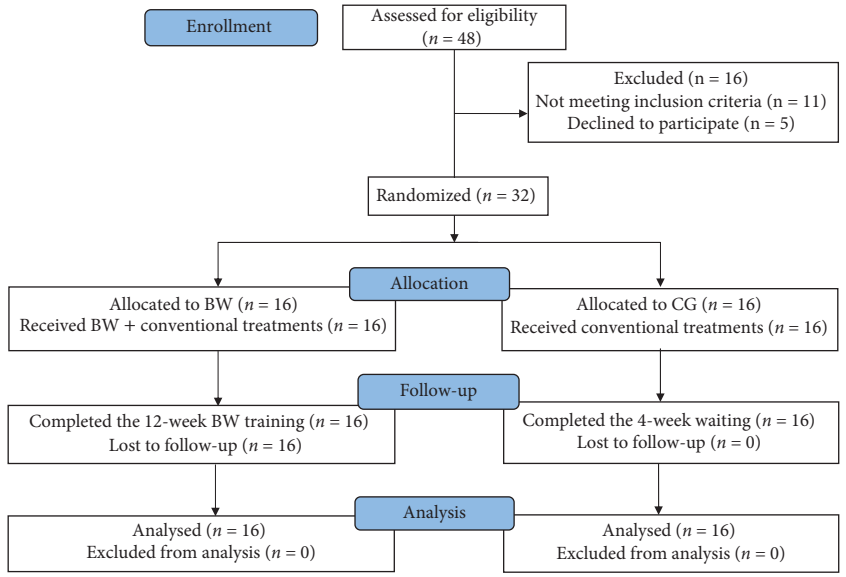


Figure 1: The flow chart of the participants in the study.

Table 1: Characteristics of the participants in the study.

| | BG ($n = 16$) | CG ($n = 16$) | $P$ |
|---|---|---|---|
| Age (years) | 60.31 ± 7.85 | 60.94 ± 6.89 | 0.812 |
| Gender (male/female) | 3/13 | 3/13 | |
| Height (cm) | 160.44 ± 6.49 | 160.75 ± 7.16 | 0.898 |
| Weight (kg) | 62.06 ± 7.62 | 63.06 ± 8.08 | 0.721 |
| BMI (kg/m$^2$) | 24.08 ± 2.24 | 24.37 ± 2.29 | 0.721 |
| K/L scale | 3.38 ± 0.619 | 3.19 ± 0.66 | 0.410 |
| Duration (month) | 38.75 ± 38.32 | 35.75 ± 34.54 | 0.691 |

BG: backward walking group; CG: control group; BMI: body mass index; K/L : Kellgren/Lawrence.

Table 2: Comparison of static stability between the two groups over time.

| Groups | Items | SL (mm) | SA (mm$^2$) |
|---|---|---|---|
| BG | Before intervention | 594.75 ± 205.13 | 949.56 ± 552.99 |
| | After intervention | 384.75 ± 106.99$^{\triangle}$ | 610.50 ± 464.26$^{\triangle}$ |
| | Mean changes | −210.00 | −339.06 |
| CG | Before intervention | 475.44 ± 156.72 | 629.00 ± 471.67 |
| | After intervention | 383.25 ± 171.88 | 538.69 ± 420.52$^{\triangle}$ |
| | Mean changes | −67.19 | −90.31$^{*}$ |
| Time effect | | 0.079 | 0.01 |
| Group◊Time effect | F | 2.063 | 1.075 |
| | P | 0.156 | 0.304 |
| | $\eta^2$ | 0.033 | 0.018 |

BG: backward walking group; CG: control group; SL: sway length; SA: sway area; $^{\triangle}$intragroup difference before intervention, $P < 0.05$; $^{*}$intergroup difference after intervention, $P < 0.05$.

and CG showed a significant reduction in ATE on left ($P = 0.045$ and $P = 0.003$, respectively) and right leg ($P = 0.003$ and $P = 0.002$, respectively) between before and after intervention, whereas the improvement in CT on both legs was not examined. However, there was no significant difference in ATE and CT at week 4 on left ($P = 0.312$ and $P = 0.136$, respectively) and right ($P = 0.171$ and $P = 0.451$, respectively) legs between both groups. Furthermore, the improvements in ATE on both legs remained closely similar between the two groups. The example of the comparison of ATE and CT in both legs before and after intervention was shown in Figures 2(b), 2(c), 2(e), and 2(f).

*3.4. NRS and WOMAC.* Table 4 detailed the outcome assessment at 4 weeks at the end of trial completion. There was no significant difference in NRS and WOMAC between intergroups before intervention, whereas a significantly

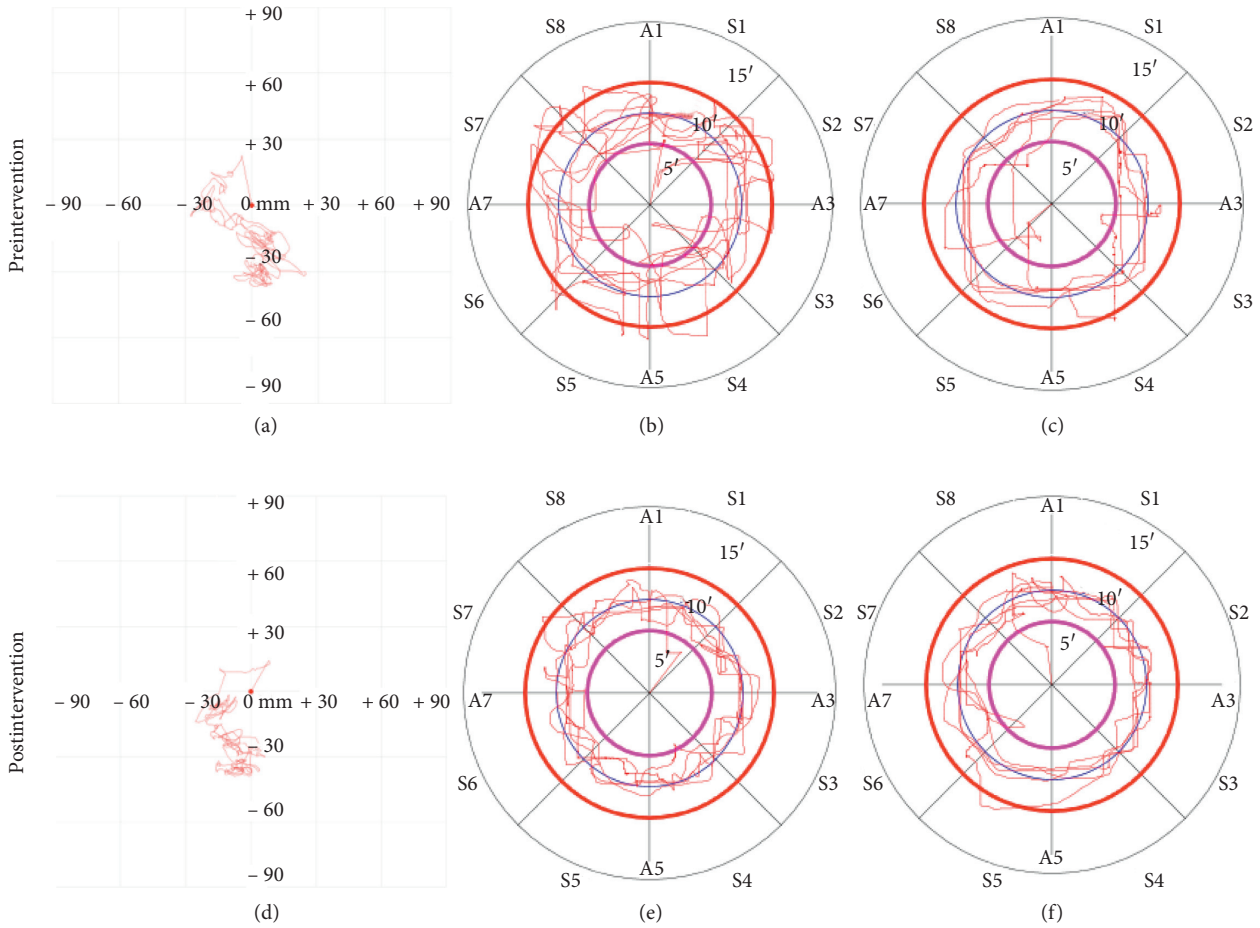


Figure 2: Example of proprioception and COP sway path tests for before and after intervention. (a) COP sway before intervention, (b) proprioception in left leg before intervention, (c) proprioception in right leg before intervention, (d) COP sway after intervention, (e) proprioception in left leg after intervention, and (f) proprioception in right leg after intervention.

lower NRS and WOMAC score were observed in the BG than those in the CG after intervention ($P = 0.02$ and $P = 0.001$, respectively). Significant group × time interaction effects were found in WOMAC ($F = 4.667$, $P = 0.035$, $\eta\,[2] = 0.072$) and function ($F = 5.363$, $P = 0.024$, $\eta\,[2] = 0.082$). Results from the simple effect test indicated that, compared to the baseline, a significant decrease in NRS and WOMAC was determined in the two groups at week 4. Regarding pain and function, compared to the baseline, BG showed a significant improvement in them after 4-week intervention, whereas pain relief was not obviously examined in the CG. Most importantly, a significantly greater reduction in NRS, WOMAC, pain, and function was observed in the BG in comparison to the CG ($P = 0.048$, $P = 0.013$, $P = 0.019$ and $P = 0.002$, respectively).

*3.5. Safety Report.* In this trial, no adverse event was reported in the two groups during the 4-week intervention period. In the CG, one patient still suffered from a moderate activity pain (NRS = 4) at week 4, and then he received the intra-articular injection of sodium hyaluronate and the pain gradually subsided.

## 4. Discussion

Static stability is considered to be one key predictor of falls among the elderly population. COP parameters measured by using force plate were always applied to assess the static postural stability, which was proved to present excellent reliability [35]. Lots of factors attribute to stability impairment, such as age, muscle strength, proprioception, axial alignment of the lower extremity, and even knee sleeve [36]. It was reported that people suffering from KOA showed static stability deficit [37]. Moreover, our previous study [5] suggested that foot posture was closely associated with static postural control. Recently, increasing number of studies reported the benefits of BW for balance improvement. The present randomized, controlled trial investigated the effect of BW on static stability, proprioception, pain, and function in patients with knee osteoarthritis. The results of this study showed that SA, NRS, WOMAC, pain, and function had a significantly greater change after 4-week intervention in the BG than those in the CG, which revealed that, compared to the CG treated with conventional methods alone, BW as an adjunctive intervention in coordination with conventional treatments had a more favorable effect on static stability

Table 3: Comparison of proprioception between the two groups over time.

| Groups | Items | Left side | | Right side | |
|---|---|---|---|---|---|
| | | ATE (%) | CT (s) | ATE (%) | CT (s) |
| BG | Before intervention | 34.63 ± 13.20 | 85.94 ± 12.29 | 36.25 ± 11.58 | 85.88 ± 15.52 |
| | After intervention | 29.75 ± 8.07$^{\triangle}$ | 80.88 ± 8.28 | 28.19 ± 7.96$^{\triangle}$ | 85.88 ± 11.02 |
| | Mean changes | −4.88 ± 13.62 | −5.06 ± 9.72 | −8.06 ± 9.04 | 0.00 ± 16.45 |
| CG | Before intervention | 34.06 ± 10.97 | 90.38 ± 17.88 | 34.19 ± 14.03 | 87.56 ± 19.52 |
| | Afterintervention | 27.06 ± 6.64$^{\triangle}$ | 88.19 ± 16.98 | 23.88 ± 9.39$^{\triangle}$ | 89.63 ± 12.93 |
| | Mean changes | −7.00 ± 7.98 | −2.19 ± 9.68 | −10.31 ± 11.27 | 2.06 ± 15.43 |
| Time effect | | 0.021 | 0.318 | 0.01 | 0.785 |
| | F | 0.179 | 0.160 | 0.168 | 0.075 |
| Group◊Time effect | P | 0.674 | 0.691 | 0.684 | 0.785 |
| | $\eta^2$ | 0.003 | 0.003 | 0.003 | 0.001 |

BG: backward walking group; CG: control group; ATE: average trajectory error; CT: completion time; $^{\triangle}$intragroup difference before intervention, $P < 0.05$; $^{*}$intergroup difference after intervention, $P < 0.05$.

Table 4: Comparison of pain and function between the two groups over time.

| Groups | Items | NRS | WOMAC | | | |
|---|---|---|---|---|---|---|
| | | | Total | Pain | Stiffness | Function |
| BG | Before intervention | 3.69 ± 0.79 | 21.56 ± 6.18 | 5.63 ± 1.93 | 1.31 ± 1.58 | 14.63 ± 3.56 |
| | After intervention | 1.56 ± 0.63$^{\triangle}$ | 11.69 ± 2.50$^{\triangle}$ | 2.63 ± 0.81$^{\triangle}$ | 0.88 ± 1.09$^{\triangle}$ | 8.19 ± 1.87$^{\triangle}$ |
| | Changes | −2.13 ± 1.09 | −9.88 ± 4.99 | −3.00 ± 1.67 | −0.44 ± 0.73 | −6.44 ± 3.69 |
| CG | Before intervention | 3.63 ± 0.96 | 21.13 ± 4.87 | 5.19 ± 1.56 | 0.94 ± 1.18 | 15.00 ± 3.31 |
| | After intervention | 2.25 ± 0.86$^{\triangle *}$ | 16.19 ± 3.94$^{\triangle *}$ | 3.31 ± 1.20 | 0.75 ± 0.93 | 12.13 ± 3.28$^{\triangle *}$ |
| | Changes | −1.38 ± 0.89$^{*}$ | −4.94 ± 2.41$^{*}$ | −1.88 ± 1.03$^{*}$ | −0.19 ± 0.40 | −2.88 ± 1.78$^{*}$ |
| Time effect | | 0.001 | 0.001 | 0.001 | 0.309 | 0.001 |
| | $F$ | 3.364 | 4.667 | 2.462 | 0.168 | 5.363 |
| Group◊Time effect | $P$ | 0.072 | 0.035 | 0.122 | 0.683 | 0.024 |
| | $\eta^2$ | 0.053 | 0.072 | 0.039 | 0.003 | 0.082 |

BG: backward walking group; NRS: numerical rating scale; WOMAC: the Western Ontario and McMaster Universities Osteoarthritis Index; $^{\triangle}$intragroup difference before intervention, $P < 0.05$; $^{*}$intergroup difference after intervention, $P < 0.05$.

enhancement, pain relief, and function improvement in KOA patients. However, even though BG and CG showed a significant improvement in proprioception, the advantage of BW was not obviously observed for proprioception improvement by comparing the two groups.

BW, unsimilar to forward walking, requires specialized control circuits, in addition to rhythm circuitry [38]. The toes contact the ground first and the heel is lifted off the ground at the end during BW stance phase, which leads to different muscles activation patterns and gait characteristics. Motor systems could initiate timely, then appropriate, responses and consequently counteract various disturbances [39], contributing to achievement of equilibrium condition through modifying the biomechanical state. BW training caused changes in movement control system and gait characteristics and exerted a positive effect on postural stability. Furthermore, because of little dependence on vision, BW training participants had to rely more on neuromuscular proprioceptive and vestibular senses to maintain postural stability [40]. It was proved that BW training is more effective in improving gait speed and stride length [41]. In addition, it was previously reported [18–20, 26, 42] that BW could reduce pain, increase quadriceps muscle strength, enhance hamstring flexibility, and improve physical function for individuals with KOA. Gondhalekar et al. [19] indicated that after a minimal effective dosage of 3 weeks, combination of BW and the routine physiotherapy significantly improved function in KOA patients. Those findings were in agreement with the results regarding NRS, WOMAC, pain, and function in the present study.

As a simple, practicable, and effective training, BW was used to improve the balance performance for stroke [25] and children with hemiparetic cerebral palsy [34], and the favorable effects of BW on proprioception in nonathletic males [43] and subjects with anterior cruciate ligament reconstruction [44] were observed. Nevertheless, to the best of our knowledge, this is the first time to evaluate the effect of BW on static stability and proprioception in people affected by KOA. With regard to SA, we found that both groups showed a significant reduction, and there was a significantly greater change in the BG after a 4-week BW intervention period than the waitlist control, which echoed the recently published meta-analysis [40]. However, after 4-week intervention, BG had no significant decrease in SL compared to before intervention, and there was on significant difference in SL between the two groups. It seemed that obvious difference was more likely to be detected in SA rather than SL. Similarly, Ye J et al. [17] thought SA was more sensitive in terms of reflecting a postural stability than SL. Additionally, better proprioception was examined both in BG and CG, but the

improvement of proprioception was similar between BG and CG, which meant the effect of BW on proprioception was unobvious. This result was not the same as the results reported by Sedhom et al. [43] and Shen M et al. [44]. On one hand, proprioception improvement in the two groups should be attributed to acupotomy and routine exercise. It had been proved that acupotomy was beneficial to reduce pain and improve joint function for KOA patients [45], which resulted in a better proprioception before intervention. On the other hand, the proprioception mainly includes the sense of position and movement and the sense of effort, force, and heaviness [46], and tendons and muscle spindles are the two major mechanoreceptors [47]. As was reported, muscle weakness or atrophy appeared in patients with OA as one of the earliest symptoms [48]. Due to the muscle problem, it could be difficult to obtain proprioception recovery for the KOA patients, which might be the reason for the results that patients in the BG showed no significant improvement in proprioception more than those in the CG at postintervention week 4, or a longer term of BW intervention was required. Of note, the results derived from this study showed that, at postintervention week 4, the SA presented no significant difference between BG and CG, whereas a significantly bigger change of it was observed in the BG than those in the CG when it was compared between before and after the intervention. It could be explained by the high intragroup variability in SA, which resulted in no significant intergroup difference before and after intervention. Nevertheless, changes of SA, NRS, WOMAC, pain, and function in the BG were significantly larger than those in the CG, which was obvious enough to prove the superiority of BW combined with conventional treatments for enhancing static stability, reducing pain, and improving function in KOA patients compared to conventional treatments used alone.

There are some limitations in the present study: firstly, even though 4-weeks BW intervention was proved to be the effective dosage for pain, function, and balance in KOA patients, a longer intervention period and follow-up might bring greater changes in the outcomes; secondly, the small number of cases was included because it was difficult to complete the task of proprioception test for the KOA patients with impaired proprioception, so a more practicable and easier method for proprioception measurement would be helpful to conduct a clinical trial with larger scale; thirdly, the included patients showed a high variability in SA which resulted in unobvious benefits from BW for KOA patients after intervention; hence, inclusion criteria should have restrictions on static stability in future study.

## 5. Conclusion

In conclusion, the current study indicated that, compared to the waitlist control, KOA patients treated with 4-week BW training in combination with conventional treatment showed a greater reduction in pain and functional disability and had a greater improvement in static stability. However, for KOA patients, 4-week BW combined with conventional therapy presented no significantly greater improvement in proprioception than conventional therapy used alone.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

ZH-C and XM-X designed the study. XM-X did the acupotomy work. WJ-C did the evaluation of static stability and proprioception. ZH-C and Z-S conducted the NRS and WOMAC assessment. ZH-C and Z-S collected the data. T-J and WJ-C did statistical analyses. ZH-C and Z-S drafted the manuscript. JT-W, Y-W, and H-W revised the manuscript. XL-Y and Y-W edited the language. XM-X supervised the study. All authors read and approved the submitted version.

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*Research Article*

# The Effect of Rhythmic Breathing on the Severity of Sternotomy Pain after Coronary Artery Bypass Graft Surgery: A Randomized Controlled Clinical Trial

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*Background*. Moderate-to-severe pain is reported in up to 75% of the patients in the first 48 hours after cardiac surgery. Evidence suggests that distraction is an effective nursing intervention for controlling short-term and transient pain. Distraction can be achieved by various techniques, including progressive muscle relaxation, meditation, and rhythmic breathing (RB). The present research aimed at evaluating the impacts of RB on the severity of sternotomy pain after Coronary Artery Bypass Graft (CABG). *Methods*. This randomized, controlled clinical trial was conducted on 60 patients after CABG surgery at the open-heart surgery Intensive Care Unit (ICU) of Kowsar Hospital, affiliated to Semnan University of Medical Sciences in Semnan, Iran. The patients were selected through convenience sampling and randomly assigned to two groups, including (1) intervention or RB and (2) control groups. RB was performed in the intervention group every 12 hours (9 a.m. and 9 p.m.) for three consecutive days after the surgery. The control group received only routine care for pain control (opioid analgesics) with no additional interventions. The severity of pain was measured every day in both groups of patients before and after the interventions using the Visual Analog Scale (VAS). *Results*. The mean postintervention pain scores were significantly different from the mean preintervention scores in the intervention group ($p < 0.05$). The changes in the mean pain score in the intervention group were also significantly different from the corresponding changes in the controls ($p < 0.05$). *Conclusion*. Based on the results, the severity of pain after the intervention was significantly lower in the RB group compared to the control. RB was found to be an effective technique for reducing the patients' pain and is therefore recommended as a post-CABG pain control technique. Iranian Registry of Clinical Trials: this trial is clinically registered with IRCT20120109008665N7, registered 3 September 2018.

## 1. Introduction

Open-heart surgery is currently performed in many countries to increase the survival and quality of life of patients with cardiac diseases [1]. Coronary Artery Bypass Graft (CABG) is the most common open-heart surgery in the US with 156,931 cases in 2016, which shows an increase of 6.1% compared to the preceding four years, and 63% of them were nonelective [2]. In Iran, over 30,000 open-heart surgeries are performed in different medical centers every year [3], of which 60% are CABG [4]. Despite being a successful cardiac treatment, heart surgery is still a traumatic event that can lead to a wide range of complications, including severe pain in the surgery site, stroke, pulmonary edema, pericarditis, and postsurgery depression [5–9].

Several factors contribute to the pain following open-heart surgery, including sternotomy, harvesting saphenous vein, harvesting internal thoracic artery, and inserting various drains [10]. The patients' pain can prevent their effective coughing, deep breathing, and cooperation in

physiotherapy; cause the retention of pulmonary secretions; and lead to complications such as pulmonary atelectasis, pneumonia, and respiratory failure [11, 12]. By stimulating the sympathetic nervous system and raising epinephrine and norepinephrine levels, pain can also increase cardiac workload, create an imbalance in oxygen supply and demand, and consequently lead to ischemia and myocardial infarction [13]. Improper pain control after cardiac surgery can cause a high incidence of Poststernotomy Pain Syndrome (PSPS) [14]. PSPS is hard to treat and affects the quality of life of the patients and their relatives in the long term [15–17].

According to medical personnel, patients are not in great pain after sternotomy because the tissue damage that occurred is negligible and the patients are minimally mobile after surgery. Nevertheless, Lahtinen reported moderate-to-severe pain (pain score of 4 to 10) in up to 75% of the patients in the first 48 hours after cardiac surgery [18]. Thus, effective pain management will result in faster recovery, reduced postsurgery complications and length of stay, and increased patient satisfaction.

A variety of medicinal and nonmedicinal therapies have been proposed for pain management. Medicinal methods such as opioids are currently used to alleviate pain in cardiac patients but are not desirable as the first line of treatment because of their costs and adverse effects on different body parts, which increase patient mortality and morbidity [19]. Hence, nonmedicinal pain control interventions are preferred because they reduce the need for medications [20]. As such, various nursing measures have been used as Complementary Therapy Methods (CTM) to help patients [21, 22].

Studies indicate that distraction is an effective nursing intervention for controlling short-term and transient pain by increasing endorphins [23, 24]. Quoting several studies, Malloy and Milling reported distraction as one of the oldest psychological interventions for pain relief with remarkable effects [25]. By balancing the anterior and posterior hypothalamus and reducing the activity of the sympathetic nervous system and the secretion of catecholamine, distraction can mitigate stress-induced muscle tension and physiological side-effects such as hypotension, heart rate, and muscular spasms [26]. Another advantage of distraction in clinical settings is that patients can perform this technique independently. Thus, in combination with analgesics, it provides comprehensive pain relief. Distraction can be achieved by various techniques, including progressive muscle relaxation, mandibular relaxation, meditation, and rhythmic breathing (RB) [27].

RB is one of the distraction methods that make patients voluntarily distract themselves from a painful stimulus and thus help control their pain [28, 29]. Distraction is based on the idea that, with diverse and sufficient sensory stimuli, the reticular formation in the brainstem can choose to inhibit or ignore the transmission of such feelings as pain [30]. In this technique, the perception of pain is minimized through distraction-induced reduced alertness [31]. Moreover, RB mitigates pain, anxiety, and stress by increasing the activity of the parasympathetic nervous system via the vagus nerve and increasing the inhibitory function of the Gamma-Aminobutyric Acid (GABA) receptors in the brain pathways that are vital to perceiving fear, emotional regulation, and stress response. The advantages of using this technique include its simplicity, low costs, noninvasiveness, safety, and long-term application [28, 32].

Several studies have reported the effect of RB on reducing pain [28, 33–37]. For example, Farzin Ara et al. showed that RB reduced pain from $7.2 \pm 3.7$ to $5.9 \pm 1.1$ following orthopedic surgeries [33]. Borzou et al. showed that RB can help reduce pain and the frequency of analgesic administration in patients after orthopedic surgeries [34]. Furthermore, Borzou et al. showed that RB is an effective method for relieving pain due to hemodialysis vascular needles [28]. Lalegani et al. (2013), Bozorg-Nejad et al., and Park et al. showed that RB can significantly reduce the severity of pain during the dressing of burns [35–37].

Sternotomy site pain is the patients' most common complaint and probably the most severe postoperative pain experienced, which can be associated with increased patient morbidity and mortality [38]. Patients undergoing open-heart surgery experience the worst possible pain during coughing and deep breathing, and their pain is inadequately controlled and prevents them from deep breathing and effective coughing despite medicinal interventions such as the administration of nonsteroidal anti-inflammatory medications and opioids [39]. Given the lack of research on the effect of this technique on patients' pain following CABG, the present study was designed and conducted to determine the effect of RB on post-CABG sternotomy pain control.

## 2. Methods

*2.1. Study Design and Participants.* This parallel randomized, controlled nonmasked clinical trial was conducted between September 2018 and September 2019 on patients undergoing CABG surgery at Kowsar Hospital, affiliated to Semnan University of Medical Sciences in Iran. Following a preliminary study with ten patients from each group (RB and control), the mean and standard deviation of the severity of pain were found as $1.3 \pm 0.65$ in the RB group and $2 \pm 0.8$ in the control group. Then, taking into account 95% confidence interval and 80% test power and using equation $(n=((z_{1-\alpha/2} + z_{1-\beta})^2 \times (\delta_1^2 + \delta_2^2))/(\mu_1 - \mu_2)^2)$, the sample size was determined as 30 patients per group. Using $G*$power, the effect size was estimated as 0.96 (means: difference between two independent means). A total of 67 patients entered the study, of whom seven withdrew for various reasons, and the data from 60 patients (30 per group) were ultimately analyzed. The patients who fulfilled the inclusion criteria were included in the study and were then randomly assigned to the groups using random blocks of A for the experimental group (RB) and B for the control group. Since patients who practice RB are easily distinguished and the researcher taught the patients how to breathe rhythmically, it was not possible to perform blinding. Figure 1 presents the flow chart of the participants.

The study inclusion criteria were as follows: undergoing elective CABG surgery, age 35–80 years, stable

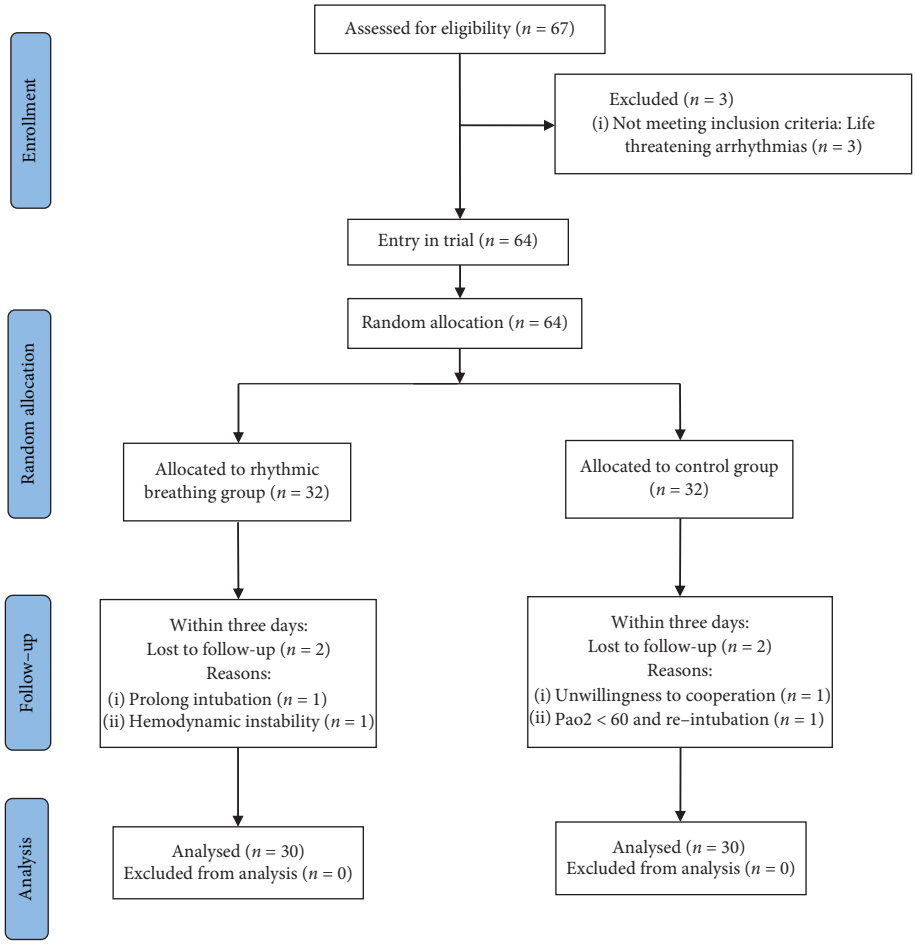


Figure 1: CONSORT flowchart of the study.

hemodynamic status (blood pressure>90 mmHg, and 50 < pulse rate < 110), no previous history of CABG, no life-threatening arrhythmias, and no use of Cardiopulmonary Bypass (CPB). The exclusion criteria were as follows: having a history of diabetes, prolonged intubation (more than 24 hours after surgery), dependence on inotropic agents for hemodynamic stability after extubation, $PaO_2 < 60$ mmHg without receiving oxygen via the nasal cannula or mask, renal dysfunction before and after surgery, history of chronic pain, and the need for other pain control methods such as music or massage therapy.

*2.2. Ethical Considerations.* In terms of ethical considerations, the Ethics Committee of Semnan University of Medical Sciences approved the present research (IR.SEMUMS.REC.1397.138). This study was also registered in the Iranian Registry of Clinical Trials (IRCT20120109008665N7). After introducing himself to the patients, the researcher briefed the participants on the research objectives and procedures, assured them of the confidentiality of their information and their right to withdraw from the study at their own discretion and responded to their questions. The subjects then signed written informed consent forms for participation.

*2.3. Interventions.* RB technique was performed for the intervention group. RB was individually taught to the intervention group before surgery until they could perform it independently and correctly. The day after surgery, upon gaining full consciousness and hemodynamic stability, the patients were asked to close their eyes in the supine position, inhale through the nose, then hold their breath, and exhale through the mouth, while counting from 1 to 3 in each step. All the patients in the intervention group were trained to focus only on air entry and exit while breathing, and they were asked to perform RB once every five minutes, lasting one minute each time, for 20 minutes (totally four times every 20 minutes) [32]. The patients received supplemental oxygen during the intervention if required. Supervised by the researcher, RB was performed every 12 hours (9 a.m. and 9 p.m.) for three consecutive days. The control group received only routine care (opioids, such as morphine) with no additional interventions. The routine care given to both groups was the same.

*2.4. Data Collection.* Data were collected using a two-part questionnaire. The first part dealt with the patients' demographic details, including age, gender, education, body mass index (BMI), underlying diseases, duration of

hospitalization before and after surgery, history of smoking and opioid use, amount of opioid injection, on-pump duration, ejection fraction, duration of mechanical ventilation, and the number of grafts and chest tubes. Surgery was performed using a cardiopulmonary pump in both groups. Also, the method of anesthesia, anesthesia medications, and fluid therapy during and after surgery were the same for all the patients. Any changes in routine instructions were recorded by the researcher.

In the second part, the patients' pain score data were collected by the Visual Analog Scale (VAS). Pain was measured at 9 a.m. and 9 p.m. before and after RB performance in both groups for three days. The patients were asked to express their pain severity by choosing a number between 0 (no pain) and 10 (most severe pain). VAS categorizes the severity of pain into four groups: 0 = no pain, 1–3 = mild pain, 4–7 = moderate pain, and 8–10 = severe pain. In one study, Alghadir et al. confirmed the reliability of VAS with an intraclass correlation coefficient of 0.97 [40]. In the present study, the reliability of VAS was confirmed in a pilot study on ten patients with Cronbach's alpha of 0.94.

*2.5. Statistical Analysis.* Data were analyzed with a per-protocol approach in SPSS-19 (SPSS Inc., Chicago, IL, USA) at a significance level of 0.05. The study data were described, grouped, and compared in absolute and relative frequency tables. The results of the Kolmogorov–Smirnov test confirmed the normal distribution of the data. The independent sample *t*-test was used to compare the two groups in terms of age, BMI, smoking, on-pump duration, ejection fraction, mechanical ventilation, hospitalization before and after surgery, mean severity of pain before and after intervention, difference between the mean values preintervention and postintervention in each group, and the amount of morphine used. The Chi-square test was used to compare the absolute and relative frequencies in terms of the patients' gender, education, drug use, underlying diseases, number of grafts and chest tubes, and pain intensity between the two groups. The repeated-measures analysis of variance was used to assess the effect of time and the interaction effect of group and time on the mean severity of pain three days after surgery.

For analysis of variance with repeated measures, the following statistical presuppositions were examined first: quantitativeness of the dependent variable, elimination of outliers, normal distribution of the dependent variable distribution, and confirmation of sphericity of the groups with Mauchly's statistics, and three factors of intervention (versus control), time point during the day (difference between before and after scores at 9 a.m. and 9 p.m.), and days (first, second, and third) were entered into the analysis.

## 3. Results

*3.1. Participants' Characteristics.* The present study recruited 60 patients after CABG surgery. The patients' mean age was 61.58 ± 9.7 years, and the majority (80%) were male. Table 1 presents the demographic and surgery details of the patients in both groups. No significant difference was found between the two groups in terms of demographic and surgery details ($p > 0.05$). One-third of the patients in the intervention group and more than half in the control group had several underlying diseases, and hypertension was the single most frequent underlying disease in them.

*3.2. Severity of Pain.* The independent *t*-test results showed no significant difference between the two groups in the severity of pain in the first three days after surgery before beginning the intervention, except on the third night ($p > 0.05$). Meanwhile, the severity of pain after the intervention, as measured on both occasions (9 a.m. and 9 p.m.) in all three days, was significantly lower in the RB group compared to the controls ($p < 0.05$) (Table 2, Figure 2). The results of the Chi-square test revealed significant differences between the two groups in terms of the severity of pain (no pain, mild pain, and moderate pain) before and after the intervention ($p < 0.05$). None of the patients in both groups had severe pain (Table 3). There were significant differences between the two groups in terms of the mean difference in the severity of pain before and after the intervention ($p < 0.05$) (Table 4). The results of the repeated-measures ANOVA showed a significant difference between the two groups in the mean difference in severity of pain after the intervention, such that the severity of pain was lower in the RB group compared to the controls. In other words, the intervention reduced the severity of pain significantly over time ($F(1,58) = 137.3$, $p < 0.001$) (Table 5). The mean and standard deviation of the dose of morphine administered was 3.40 ± 1.97 mg in the RB group and 5.30 ± 2.57 mg in the control group. The *t*-test results showed no significant difference between the two groups in the dose of morphine administered ($p = 0.604$).

## 4. Discussion

The present findings on the effect of RB on post-CABG sternotomy pain control confirmed that RB was effective in reducing the severity of sternotomy pain after surgery in the intervention group. To the best of the authors' knowledge, this is the first study to investigate the effect of RB on post-CABG sternotomy pain.

After the intervention, the severity of pain, as measured on both occasions (9 a.m. and 9 p.m.) in three consecutive days following the surgery, was significantly lower in the intervention group compared to the controls. The results of the repeated-measures ANOVA showed a significant difference between the two groups in the mean difference in the severity of pain over the three days, which was lower in the intervention group. In agreement with this result, several studies conducted on the effect of RB on pain have also shown that RB can help reduce pain and the number of analgesics administered after surgery [28, 33, 34]. The results of a study conducted in Iran by Farzin Ara et al. (2018) to compare the effect of reciting the word "*Allah*" and performing RB on postoperative pain in orthopedic patients showed that, compared to the control group, the group

Table 1: The demographic and surgical-related characteristics of the participants in the rhythmic breathing/control groups.

| Groups characteristics | RB ($n = 30$) $N$ (%) | Control ($n = 30$) $N$ (%) | $p$ value* |
|---|---|---|---|
| *Gender* | | | |
| Male | 24 (80) | 27 (90) | $X^2 = 1.17,\ 1,\ p = 0.278$ |
| Female | 6 (20) | 3 (10) | |
| *Level of education* | | | |
| Below high school diploma | 28 (93.3) | 28 (93.3) | $X^2 = 0.000,\ 1,\ p = 1$ |
| Higher education | 2 (6.7) | 2 (6.7) | |
| *Opium consumption* | | | |
| Yes | 9 (30) | 15 (50) | $X^2 = 2.50,\ 1,\ p = 0.114$ |
| No | 21 (70) | 15 (50) | |
| *Underlying disease*[a] | | | |
| Yes | 24 (80) | 28 (93.3) | $X^2 = 2.30,\ 1,\ p = 0.129$ |
| No | 6 (20) | 2 (6.7) | |
| *Number of grafts* | | | |
| 2 | 1 (3.3) | 1 (3.3) | |
| 3 | 10 (33.3) | 9 (30) | $X^2 = 0.07,\ 2,\ p = 0.962$ |
| ≥4 | 19 (63.4) | 20 (66.7) | |
| *Number of chest tubes* | | | |
| 2 | 24 (80) | 27 (90) | $X^2 = 1.17,\ 1,\ p = 0.278$ |
| 3 | 6 (20) | 3 (10) | |
| | (Mean ± SD) | (Mean ± SD) | $p$ value** |
| Age (years) | 60.80 ± 9.4 | 62.37 ± 10.1 | $t(58) = -0.61,\ p = 0.539$ |
| BMI (kg/m2) | 26.73 ± 4.4 | 26.03 ± 4.1 | $t(58) = 0.63,\ p = 0.526$ |
| Smoking (butts/day) | 4.73 ± 10.9 | 9.83 ± 15.3 | $t(52.41) = -1.48,\ p = 0.204$ |
| On-pump duration (minute) | 109.70 ± 25.3 | 111.90 ± 28 | $t(58) = -0.32,\ p = 0.751$ |
| Ejection fraction (%) | 46.50 ± 11.2 | 50.83 ± 5.5 | $t(42.51) = -1.89,\ p = 0.065$ |
| Medical ventilation time (hour) | 9.63 ± 4.2 | 10.93 ± 4.3 | $t(58) = -1.16,\ p = 0.249$ |
| Duration of preoperative hospitalization (day) | 4.5 ± 2 | 5.53 ± 3.2 | $t(49.2) = -1.46,\ p = 0.150$ |
| Duration of postoperative hospitalization (day) | 5.67 ± 1.4 | 6.03 ± 1.3 | $t(58) = -1.01,\ p = 0.315$ |

RB: rhythmic breathing; BMI: body mass index; *Chi-square test; **independent $t$-test. [a]Including: hypertension, hyperlipidemia.

Table 2: The comparisons of mean scores of the pain severity in the RB and control groups.

| Day | Hours | Measurement times | Groups | Mean ± SD | Min | Max | value* |
|---|---|---|---|---|---|---|---|
| Fist day | 9 a.m. | Before intervention | RB | 2.37 ± 0.99 | 1 | 5 | $t(52.6) = 0.213,\ p = 0.832$ |
| | | | Control | 2.30 ± 1.39 | 1 | 6 | |
| | | After intervention | RB | 1.07 ± 0.64 | 0 | 3 | $t(58) = -2.90,\ p = 0.005$ |
| | | | Control | 1.50 ± 0.5 | 1 | 2 | |
| | 9 p.m. | Before intervention | RB | 2.10 ± 0.84 | 1 | 4 | $t(58) = 0.91,\ p = 0.363$ |
| | | | Control | 1.90 ± 0.84 | 1 | 4 | |
| | | After intervention | RB | 0.87 ± 0.50 | 0 | 2 | $t(57.2) = -4.78,\ p < 0.001$ |
| | | | Control | 1.53 ± 0.57 | 1 | 3 | |
| Second day | 9 a.m. | Before intervention | RB | 1.90 ± 0.54 | 1 | 3 | $t(40) = -040,\ p = 0.687$ |
| | | | Control | 2 ± 1.23 | 1 | 5 | |
| | | After intervention | RB | 0.83 ± 0.46 | 0 | 2 | $t(53.3) = -5.17,\ p < 0.001$ |
| | | | Control | 1.57 ± 0.62 | 1 | 3 | |
| | 9 p.m. | Before intervention | RB | 1.97 ± 0.49 | 1 | 3 | $t(54.3) = 1.58,\ p = 0.119$ |
| | | | Control | 1.73 ± 0.64 | 1 | 3 | |
| | | After intervention | RB | 0.97 ± 0.49 | 0 | 2 | $t(55.5) = -4.91,\ p < 0.001$ |
| | | | Control | 1.67 ± 0.60 | 1 | 3 | |
| Third day | 9 a.m. | Before intervention | RB | 1.73 ± 0.58 | 1 | 3 | $t(58) = 1.78,\ p = 0.079$ |
| | | | Control | 1.47 ± 0.57 | 1 | 3 | |
| | | After intervention | RB | 0.70 ± 0.53 | 0 | 2 | $t(58) = -5.46,\ p < 0.001$ |
| | | | Control | 1.43 ± 0.50 | 1 | 2 | |
| | 9 p.m. | Before intervention | RB | 1.77 ± 0.62 | 1 | 3 | $t(58) = 2.27,\ p = 0.027$ |
| | | | Control | 1.43 ± 0.50 | 1 | 2 | |
| | | After intervention | RB | 0.70 ± 0.53 | 0 | 2 | $t(58) = -5.46,\ p < 0.001$ |
| | | | Control | 1.43 ± 0.50 | 1 | 2 | |

RB: rhythmic breathing; SD: standard deviation; Min: minimum; Max: maximum; *independent $t$-test.

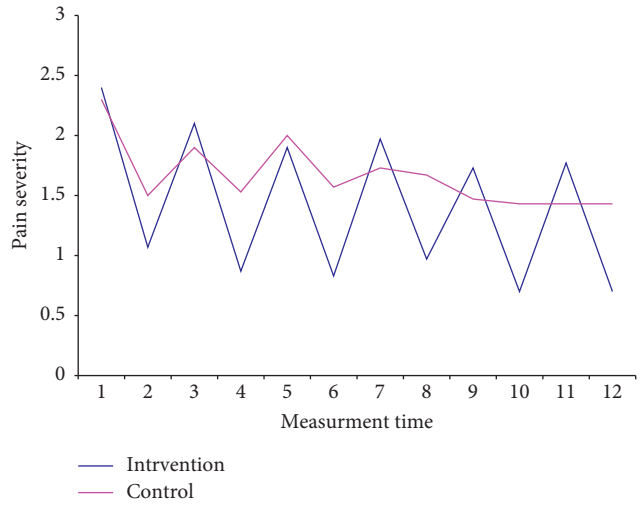


Figure 2: Mean scores of the pain severity after CABG.

Table 3: The comparison of the patients in the RB and control groups in terms of pain severity.

| Day | Hours | Measurement times | Groups | No pain | Mild pain | Moderate pain | $p$ value* |
|---|---|---|---|---|---|---|---|
| Fist day | 9 a.m. | Before intervention | RB | 0 | 25 | 5 | $X^2 = 0.109,\ 1,\ p = 0.741$ |
| | | | Control | 0 | 24 | 6 | |
| | | After intervention | RB | 4 | 26 | 0 | $X^2 = 4.21,\ 1,\ p = 0.040$ |
| | | | Control | 0 | 30 | 0 | |
| | 9 p.m. | Before intervention | RB | 0 | 27 | 3 | $X^2 = 1.05,\ 1,\ p = 0.305$ |
| | | | Control | 0 | 29 | 1 | |
| | | After intervention | RB | 6 | 24 | 0 | $X^2 = 6.55,\ 1,\ p = 0.010$ |
| | | | Control | 0 | 30 | 0 | |
| Second day | 9 a.m. | Before intervention | RB | 0 | 30 | 0 | $X^2 = 5.36,\ 1,\ p = 0.052$ |
| | | | Control | 0 | 25 | 5 | |
| | | After intervention | RB | 6 | 24 | 0 | $X^2 = 6.55,\ 1,\ p = 0.010$ |
| | | | Control | 0 | 30 | 0 | |
| | 9 p.m. | Before intervention | RB | 0 | 30 | 0 | — |
| | | | Control | 0 | 30 | 0 | |
| | | After intervention | RB | 4 | 26 | 0 | $X^2 = 4.21,\ 1,\ p = 0.040$ |
| | | | Control | 0 | 30 | 0 | |
| Third day | 9 a.m. | Before intervention | RB | 0 | 30 | 0 | — |
| | | | Control | 0 | 30 | 0 | |
| | | After intervention | RB | 10 | 20 | 0 | $X^2 = 11.80,\ 1,\ p = 0.001$ |
| | | | Control | 0 | 30 | 0 | |
| | 9 p.m. | Before intervention | RB | 0 | 30 | 0 | — |
| | | | Control | 0 | 30 | 0 | |
| | | After intervention | RB | 10 | 20 | 0 | $X^2 = 11.80,\ 1,\ p = 0.001$ |
| | | | Control | 0 | 30 | 0 | |

RB: rhythmic breathing; *Chi-square (linear-by-linear association).

reciting *Allah*, followed by the group performing RB, had lower mean severities of pain. They concluded that both methods can be used to reduce pain after orthopedic surgery [33]. The results obtained by Borzou et al. in Malayer, Iran, also showed the effectiveness of RB on the severity of pain after orthopedic surgery and the dose of analgesics administered [34]. The results obtained by Borzou et al. in Hamadan, Iran, showed the effect of RB in reducing the severity of pain caused by hemodialysis vascular needles, as well [28]. Lalegani et al., Bozorg-Nejad et al., and Park et al. confirmed the positive effect of RB on pain reduction during redressing of burns [35–37]. The results reported by Marsdin et al. in their study titled "The Effect of Audio and Video Distractions on Reducing Lithotripsy Pain" showed that there was a significant difference in the perception of pain and distress between the distraction and control groups [41]. The results obtained by Esmaeili et al. and Valizadeh et al. on the effect of regular breathing exercise and music on the pain of inserting intravenous (IV) lines during blood infusion showed that both these methods significantly reduced children's pain, although music was more effective than breathing exercise [42, 43]. Bageriyan et al. compared the effects of bubbling and regular breathing exercise on reducing venipuncture pain in school children admitted to the

Table 4: The comparison of mean differences scores of the pain severity before and after intervention in the RB and control groups.

| Day | Measurement times | Groups | Mean ± SD | Min | Max | $p$ value* |
|---|---|---|---|---|---|---|
| Fist day | 9 a.m. | RB | 1.3 ± 0.59 | 1 | 3 | $t(45.5) = 2.24,\ p = 0.028$ |
| | | Control | 0.8 ± 1.06 | 0 | 4 | |
| | 9 p.m. | RB | 1.23 ± 0.56 | 1 | 3 | $t(58) = 5.67,\ p < 0.001$ |
| | | Control | 0.36 ± 0.61 | 0 | 2 | |
| Second day | 9 a.m. | RB | 1.06 ± 0.25 | 1 | 2 | $t(32.9) = 3.45,\ p = 0.002$ |
| | | Control | 0.43 ± 0.97 | 0 | 3 | |
| | 9 p.m. | RB | 1/0 ± 0.0 | 1 | 1 | $t(29) = 20.15,\ p < 0.001$ |
| | | Control | 0.06 ± 0.25 | 0 | 1 | |
| Third day | 9 a.m. | RB | 1.03 ± 0.18 | 1 | 2 | $t(58) = 21.2,\ p < 0.001$ |
| | | Control | 0.03 ± 0.18 | 0 | 1 | |
| | 9 p.m. | RB | 1.06 ± 0.25 | 1 | 2 | $t(29) = 23,\ p < 0.001$ |
| | | Control | 0.0 ± 0.0 | 0 | 0 | |

RB : rhythmic breathing; SD: standard deviation; Min : minimum; Max : maximum; *independent $t$-test.

Table 5: Results of repeated-measures ANOVA in terms of pain severity in the RB and control groups.

| Source of change | Variables | Sum of squares | df* | Mean squares | F | $p$ value |
|---|---|---|---|---|---|---|
| Within-subjects | Time[a] | 13.10 | 1 | 13.10 | 10.56 | 0.002 |
| | Time × group | 3.66 | 1 | 3.66 | 2.95 | 0.09 |
| | Error | 71.90 | 58 | 1.24 | | |
| Between-subject | Constant | 176.4 | 1 | 176.4 | 387.05 | <0.001 |
| | Group | 62.50 | 1 | 62.50 | 137.13 | <0.001** |
| | Error | 26.43 | 58 | 0.456 | | |

*Lower bound; **adjusted for time, and interaction of time and group. Time[a]: day 1 ($T1$, $T2$); day 2 ($T1$, $T2$); and day 3 ($T1$, $T2$). T1 indicates the difference in pain severity between before and after the intervention at 9 a.m., and $T2$ indicates the difference in pain severity between before and after the intervention at 9 p.m..

thalassemia center of Kerman, Iran. Their results showed no significant difference between the two groups in the mean score of pain [44]. The results of a study conducted by Vakilian and Keramat to compare the effects of aromatherapy with lavender and breathing techniques on reducing labor pain showed that the mean change in the severity of pain before and after the intervention was significantly different in the breathing technique group [45].

Nevertheless, the results obtained by Slade showed that these techniques are less effective than expected in reducing pain [46]. According to Mehdizadeh, most studies have highlighted the beneficial and positive effects of breathing and neuromuscular techniques, but there are reports indicating their ineffectiveness [47], including one by Pugh et al., which argued that breathing techniques exhaust the mother and delay childbirth [48].

The results showed no significant difference between the two groups in the dose of morphine administered (1.97 mg in the RB group and 2.57 mg in the control group), but this difference was clinically significant. Other studies reported a significant difference between their intervention and control groups in the number of analgesics received after surgery [33, 34], which disagrees with the present findings. This disagreement can be attributed to the different target populations, patients' gender, and type of intervention or surgery.

In the present study, the severity of pain was significantly higher in the RB group than the control group at 9 p.m., three days before the intervention, but after the intervention, a significant reduction was observed in the severity of pain in the RB group compared to the controls. This important finding is indicative of the effect of RB on reducing pain, which was also confirmed by the repeated-measures ANOVA results.

The results of pain intensity in patients in both groups also revealed that the pain intensity in the RB group significantly decreased after the intervention compared to that in the control group at 9 a.m. and 9 p.m. for three consecutive days. According to the results, although the effect of time on reducing patients' pain intensity should not be ignored, the intervention (RB) significantly affected patients' pain intensity.

According to the researchers, it is necessary to consider factors affecting the perception of the severity of pain. The nature and severity of postoperative pain depend on the size and amount of incision and type of surgery. In addition, the perception of pain depends on ethnicity, culture, beliefs, personal experience of pain, and personality [49]. The factors affecting pain in patients undergoing CABG include the duration of CPB, gender, age (less than 60 years), duration of surgery (more than two hours), and surgery site (thoracic surgery) [16]. Not much evidence supports that the duration of CPB<60 minutes reduces pain. Nonetheless, the release of various cytokines (known as proinflammatory mediators) caused by CPB contributes to pain [50]. Further studies are recommended to further investigate these items.

*4.1. Study Limitations.* The main limitation of the present research included the reluctance of the patients to follow the

instructions provided for performing the breathing exercises owing to their improper psychological status. Some measures were thus taken to encourage them to cooperate. A natural limitation of the present study was also associated with the subjective nature of pain, which caused differences in the degree of pain reported by different individuals because pain severity is a patient-reported outcome. The unicenter type of this study and its small sample constituted other limitations, which restricts the external validity of the findings and prohibits their generalizability to other centers. It is recommended that further research be performed with larger samples and more prolonged follow-ups to obtain more accurate results on the effects of rhythmic breathing on pain after CABG with sternotomy.

## 5. Conclusions

The results confirmed that RB is effective in reducing the severity of sternotomy pain after CABG surgery. Given the importance of the management of pain as the fifth vital sign and to prevent the side-effects and problems caused by the lack of proper pain control, especially in patients after CABG, RB can be recommended as a simple, safe, and inexpensive method in the form of an independent nursing activity in conjunction with other medical measures for reducing the severity of pain in patients after CABG surgery.

## Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethical Approval

This study was approved by the Ethics Committee of Semnan University of Medical Sciences (approval code: IR.SEMUMS.REC.1397.138).

## Consent

Informed written consent was obtained from each participant.

## Disclosure

All the authors have read and approved the final manuscript.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Authors' Contributions

HB, MK, and AE contributed to the conception and design of the study and the study protocol. MK and HB managed the running of the study. HB and AE conducted data analysis and all authors helped with data interpretation. MK and HB wrote this manuscript. All authors read and approved the final version of the manuscript.

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*Review Article*

# Efficacy and Safety of Traditional Chinese Medicine in the Treatment of Immune Infertility Based on the Theory of "Kidney Deficiency and Blood Stasis": A Systematic Review and Meta-Analysis

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*Objective*. This study aims to evaluate the efficacy and safety of traditional Chinese medicine (TCM) therapy of tonifying kidney and activating blood circulation (TKABC) based on the theory of "kidney deficiency and blood stasis" for the treatment of immune infertility. *Methods*. Six electronic databases, including the Cochrane Library, PubMed, EMBASE, the China National Knowledge Infrastructure, Wanfang Data, and VIP information database, were searched from inception to January 2021 to identify eligible studies of randomized controlled trials (RCTs). The primary outcome measurements were the total effective rate and pregnancy rate, and the secondary outcome measurements included the negative conversion rate of serum antibodies and the incidence of adverse effects. The quantitative synthesis was performed using the Review Manager 5.3 software. The chi-square statistic and $I^2$ statistic were employed to investigate statistical heterogeneity. The fixed-effects model was used for a low heterogeneity ($I^2 < 50\%$), and the random-effects model was applied if heterogeneity was moderate ($50\% < I^2 < 75\%$). Funnel plots were used to evaluate potential reporting bias when more than ten eligible studies were included. *Results*. Thirteen RCTs involving 1298 patients with immune infertility of kidney deficiency and blood stasis were included. Compared with conventional group, TCM TKABC therapy showed a significant improvement on the total effective rate (RR: 1.38; 95% CI: 1.30,1.47; and $I^2 = 0\%$), pregnancy rate (RR: 2.04; 95% CI: 1.73, 2.40; and $I^2 = 30\%$), negative conversion rates of AsAb (RR: 1.42; 95% CI: 1.12,1.79; and $I^2 = 62\%$), AEmAb rates (RR: 1.21; 95% CI: 1.04,1.41; and $I^2 = 0\%$), and AhCGAb with less adverse effects (RR: 0.24; 95% CI: 1.73, 2.40; and $I^2 = 55\%$). However, the negative conversion rate of AoAb and ACAb showed no significant statistical difference. *Conclusions*. Our review suggests that TCM TKABC therapy based on the theory of kidney deficiency and blood stasis appears to be an effective and safe approach for patients with immune infertility. However, the methodological quality of included RCTs was unsatisfactory, and it is necessary to verify its effectiveness with more well-designed and high-quality multicenter RCTs.

## 1. Introduction

Immune infertility is defined as the presence, in one or both partners, of an antisperm immune reaction capable of impairing fertility variables [1]. It has become a serious health issue as approximately 10 to 20 percent of the sterility cases are immunological [2]. Although the definitive cause of immune infertility remains ambiguous, the presence of antireproductive antibodies in serum has been elucidated as one of the major causes of immune infertility. It has been reported that the presence of such antibodies as antisperm (AsAb), antiendometrium (AEmAb), antiovary (AoAb),

antihuman chorionic gonadotropin (AhCGAb), antizona pellucida (AZPAb), antitrophoblast (ATB), and anticardiolipin (ACA) may affect fertilization and implantation process, resulting in infertility [3]. The primary conventional treatment choices include immunosuppressive drugs, anticoagulants, intrauterine insemination, and *in vitro* fertilization. However, long-term usage of immunosuppressive therapy may cause side effects, and assisted reproduction treatment is expensive with a low success rate [3, 4]. Hence, in recent years, the interest in complementary and alternative medicine has increased.

Traditional Chinese medicine (TCM) has been commonly used to treat infertility in Asian countries. TCM is featured by the concept of holism and treatment based on syndrome differentiation. From the perspective of TCM, immune infertility is often attributable to kidney deficiency and blood stasis [5]. Previous studies reported that TCM therapy of tonifying kidney and activating blood circulation (Bushen Huoxue, TKABC) is essential for treating this illness [5, 6]. A large number of studies have reported that TKABC may remarkably reduce serum levels of such antibodies as AsAb, eliminate testicular immunological complexes, regulate the ratio of $CD_4/CD_8$ T cells, and eliminate inflammatory cytokines to cure immune-induced infertility [7–10]. In recent years, a growing body of random controlled trials (RCTs) has been conducted to assess the effectiveness and safety of TKABC therapy for the treatment of immune infertility, and the results have suggested it might be an effective and safe therapeutic approach. However, currently no systematic review and meta-analysis have been reported for this specific ailment. Thus, we performed this study to evaluate the efficacy and safety of TCM TKABC therapy based on the theory of "kidney deficiency and blood stasis" for the treatment of immune infertility. Hopefully, the findings of this review may provide helpful evidence for the decision-making process of the patients, physicians, and investigators concerned.

## 2. Methods

This meta-analysis was conducted using Review Manager following the Cochrane Handbook for Systematic Reviews of Interventions (version 5.3.3) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. The protocol of this review was registered in INPLASY (INPLASY202110098).

*2.1. Search Strategy.* Six electronic databases, including China National Knowledge Infrastructure (CNKI), Wanfang Data, Chinese Scientific Journals Database (VIP), PubMed, EMBASE, and Cochrane Library were searched from inception to January 2021 for identifying eligible studies. No restriction on language or publication status was imposed. The following terms were used in a combination for the electronic search: immune infertility, immunological infertility, infertility, traditional Chinese medicine, complementary and alternative medicine, Chinese medicine, herbal medicine, prescription, formula, kidney deficiency, blood stagnation, blood stasis, supplementing kidney, tonifying kidney, activating blood circulation, randomized control, randomization, randomized clinical trials, RCT, and trials. Any inconsistency was solved by a third reviewer. Manual searches were performed to identify relevant studies in the reference lists of the included studies.

*2.2. Eligibility Criteria.* The inclusion criteria were prespecified as (1) types of participants: patients diagnosed with immunity infertility using any recognized diagnostic criteria, regardless of age, gender, source of cases, duration of disease, ethnicity, or nationality; (2) types of interventions: TCM therapy of TKABC prescription based on the theory of "kidney deficiency and blood stasis" clearly stated in the trial group either alone or in combination with conventional treatments; no restriction was imposed on the prescription name, administration mode, dosage, and course of treatment; (3) types of comparator(s)/control: patients treated with conventional (the same conventional regimen as intervention group in the same original study), placebo, or no treatment; (4) types of outcome measures: the total effective rate for immune infertility, pregnancy rate, negative conversion rate of antibodies, and adverse effects; and (5) types of study: RCT. The exclusion criteria included (1) non-RCTs, reviews, animal-based research, conference proceedings, and literature review; (2) unclear diagnostic criteria and outcome measurements; (3) unable to get original data; (4) duplicated publications; and (5) other TCM treatments involving acupuncture and massage.

*2.3. Outcome Measurements.* Primary outcomes included the total effective rate and pregnancy rate. The secondary outcomes were defined as the negative conversion rates of antibodies (AsAb, AEmAb, AoAb, AhCGAb, and ACAb) and incidence of adverse effects.

*2.4. Data Extraction.* Two reviewers (YLB and HZW) independently screened the titles and abstracts of eligible studies and then reviewed the full text following the prespecified eligibility criteria. They independently extracted the following information by a predesigned and standardized data extraction form: first author, year of publication, sample size, gender and age, course of the disease, TCM pattern differentiation, TCM treatment interventions and control groups, treatment duration, and primary and secondary outcome measurements. Any conflict was resolved by a third author (YHC). All data were cross-checked and transferred to RevMan software (V.5.3).

*2.5. Quality Assessment.* Two reviewers (YLB and LLH) independently used the Cochrane Handbook for Systematic Reviews of Interventions to evaluate the risk of bias for the included studies in the following seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective reporting, and other sources of bias. Each domain was assessed and graded as

"low risk," "unclear," and "high risk." Any disagreement was referred to a third investigator (YHC).

*2.6. Statistical Analysis.* The quantitative synthesis was performed using the Review Manager 5.3 software (The Cochrane Collaboration, NCC, CPH, Denmark). Relative risk (RR) with 95% confidence intervals (CIs) was used for binary variables, while the standard mean differences (SMD) with 95% CIs was applied for continuous variables. The chi-square statistic and $I^2$ statistic were employed to investigate statistical heterogeneity. The fixed-effects model was used for a low heterogeneity ($I^2 < 50\%$), and the random-effects model was applied if heterogeneity was moderate ($50\% < I^2 < 75\%$). Subgroup analyses were carried out to identify the potential source of high heterogeneity. Funnel plots were used to evaluate the potential reporting bias when more than ten eligible studies were included. Sensitivity analysis was conducted to assess the robustness of the pooled effects of the included studies.

## 3. Result

*3.1. Results of Literature Search.* Initially, potential 132 relevant studies were identified based on the search strategy. After excluding duplicate studies, the abstract and title of 86 studies were reviewed. Then, 48 articles were evaluated by full text, and 35 trials were excluded for the following reasons: three non-TKABC studies, 15 articles lack of control group, four studies without consistent intervention measures, three articles lack of eligible outcome measurements, six articles without the eligible type of prescription, and four articles with duplicate publication. Eventually, 13 studies were included for meta-analysis [6–18]. The flowchart of the selection process is shown in Figure 1.

*3.2. Basic Characteristics of the Included Studies.* Table 1 summarizes the basic characteristics of the included 13 trials. All the studies were conducted in China. A total of 1298 patients with immunity infertility were included [6–18], 730 in the trial group and 568 in the control group. The diagnosis of immunity infertility was clearly identified in all studies. Twelve studies were treated with herbal decoction [6–15, 17, 18], and one study was cured with Chinese patent medicine [16]. Patients in the control group were treated with Western medicine in all studies. For the outcome measurements, 12 trials presented the total effective rate [6, 8–18], 12 trials reported pregnancy rates [7–18], five trials mentioned AsAb [8, 11, 13, 14, 16], three trials presented AEmAb [12–14], one trial evaluated AoAb [13], one trial mentioned AhCGAb [13], two trials stated ACAb [7–14], and two trials reported adverse effects [11, 13]. The composition of TCM TKABC prescription in the included studies is shown in Supplementary Table 1.

*3.3. Risk of Bias Assessment.* Eleven studies of the 13 studies were classified as unclear risk because they just mentioned "random" and did not describe the methods for generating method [6, 8, 9, 11–18], and two studies were considered as high risk [7, 10]. None of the studies reported the process of allocation concealment and blinding. Thus, they were rated as high risk. All the studies had complete data; hence, the attrition bias was assessed as low risk. Reporting bias and other biases were classified as unclear due to insufficient information to evaluate the risk. In summary, the quality of included RCTs was poor (Figure 2).

*3.4. Total Effective Rate.* Twelve studies reported the total effective rate of TCM TKABC therapy in patients with immune infertility [6, 83.4; total effective rate: 18]. The pooled data of meta-analysis showed that the experimental group had a significantly higher total effective rate than that of the control group (RR: 1.38; 95% CI: 1.30, 1.47; and $I^2 = 0\%$) (Figure 3).

*3.5. Pregnancy Rate.* Twelve studies reported pregnancy rate [7–18]. The pooled effect of meta-analysis demonstrated that the pregnancy rate in the experimental group was significantly higher than that of the control group (RR: 2.04; 95% CI: 1.73, 2.40; and $I^2 = 30\%$) (Figure 4).

*3.6. Negative Conversion Rate of Serum Antibody.* All studies reported the negative conversion rate of serum antibodies. The pooled data of meta-analysis demonstrated that the negative conversion rates of serum antibodies were significantly improved in the experimental group (RR: 1.39; 95% CI: 1.26, 1.53; and $I^2 = 52\%$) (Figure 5). Subgroup analyses were performed on different comparators, as the control groups in four trials were treated with prednisone, three trials were intervened with the combination of enteric-coated aspirin, prednisone, and vitamin C, and two trials received dexamethasone therapy. The pooled data of meta-analysis revealed that the negative conversion rates of serum antibodies were significantly ameliorated in the experimental groups when compared with prednisone (RR: 6.55; 95% CI: 2.38, 18.04; and $I^2 = 72\%$) and enteric-coated aspirin, prednisone, and vitamin C (RR: 7.94; 95% CI: 2.52, 25.01; and $I^2 = 62\%$). No significant difference was evident upon comparison with the dexamethasone intervention (RR: 2.85; 95% CI: 1.40, 5.80; and $I^2 = 22\%$). The results of subgroup analyses are summarized in Figure 6. Further, subgroup analyses were carried out for serum antibodies. AsAb level was assessed in five trials, AEmAb level was measured in three trials, and ACAb level was evaluated in two trials. The pooled data of meta-analysis demonstrated that compared with the control groups, the negative conversion rates of AsAb (RR: 1.42; 95% CI: 1.12, 1.79; and $I^2 = 62\%$), AEmAb rates (RR:1.21; 95% CI: 1.04,1.41; and $I^2 = 0\%$), and AhCGAb were significantly higher in the experimental groups. No significant difference in the negative conversion rate of AoAb and ACAb (RR: 1.87; 95% CI: 0.81, 4.31; and $I^2 = 0\%$) was revealed. The results of subgroup analyses are summarized in Figure 7.

*3.7. Adverse Effects.* Two trials reported adverse effects [11, 13], including weight gain, indigestion, nausea, abdominal distension, mood changes, acne, full moon face, and

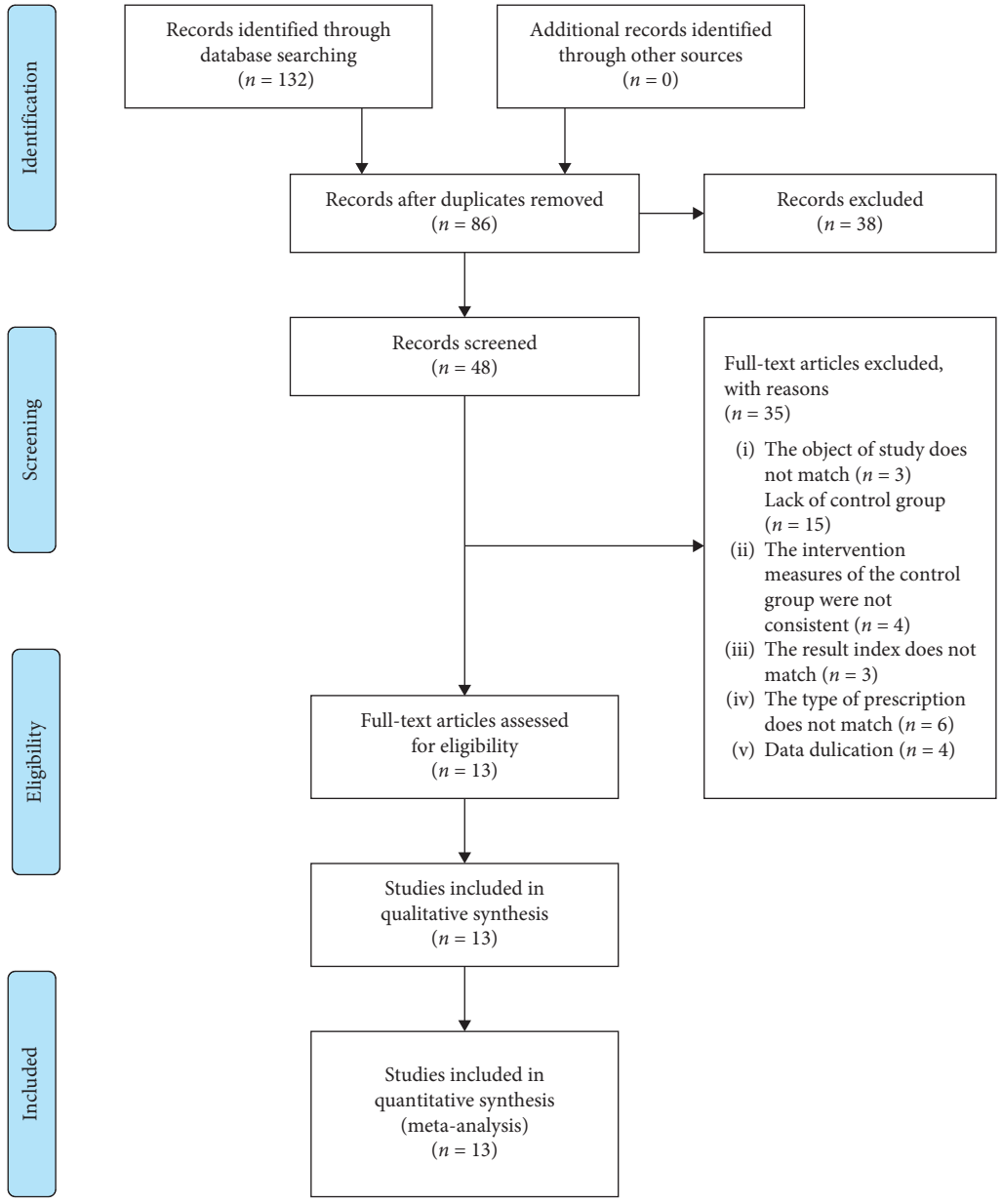


Figure 1: The PRISMA flowchart of the selection process.

flushing. The pooled effect of meta-analysis showed that compared with the control group, the adverse effects of the experimental group were significantly lower (RR: 0.24; 95% CI: 1.73, 2.40; and $I^2 = 55\%$) (Figure 8).

*3.8. Publication Bias.* Funnel plots were used to measure the publication bias. The total effective rate, antibody negative conversion rate, and pregnancy rate were in asymmetric distribution, indicating that publication bias might exist (Figure 9).

*3.9. Sensitivity Analysis.* Sensitivity analysis was performed for the total effective rate, the negative conversion rate of antibody, and pregnancy rate. The effect remained unchanged, indicating the robustness of the pooled results.

## 4. Discussion

According to TCM theory, the etiology and pathogenesis of immune infertility are dominated by kidney deficiency and blood stasis. The kidney is considered as "the origin of congenital constitution." It is the origin of yin-yang, the source of life, stores the essence, and acts as the primary material foundation for the growth, development, and reproduction of human beings. Long-term kidney deficiency may cause blood stasis, and blood stasis may aggravate kidney deficiency [19–22]. Therefore, the fundamental therapeutic principles for immune infertility treatment are to tonify kidney, activate blood circulation, remove blood stasis, and dredge collaterals. Correlation analyses revealed that kidney-tonifying and blood circulation-activating prescriptions and herbs are commonly used to treat immune

Table 1: Basic characteristics of the included study.

| Author(s) | Sample size Expt./Ctrl. | Average age ($y$) Expt./Ctrl. | Gender | Diagnostics | Intervention measures | | Duration treatment | Outcome measures |
|---|---|---|---|---|---|---|---|---|
| | | | | | Expt. | Ctrl. | | |
| Wu [6] | 31/31 | 32.6/31.4 | Female | A + B + C | Bushen Huoxue decoction + WM | Prednisone + clomiphene + vitamins C, E | 3m * 2 | ① |
| Lu and Gong [7] | 28/28 | 26.34/27.12 | Female | A + C | Xiaokang II decoction | Aspirin | 2m * 2 | ⑥⑦ |
| Chen and Xu [8] | 30/30 | 27.03/26.67 | Female | A + B + C | Bushenyikang decoction | Prednisone | 1m * 3 | ①②⑦ |
| Ma and Zhang [9] | 46/44 | 31.2/31.4 | Female | A + B + C | Yikang Zhuyun decoction | Prednisolone | 14d * 3 | ①⑦ |
| Liu et al. [10] | 176/82 | 33.5/33.5 | Female | A + B + C | Bushen Huoxue decoction + WM | Vitamin C + aspirin + prednisone | 21d * 2 | ①⑦ |
| Liu [11] | 35/35 | 29.33/29.25 | Female | A + B + C | Bushen Huoxue decoction + WM | Prednisone | 3m * 3 | ①②⑦⑧ |
| Cai et al. [12] | 40/40 | 28.76/30.72 | Female | A + B + C | Huoxue Xiaokang decoction | Prednisone + acetate aspirin | 45d * 2 | ①③⑦ |
| Zhong et al. [13] | 70/65 | 27.8/27.8 | Female | A + B + C | Bushen Huoxue decoction | Prednisone | 1m * 3 | ①②③④⑤⑦⑧ |
| Qi et al. [14] | 78/60 | 29.5/29.5 | Female | A + B + C | Yulin Qingkang decoction | Dexamethasone | 1m × 3 | ①②③⑥⑦ |
| Wu [15] | 69/50 | 28.35/28.35 | Female | A + B + C | Assisting-pregnancy decoction | Dexamethasone | 2m × 1 | ①⑦ |
| Zhao [16] | 56/35 | 28.35/28.35 | Female | A + B + C | Anti-immunity I tablet | Prednisone | 2m * 3 | ①②⑦⑧ |
| Fu [17] | 23/21 | 28.1/28.1 | Female | A + B + C | Bushen Huoxue Xiaokang decoction + WM | Enteric-coated aspirin + prednisone + vitamin C | 14d * 1 | ①⑦ |
| Liang and Yuan [18] | 48/47 | — | Female | A + B + C | Bushen Huoxue Xiaokang decoction + WM | Enteric-coated aspirin + prednisone + vitamin C | 14d * 2 | ①⑦ |

Expt.: experimental group; Ctrl.: control group; A: diagnostic criteria for infertility: unable to conceive after one year or longer of unprotected sex; B: ruling out infertility due to other factors, such as tubal obstruction, ovulation disorders, and endometriosis; C: positive for at least one of the following serum antibody tests: AsAb, AEmAb, ACAb, AoAb, AZPAb, and AhCGAb. ① Total effective rate; ② serum AsAb negative conversion rate; ③ serum AEmAb negative conversion rate; ④ serum AoAb negative conversion rate; ⑤ serum AhCGAb negative conversion rate; ⑥ ACAb negative conversion rate; ⑦ pregnancy rate; and ⑧ adverse effects.

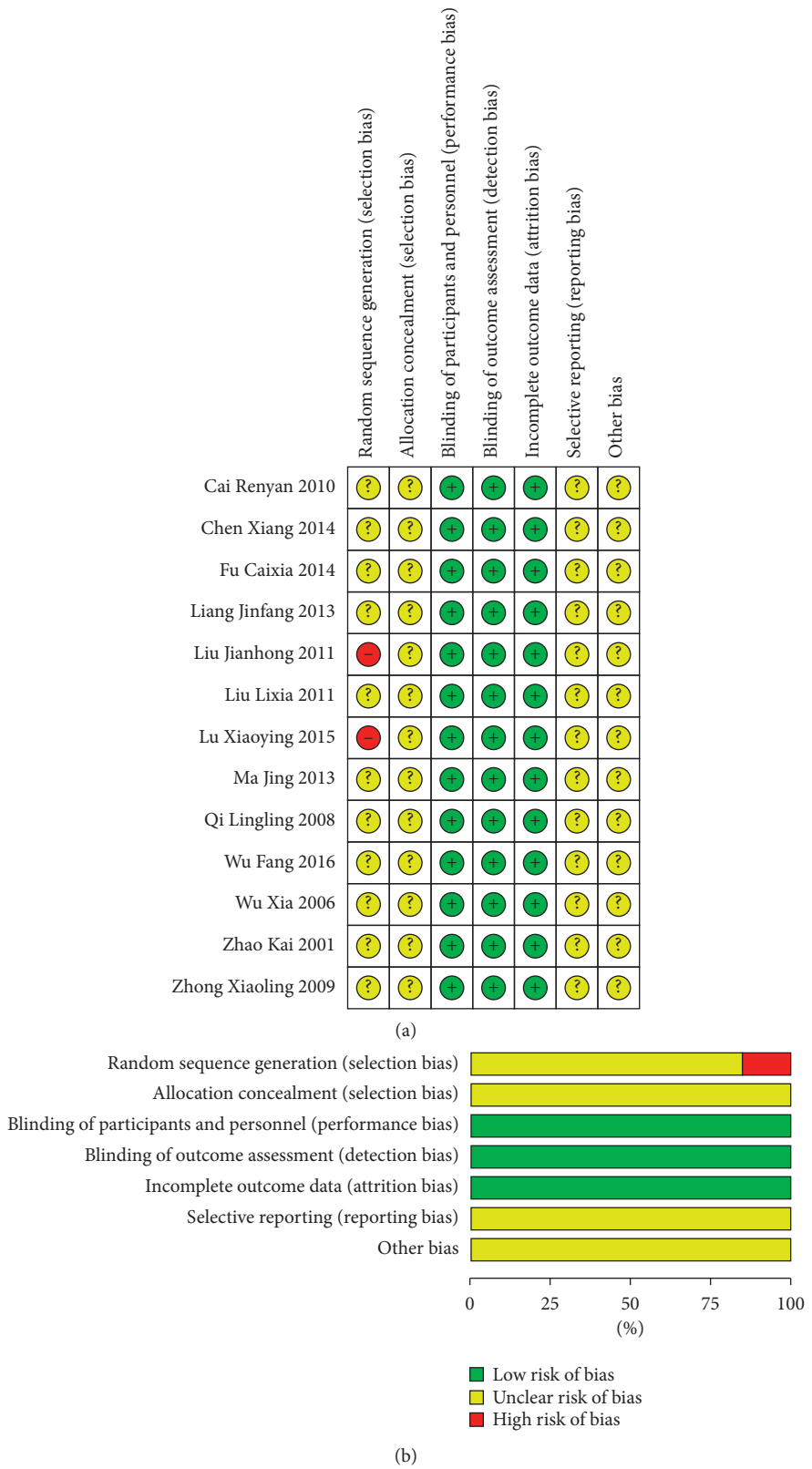


Figure 2: Summary of the risk of bias. The risk of bias assessment revealed that the RCTs were of poor methodological quality.

| Study or subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight (%) | Risk ratio M-H, fixed, 95% CI |
|---|---|---|---|---|---|---|
| Cai Renyan 2010 | 32 | 40 | 27 | 40 | 5.8 | 1.19 [0.91, 1.54] |
| Ma Jing 2013 | 42 | 46 | 32 | 44 | 7.0 | 1.26 [1.03, 1.54] |
| Liang Jinfang 2013 | 44 | 48 | 34 | 47 | 7.4 | 1.27 [1.04, 1.54] |
| Fu Caixia 2014 | 21 | 23 | 15 | 21 | 3.4 | 1.28 [0.95, 1.72] |
| Zhong Xiaoling 2009 | 63 | 70 | 45 | 65 | 10.1 | 1.30 [1.09, 1.56] |
| Wu Xia 2006 | 63 | 69 | 35 | 50 | 8.7 | 1.30 [1.07, 1.59] |
| Wu Fang 2016 | 29 | 31 | 22 | 31 | 4.7 | 1.32 [1.03, 1.68] |
| Liu Jianhong 2011 | 275 | 281 | 135 | 192 | 34.6 | 1.39 [1.27, 1.53] |
| Liu Lixia 2011 | 27 | 35 | 19 | 35 | 4.1 | 1.42 [1.00, 2.02] |
| Chen Xiang 2014 | 24 | 30 | 15 | 30 | 3.2 | 1.60 [1.07, 2.39] |
| Zhao Kai 2001 | 53 | 56 | 20 | 35 | 5.3 | 1.66 [1.23, 2.22] |
| Qi Lingling 2008 | 53 | 78 | 23 | 60 | 5.6 | 1.77 [1.24, 2.53] |
| *Total (95% CI)* | | *807* | | *650* | *100.0* | *1.38 [1.30, 1.47]* |
| Total events | 726 | | 422 | | | |

Heterogeneity: chi$^2$ = 7.94, df = 11 ($P$ = 0.72); $I^2$ = 0%
Test for overall effect: $Z$ = 10.45 ($P$ < 0.00001)

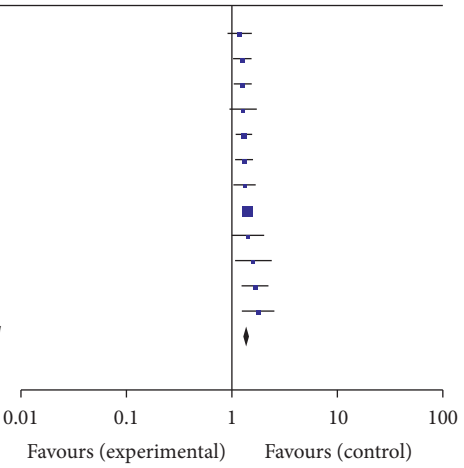


Figure 3: Forest plot for total effective rate between the experimental and control groups.

| Study or subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight (%) | Risk ratio M-H, fixed, 95% CI |
|---|---|---|---|---|---|---|
| Cai Renyan 2010 | 14 | 40 | 4 | 40 | 2.8 | 3.50 [1.26, 9.72] |
| Chen Xiang 2014 | 12 | 30 | 4 | 30 | 2.8 | 3.00 [1.09, 8.25] |
| Fu Caixia 2014 | 19 | 23 | 13 | 21 | 9.4 | 1.33 [0.91, 1.96] |
| Liang Jinfang 2013 | 25 | 48 | 12 | 47 | 8.4 | 2.04 [1.17, 3.57] |
| Liu Jianhong 2011 | 82 | 176 | 19 | 82 | 18.0 | 2.01 [1.31, 3.07] |
| Liu Lixia 2011 | 15 | 35 | 7 | 36 | 4.8 | 2.20 [1.02, 4.75] |
| Lu Xiaoying 2015 | 16 | 28 | 5 | 28 | 3.5 | 3.20 [1.36, 7.54] |
| Ma Jing 2013 | 24 | 46 | 13 | 44 | 9.2 | 1.77 [1.04, 3.01] |
| Qi Lingling 2008 | 20 | 78 | 7 | 60 | 5.5 | 2.20 [1.00, 4.85] |
| Wu Xia 2006 | 40 | 69 | 12 | 50 | 9.7 | 2.42 [1.42, 4.11] |
| Zhao Kai 2001 | 30 | 56 | 5 | 35 | 4.3 | 3.75 [1.61, 8.75] |
| Zhong Xiaoling 2009 | 45 | 70 | 30 | 65 | 21.6 | 1.39 [1.02, 1.91] |
| *Total (95% CI)* | | *699* | | *538* | *100.0* | *2.04 [1.73, 2.40]* |
| Total events | 342 | | 131 | | | |

Heterogeneity: chi$^2$ = 15.67, df = 11 ($P$ = 0.15); $I^2$ = 30%
Test for overall effect: $Z$ = 8.46 ($P$ < 0.00001)

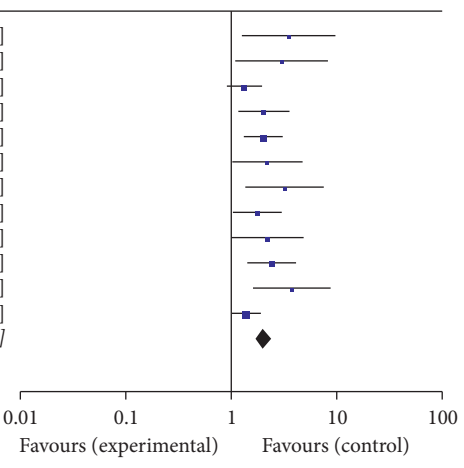


Figure 4: Forest plot for pregnancy rate between the experimental and control groups.

| Study or subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight (%) | Risk ratio M-H, random, 95% CI |
|---|---|---|---|---|---|---|
| Cai Renyan 2010 | 32 | 40 | 27 | 40 | 7.7 | 1.19 [0.91, 1.54] |
| Chen Xiang 2014 | 24 | 30 | 15 | 30 | 4.4 | 1.60 [1.07, 2.39] |
| Fu Caixia 2014 | 21 | 23 | 15 | 21 | 6.7 | 1.28 [0.95, 1.72] |
| Liang Jinfang 2013 | 44 | 48 | 34 | 47 | 10.5 | 1.27 [1.04, 1.54] |
| Liu Jianhong 2011 | 275 | 281 | 135 | 192 | 16.0 | 1.39 [1.27, 1.53] |
| Liu Lixia 2011 | 27 | 35 | 19 | 35 | 5.3 | 1.42 [1.00, 2.02] |
| Lu Xiaoying 2015 | 25 | 28 | 12 | 28 | 3.7 | 2.08 [1.33, 3.26] |
| Ma Jing 2013 | 42 | 46 | 32 | 44 | 10.3 | 1.26 [1.03, 1.54] |
| Qi Lingling 2008 | 29 | 78 | 13 | 60 | 2.5 | 1.72 [0.98, 3.01] |
| Wu Fang 2016 | 25 | 31 | 15 | 31 | 4.3 | 1.67 [1.11, 2.49] |
| Wu Xia 2006 | 63 | 69 | 35 | 50 | 10.6 | 1.30 [1.07, 1.59] |
| Zhao Kai 2001 | 53 | 56 | 10 | 35 | 2.8 | 3.31 [1.95, 5.61] |
| Zhong Xiaoling 2009 | 123 | 131 | 97 | 129 | 15.2 | 1.25 [1.12, 1.39] |
| *Total (95% CI)* | | *896* | | *742* | *100.0* | *1.39 [1.26, 1.53]* |
| Total events | 783 | | 459 | | | |

Heterogeneity: tau$^2$ = 0.01; chi$^2$ = 24.84, df = 12 ($P$ = 0.02); $I^2$ = 52%
Test for overall effect: $Z$ = 6.73 ($P$ < 0.00001)

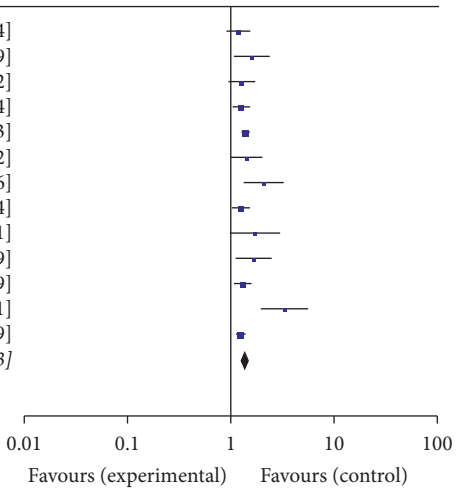


Figure 5: Forest plot for negative conversion rate of serum antibody between the experimental and control groups.

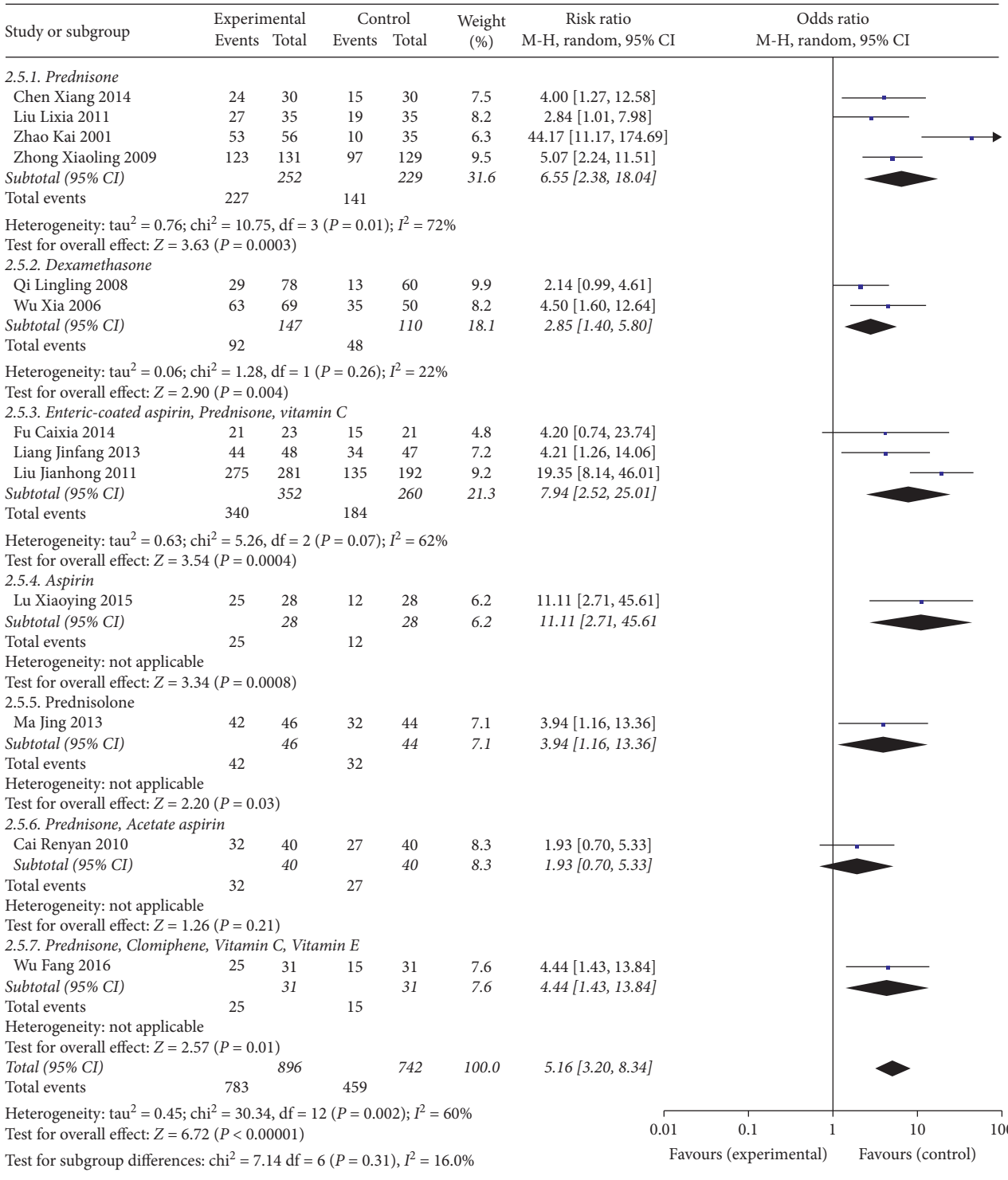


Figure 6: Subgroup analysis for the negative conversion rate of different comparators between the experimental and control groups.

infertility and can regulate the reproductive axis in a bidirectional manner, the immune function, and serum antibodies [23–25]. In immune infertility, AsAb is a complex pathological product. Sperm is an antigen that causes the body to produce AsAb when the immunity system is exposed to it. AsAb reduces sperm motility, prevents sperm from undergoing capacitation and acrosome reactions, and impacts sperm-oocyte recognition and fusion [26, 27]. In this meta-analysis, we found that TKABC therapy based on the theory of kidney deficiency and blood stasis could

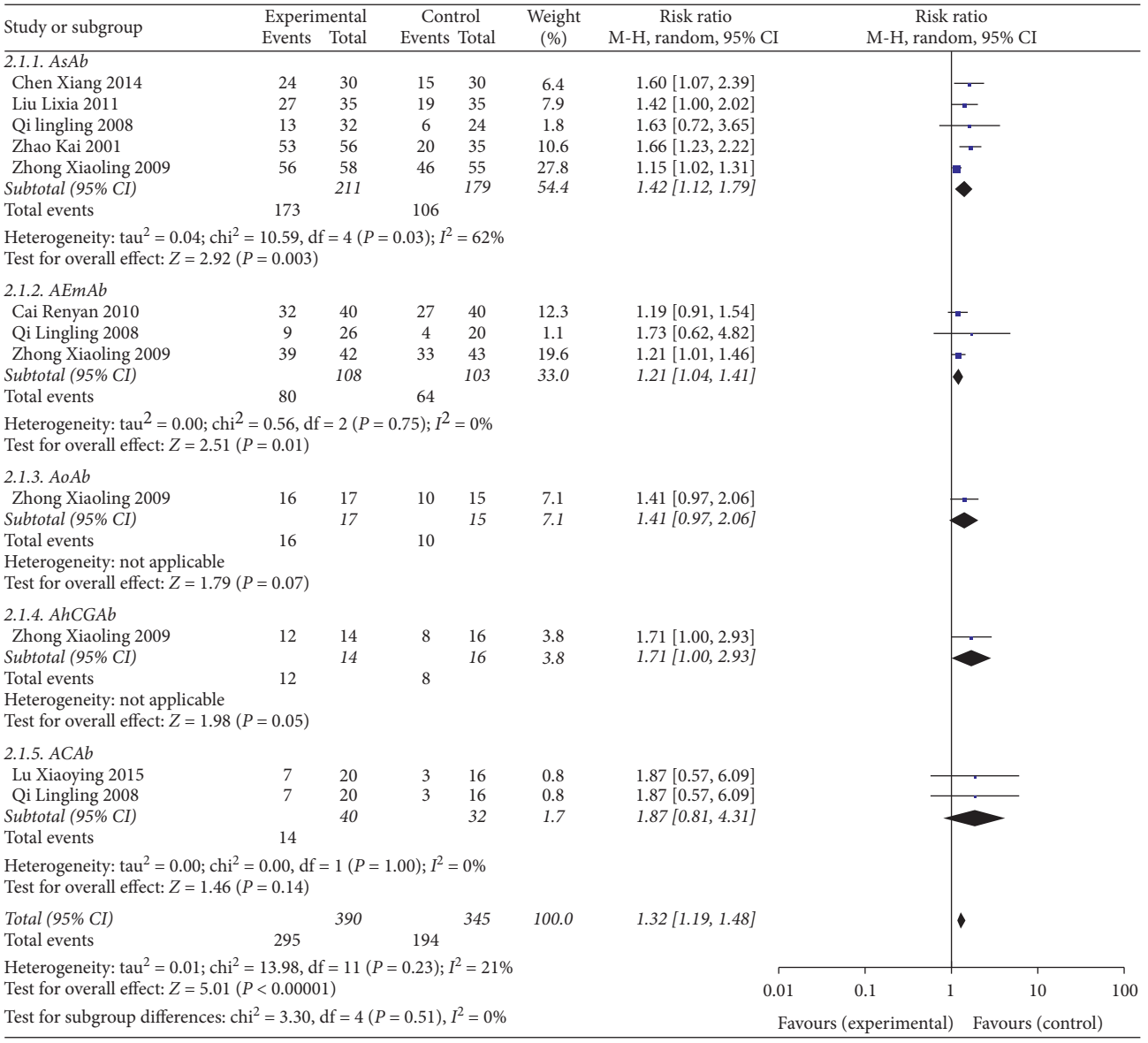


Figure 7: Subgroup analysis for the negative conversion rate of various serum antibody between the experimental and control groups.

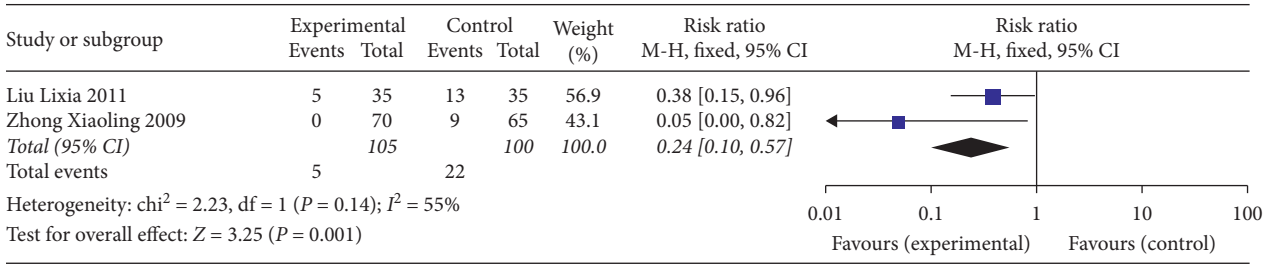


Figure 8: Forest plot for adverse effects between the experimental and control groups.

significantly improve the total effective rate, the negative conversion rate of AsAb, AEmAb, and AhCGAb, and pregnancy rate with fewer adverse effects.

Although the effectiveness and safety of TKABC on immune infertility were evaluated using a meta-analysis, this study has several limitations. (1) The number of included studies and sample size of the studies were small. (2) Some RCTs had low methodological quality and may result in overestimation of the therapeutic effect. (3) Although we searched the studies without language limitations, all the

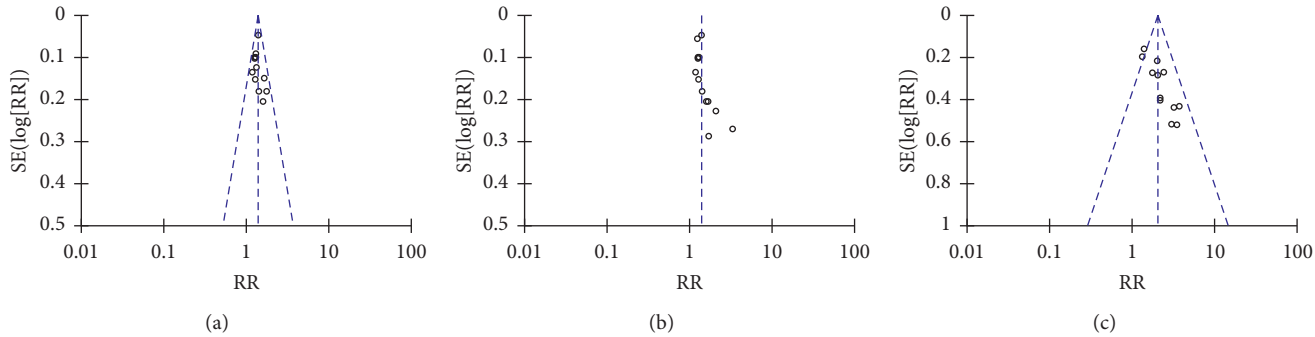


Figure 9: Funnel plots for publication bias. (a) Total effective rate. (b) Negative conversion rate of serum antibody. (c) Pregnancy rate.

publication regions were in China. (4) The herbal components of TKABC therapy were different among studies, which might cause bias. (5) The criteria for the efficacy and duration of treatment in each study were inconsistent. (6) Studies with negative results may have been published with a lower frequency and cause publication bias.

## 5. Conclusion

In summary, this study shows that TCM therapy of TKABC based on the theory of "kidney deficiency and blood stasis" may be effective and safe for immune infertility. It might be considered as a complementary and alternative treatment to conventional therapy. However, due to limited data and the low quality of methodology of the included studies, more well-designed and high-quality multicenter RCTs with a larger sample size need to be performed to confirm these results.

## Ethical Approval

As it is a systematic review and meta-analysis based on previously published literature, ethical approval is not required.

## Consent

As it is a systematic review and meta-analysis based on previously published literature, informed consent of patients is not required.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Authors' Contributions

Yi-ling Bai and Yun-hui Chen contributed equally to this work. All authors contributed substantially to the design, interpretation of the data, statistical analysis, drafting the manuscript, and approving the submission.

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## Supplementary Materials

*Supplementary Table 1*. Composition of TCM prescription in the included studies. (*Supplementary Materials*)

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*Research Article*

# Comparison of Effects between Combined Lumbar-Sacral Plexus Block plus General Anesthesia and Unilateral Spinal Anesthesia in Elderly Patients Undergoing Hip Fracture Surgery: A Pilot Randomized Controlled Trial

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*Purpose*. Hip fracture is a common injury in geriatric populations, which is associated with poor quality of life. However, the ideal anesthesia technique for this disease is yet to be identified. This study aimed to compare the combined lumbar-sacral plexus block (CLSB) plus general anesthesia (bispectral index (BIS) 60–80) with the unilateral spinal anesthesia (SA) on activity of daily living in elderly patients undergoing hip fracture surgery. *Methods*. A total of 124 elderly patients undergoing hip fracture surgery were randomly assigned to two groups. Patients in the SA group received light-specific gravity spinal anesthesia, and patients in the CLSB group received lumbar and sacral plexus block with general anesthesia (BIS 60–80). The primary outcomes were 30-day activity of daily living (ADL). The secondary outcomes were postoperative pain scores, postoperative delirium, in-hospital cost, and major complications. *Results*. The ADL scores of postoperative day 30 (POD30) in the CLSB group are higher than those in the SA group ($27.34 \pm 7.01$ versus $24.70 \pm 6.40$, $P = 0.045$). Compared to preoperative ADL scores, there were higher increased scores in the CLSB group than in POD30 (CLSB group $8.09 \pm 3.39$ versus SA group $4.87 \pm 3.90$, $P < 0.001$). Mild-to-moderate pain did not have differences between the two groups (rest pain: 3 versus 2, $P = 0.344$; motion pain: 5 versus 4, $P = 0.073$). There were no significant differences in incidence of postoperative delirium, PONV, and other complications. *Conclusion*. The unilateral SA can reduce the deterioration of ADL after hip fracture surgery and provide a better postoperative recovery.

## 1. Introduction

Hip fracture is a common injury in geriatric populations, ranking the second among the causes of hospitalization and disability for elderly patients. It has critical consequences of deteriorated function status, for example, increasing mortality and decreasing quality of life [1, 2]. A large percentage of these patients did not attain their prefracture level of independence and ambulatory status [3, 4]. Thus, no matter for orthopedists or anesthesiologists, it is important to implement occupational therapy interventions to improve the function status and the quality of life among this population.

Activity of daily living (ADL), an important tool to assess the functional status and the quality of life, has been widely used to evaluate postoperative recovery and disease progression [5]. Previous studies had investigated the potential influence of different surgical methods [6], the early operative time [7], and the nutritional status [8] on the activities of daily living after hip fracture surgery. However, evidence to clarify the influence of different types of anesthesia on postoperative ADL is still lacking.

Both the combined lumbar and sacral plexus block (CLSB) with general anesthesia (BIS 60–80) and the unilateral spinal anesthesia (SA) on the operative side had been reported to be safe and effective as regional anesthesia techniques for hip surgery [9–11]. To the authors' knowledge, there were no studies comparing the superiority of these two anesthesia methods in terms of ADL. Hence, the purpose of this study was to compare the CLSB plus general

anesthesia (BIS 60–80) and the unilateral spinal anesthesia plus monitored anesthesia care (MAC) on the postoperative ADL of elderly patients undergoing hip fracture surgery. We hypothesized that the unilateral SA plus MAC would attenuate the deterioration of daily activity compared to the CLSB plus general anesthesia.

## 2. Materials and Methods

This prospective, controlled, and two parallel-group clinical trial was approved by the ethics committee of the First Affiliated Hospital of Anhui Medical University (approval number: PJ2018-11-06) and registered in the Chinese Clinical Trial Registry (ChiCTR1900025113; principal investigator: Panpan Fang; date of registration: August 12, 2019). The study was conducted in accordance with the principles of the Helsinki Declaration at the First Affiliated Hospital of Anhui Medical University.

*2.1. Patients and Randomization.* Between August 2019 and December 2019, patients who underwent elective unilateral hip fracture surgeries, including osteosynthesis, artificial femoral head replacement, and total hip replacement, at the First Affiliated Hospital of Anhui Medical University were assessed for eligibility.

The inclusion criteria were as follows: >65 years of age and American Society of Anesthesiologists I–IV. The exclusion criteria were as follows: dementia or severe cognitive dysfunction (simple mental state questionnaire ≥8), unstable mental state or mental disease, reception of psychotropic drugs or abuse of narcotic sedation analgesics, being delirious or history of delirium, anesthesia and surgery within 6 months, other surgeries at the same time, cerebrovascular accidents such as cerebral stroke and transient ischemic attack within 3 months, and prosthesis fracture repair surgery.

The participants were randomized to the CLSB or the SA group with a 1 : 1 allocation using computer-generated randomized numbers. Patients in the CLSB group received lumbar and sacral plexus block with general anesthesia (BIS 60–80), while patients in the SA group received light-specific gravity spinal anesthesia plus MAC. Written informed consent forms were offered to all patients or their legal relatives.

*2.2. Anesthetic Management.* Heart rate, blood pressure, electrocardiograph, pulse oxygen saturation ($SpO_2$), and BIS were routinely monitored for each patient. After the venous access was available, all patients were injected with 6–8 mL/kg/h of Ringer's lactate solution or hydroxyethyl starch 130/0.4 sodium chloride before anesthesia. Then, the fluids were adjusted according to the hemodynamic monitoring and blood loss in the operating room.

*2.3. CLSB with General Anesthesia (BIS 60–80).* All CLSB procedures were conducted by an attending anesthesiologist well versed with peripheral anesthesia. Before the patient was placed in a lateral decubitus position with the operated side uppermost, flexion of uninjured hip and knee, 5 $\mu$g sufentanil was administered intravenously for pain relief.

During the CLSB procedure, oxygen was provided via a face mask with a flow of 5–8 L/min. All peripheral nerve blockades were performed under the guidance of a nerve stimulator (Stimuplex HNS 12, B. Braun Medical Inc., Germany) and ultrasound (FUJIFILM Sonosite Inc., WA, USA). A 12 cm 22-gauge nerve stimulation needle (Stimuplex D, B. Braun Medical Inc., Germany) was advanced perpendicularly to the skin between L3 and L4 transverse processes. An appropriate needle position was confirmed as quadriceps contraction after a stimulating current of 0.4 mA and a frequency of 2 Hz. After negative aspiration with blood, 20 mL of 0.25% ropivacaine was slowly injected.

The sacral plexus nerve block was performed using the transgluteal approach with the same position under ultrasound and nerve stimulation guidance. The sacral plexus was identified by the motor response of the gluteus maximus and gastrocnemius, with a stimulating current of 0.4 mA and a frequency of 2 Hz. Similarly, 20 mL of 0.25% ropivacaine was slowly injected to complete the sacral plexus block. It was considered as a failed procedure in the case of the absence of the right motor responses after three nerve block puncture attempts. Then general anesthesia was applied, and the patient was eliminated from the study.

After confirming successful block using the pinprick test, propofol (1–1.5 mg/kg), sufentanil (0.1–0.2 $\mu$g/kg), and cisatracurium besilate (0.2 mg/kg) were used for anesthesia induction [10]. A laryngeal mask (LMA Supreme, Laryngeal Mask Company Ltd., Malaysia) was used for airway management. Mechanical ventilation was set as follows: the tidal volume was set as 6–8 mL/kg, the respiratory rate was set as 10–12 breaths/min, the ratio of expiration: inspiration was set as 2 : 1, and the end-tidal carbon dioxide pressure remained at 35–40 mmHg. The effect-site concentration of propofol was adjusted to maintain the depth of sedation (BIS: 60–80) [10]. The depth of sedation was assessed by observer's assessment of alertness/sedation (OAA/S) as a supplement.

*2.4. Unilateral Spinal Anesthesia plus MAC: Light-Specific Gravity Spinal Anesthesia.* All the SA procedures were performed by a skilled attending anesthesiologist. After the patient was placed in a vertical position with the operated side uppermost and flexion of uninjured hip and knee, the puncture sites were selected at L3–4 or L2–3 vertebral interspace. The median side of the spine was opened between 0.5 and 1.0 cm for local infiltration. With both hands holding the protruding needle tip (25 G), the needle was advanced perpendicularly into the spine through the middle of the lumbar vertebral space. The recorded depth was between 3.5 and 5.0 cm. Once encountering bone, the needle tip was slightly tilted to the side of the head by about 10° to 15° and then reinserted until the clear cerebrospinal fluid reflux. Further, 4 mL of 0.25% hypobaric ropivacaine was injected into the subarachnoid space [12]. The sensory block on lower limbs was evaluated using the pinprick test, whereas the

motor blockade was evaluated by modified Bromage scale (0 = no motor block, 1 = hip blocked, 2 = hip and knee blocked, and 3 = hip, knee, and ankle blocked). Assessments of motor and sensory blocks in the operated and the nonoperated sides were made at the following times: 3, 5, 10, and 15 minutes after the injection. Successful anesthesia was defined as no pain at T12 and a modified Bromage score ≥2 only on the operated limb. Then, light depth of sedation (BIS 60–80) was maintained by adjusting the effect-site concentration of propofol. The depth of sedation was assessed by observer's assessment of alertness/sedation (OAA/S) as a supplement. The absence of the right motor response after three spinal puncture attempts was considered as failed, and then general anesthesia was applied.

Intraoperative hypotension was defined as a decrement in systolic blood pressure by more than 20% from preoperative values and/or mean arterial pressure less than 65 mmHg. Patients with intraoperative hypotension were immediately treated with phenylephrine. All operations were completed using the posterior approach and by the same arthroplasty surgeons. All patients were transferred to the postanesthesia care unit (PACU) after showing satisfactory spontaneous breathing.

The criteria for extubation were as follows: recovery of consciousness, the train-of-four ratio >0.9, the tidal volume >6 mL/kg, the breathing rate <30 beats/min, and the maintenance of pulse oximetry ($SpO_2$) at >92% under air inspiration. Postoperative analgesia was achieved by a patient-controlled analgesia (PCA) solution, which was prepared by dissolving sufentanil (2.5 $\mu$g/kg) and flurbiprofen axetil (100 mg) in 100 ml of saline. For the loading dose, one bolus was set to 2 mL and background infusion rate was set to 2 ml/h, while the lockout time was set to 15 min. The analgesic goal was postoperative VAS ≤3. If the VAS score ≥4, patients would receive rescue analgesic to relief pain.

### 2.5. Outcomes Measures

*2.5.1. Primary Endpoint.* The primary endpoint was the 30-day function status assessed by the Chinese version of the daily living scale (CADL), which includes a physical self-maintenance scale (PSMS) and an instrumental activities of daily living (IADL) scale [13]. The function status before fracture was assessed one day before surgery with CADL by interviewing the patients. The PSMS, normally used to assess the fundamental skills to live, consists of six tasks: ambulating, dressing, eating, grooming, toileting, and bathing. The IADL scale is used to assess more complicated activities that allow an individual to live independently. This scale contains eight tasks: preparing food, taking public transportation, housekeeping, doing laundry, taking medications, making phone calls, shopping, and managing finances. CADL has a total of 14 items and ranged from 14 to 56 points. The single item score is determined by a 4-point numeric rating scale (1 = can do it by oneself, 2 = have some difficulty but can still do it by oneself, 3 = need help to do it, and 4 = cannot do it at all). Higher ADL scores indicate a poor functional status, and 22 points were the cutoff score. Over the cutoff score was defined as impairment in ADL.

*2.5.2. Secondary Endpoints.* Secondary endpoints included the incidence of postoperative delirium, pain scores, the in-hospital cost, postoperative nausea and vomiting (PONV), and major postoperative complications (stroke and heart failure). All the patients had same discharge criteria: no fever, resumed a normal diet, and well incisions heal. The preoperative mental state was assessed using the simple mental state questionnaire, while perioperative delirium was evaluated by the Confusion Assessment Method (CAM) [14]. The postoperative delirium was assessed twice daily for 7 days or until discharged. Rest pain, motion pain, postoperative nausea and vomiting (PONV), and major postoperative complications were evaluated at 8 : 00 a.m. on the first three days after surgery. Evaluation for ADL score was completed via phone calls on the 30th day after surgery (postoperative day 30, POD30).

A trained researcher in our department conducted all these assessments, and he was blinded to the randomized allocation and intervention in this study.

*2.6. Statistical Analysis.* We used PASS11.0 (NCSS, LLC., Kaysville, UT, USA) for the sample size calculation. According to our pilot data, we estimated that the mean of ADL incremental value was 5 in SA group and the SD was 4; the mean of ADL incremental value was 8 in CLSB group. Assuming a 5% two-tailed type I error rate, a sample size of 92 was required to detect a significant difference with the power of 90%. Allowing for 15% noncompliance, at least 106 patients were required in the present study.

Data were expressed as mean ± standard deviation, median (interquartile range), or number (proportion, %) and analyzed by SPSS version 16.0 (SPSS Inc., IL, USA). For normal-distribution data, two-tailed Student's $t$-test was used. Continuous data that were not normally distributed were analyzed by the Mann–Whitney $U$ test. Categorical data were analyzed using the chi-square ($\chi^2$) test or Fisher's exact test where appropriate. Statistical significance was set at $P < 0.05$.

## 3. Results

Of the 159 patients assessed for eligibility, 10 patients did not meet the inclusion criteria, while 3 refused to participate in the study. Further, 22 patients were excluded from the study. Eventually, 124 patients were randomly assigned to SA and CLSB groups. In the CLSB group, four patients were lost to follow-up and three patients were switched to general anesthesia. In the SA group, two patients were lost to follow-up and five were switched to general anesthesia. Therefore, only 110 patients completed the study (Figure 1). No significant differences were observed in the demographics and baseline data between two groups (shown in Table 1).

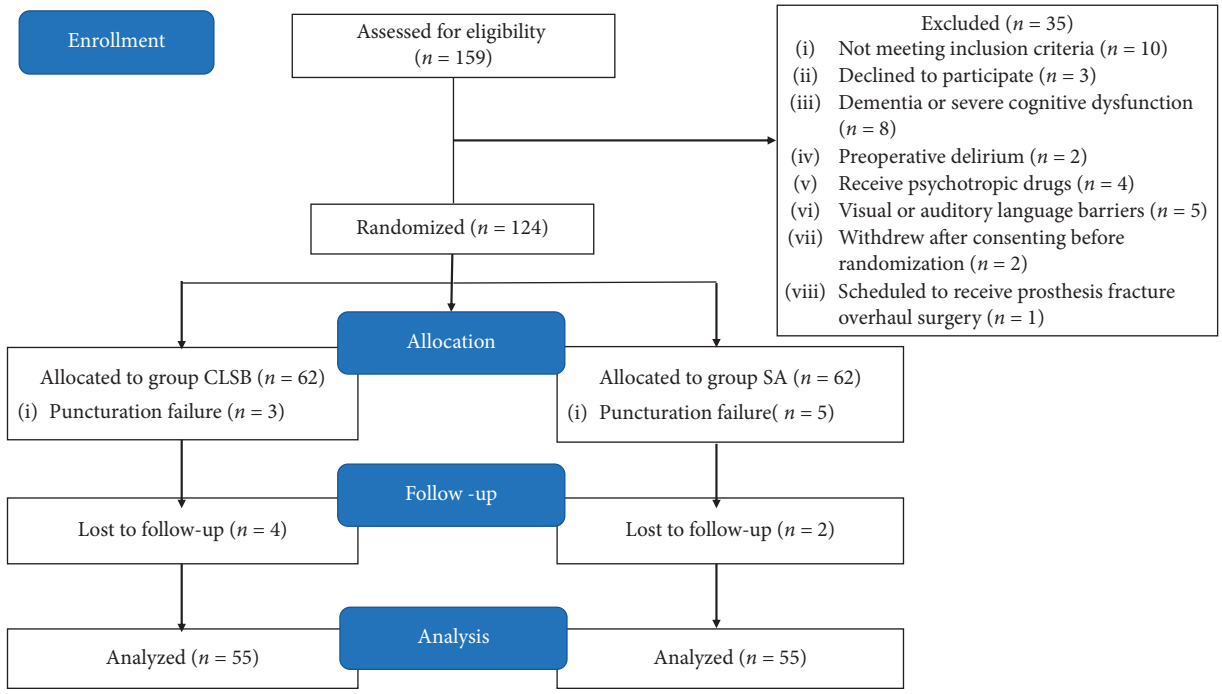


Figure 1: The consort flow chart outlining patients' assignment and treatment protocols. Patients in the SA group received light-specific gravity spinal anesthesia, while the CLSB group received lumbar and sacral plexus block with light sedation (BIS 60–80).

Table 1: Demographics and baseline data of the study population.

| | Group SA | Group CLSB | *P* value |
|---|---|---|---|
| Age (year) | 78.00 (6.45) | 76.60 (6.98) | 0.277 |
| Sex (male/female) | 16/39 | 20/35 | 0.416 |
| BMI | 21.35 (3.16) | 22.41 (3.25) | 0.086 |
| ASA (II/III/IV) | 21/31/3 | 21/29/5 | 0.818 |
| *CCI* | | | 0.205 |
| 0 | 14/55 (25.5%) | 11/55 (20.0%) | |
| 1 | 17/55 (30.9%) | 13/55 (23.6%) | |
| 2 | 20/55 (36.4%) | 25/55 (45.5%) | |
| 3 | 3/55 (5.5%) | 4/55 (7.3%) | |
| 4 | 1/55 (1.8%) | 2/55 (3.6%) | |
| *Education (year)* | | | 0.079 |
| 0 | 28/55 (50.9%) | 19/55 (34.5%) | |
| 5 | 13/55 (23.6%) | 18/55 (32.7%) | |
| 8 | 6/55 (10.9%) | 4/55 (7.3%) | |
| 11 | 7/55 (12.7%) | 9/55 (16.4%) | |
| 15 | 1/55 (1.8%) | 4/55 (7.3%) | |
| 18 | 0/55 (0.0%) | 1/55 (1.8%) | |
| Hemoglobin (g/dL) | 10.99 (1.87) | 11.34 (2.34) | 0.397 |
| Albumin (g/dL) | 3.74 (0.41) | 3.78 (0.55) | 0.619 |
| Preoperative ADL | 19.76 (5.11) | 19.60 (5.54) | 0.872 |
| Rest pain | 3 (2, 4) | 2 (2, 3) | 0.344 |
| Motion pain | 5 (4, 6) | 4 (3, 6) | 0.073 |

Statistics are presented as mean (standard deviation), median (interquartile range), or *N*/total number of patients (%) as appropriate. ADL, activity of daily living; ASA, American Society of Anesthesiologists; BMI, body mass index; CCI, Charlson comorbidity index; CLSB, combined lumbar and sacral plexus block; SA, spinal anesthesia.

*3.1. Primary Outcomes.* The ADL scores before fracture were comparable between two groups. The patients in the CLSB group had a higher POD30 ADL score ($27.34 \pm 7.01$ versus $24.70 \pm 6.4$, $P = 0.045$) (shown in Table 2). The ADL scores of POD30 in both groups were increased, compared with the ADL scores before fracture. However, patients in the CLSB group had higher incremental ADL values ($8.09 \pm 3.39$ versus $4.87 \pm 3.90$, $P < 0.001$) and a higher decremental function level (43.04% versus 25.83%, $P < 0.001$) compared with the patients in the SA group (Figure 2).

*3.2. Secondary Outcomes.* In addition, patients in the SA group had a lower in-hospital cost ($44264 \pm 9115.12$ versus $49636 \pm 9708.11$, $P < 0.003$) compared with ones in the CLSB group. There was no patient having stroke or heart failure in both groups. No significant difference was found in the incidence of PONV, postoperative delirium, blood transfusion, and the postoperative pain score between the two groups (shown in Table 2).

No significant difference was observed between two groups in terms of intraoperative crystal, colloid infusion, and intraoperative blood loss. Compared with the CLSB group, patients in the SA group had a shorter residence time in PACU ($51.27 \pm 14.51$ versus $39.82 \pm 10.38$, $P < 0.001$). Time of surgery, time of anesthesia, and the incidence of hypotension were comparable between two groups. The type of surgery was comparable between the two groups (Table 3).

## 4. Discussion

The results of this study showed a fewer loss of the daily activity in the SA group, compared with the patients in the CLSB group, although the patients in both groups did not return to the preoperative functional status 30 days after surgery. Furthermore, the rate of ADL decrement in the CLSB group was higher than that in the SA group. More

Table 2: Postoperative outcomes.

| | Group SA | Group CLSB | *P* value |
|---|---|---|---|
| POD30 ADL | 24.70 (6.40) | 27.34 (7.01) | 0.045 * |
| ADL increased value | 4.87 (3.90) | 8.09 (3.39) | <0.001 * |
| POD1 rest pain | 0 (0, 2) | 1 (0, 2) | 0.131 |
| POD1 motion pain | 2 (1, 4) | 3 (2, 4) | 0.208 |
| POD2 rest pain | 0 (0, 2) | 1 (0, 2) | 0.132 |
| POD2 motion pain | 2 (1, 3) | 3 (2, 3) | 0.058 |
| POD3 rest pain | 0 (0, 1) | 0 (0, 1) | 0.068 |
| POD3 motion pain | 2 (1, 2) | 2 (1, 2) | 0.143 |
| Postoperative delirium | 6/55 (5.5%) | 8/55 (7.3%) | 0.567 |
| POD1 PNOV | | | 0.152 |
| 1[a] | 47/55 (85.5%) | 41/55 (74.5%) | |
| 2[a] | 6/55 (10.9%) | 10/55 (18.2%) | |
| 3[a] | 2/55 (3.6%) | 4/55 (7.3%) | |
| POD2 PONV | | | 0.243 |
| 1[a] | 53/55 (96.4%) | 50/55 (90.0%) | |
| 2[a] | 2/55 (3.6%) | 5/55 (9.1%) | |
| 3[a] | 0/55 (0.0%) | 0/55 (0.0%) | |
| Blood transfusion | 6/55 (10.9%) | 9/55 (16.4%) | 0.405 |
| In-hospital cost (RMB) | 44264 (9115.12) | 49636 (9708.11) | 0.003* |

Statistics are presented as mean (standard deviation), median (interquartile range), or *N*/total number of patients (%) as appropriate. * $P < 0.05$, CLSB group compared with SA group. ADL, activity of daily living; CLSB, combined lumbar and sacral plexus block; POD, postoperative day; PONV, postoperative nausea and vomiting; SA, spinal anesthesia. Grade of PONV: 1[a], no nausea or vomiting; 2[a], mild nausea and vomiting without no vomitus; 3[a], severe nausea and vomiting with vomited matter.

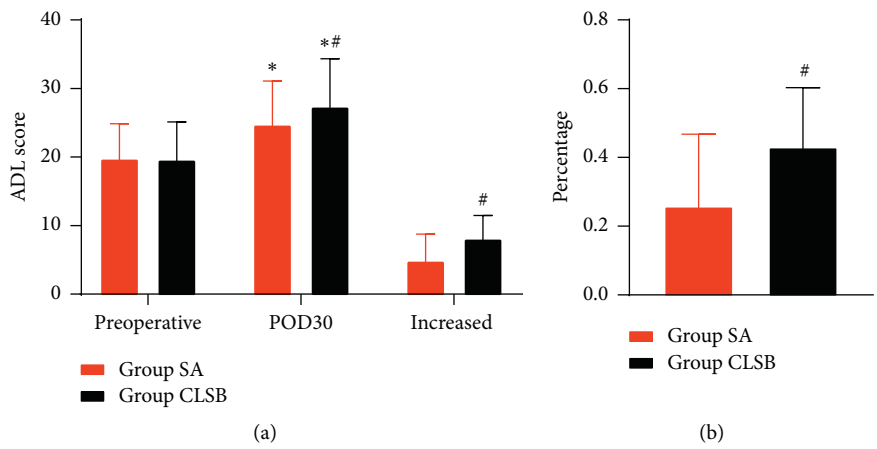


Figure 2: ADL score, percentage of increased ADL scores between the two groups. (a) ADL scores of two groups. (b) Percentage of two groups. * $P < 0.05$, compared with preoperative ADL scores; # $P < 0.05$, the CLSB group compared with the SA group; ADL, activity of daily living; CLSB, combined lumbar and sacral plexus block; POD, postoperative day; SA, spinal anesthesia. A higher ADL score means a worse quality of life.

items' scores were 3 (need help to do it) to 4 (cannot do it at all) in the CLSB group. Moreover, the patients in the SA group exhibited a shorter residence time in PACU and a lower in-hospital cost.

The functional status and the quality of life after hip fracture surgery are common concerns for orthopedics and anesthesiologists. It has been reported that nearly 33.3% of patients failed to return their prefracture ADL at six months after hip surgery [3]. Kristensen [4] reported that 13% older patients with hip fracture are no longer able to live alone. In line with previous studies, our data demonstrated that POD30 ADL did not return to the preoperative state through aggressive and proper surgical treatments.

In the current study, the items in PSMS are necessary for basic functional living and the ability to implement IADLs can significantly improve the quality of life. A higher CADL score (impaired ADL) may indicate the need for home healthcare or more medical resources [15]. Our study demonstrated that the CADL score in the CLSB group was also higher than that in the SA group at POD30. This indicates that the patients in the CLSB group perceived greater physical functional disability. Segev-Jacubovski et al.

Table 3: Intraoperative information.

| | Group SA | Group CLSB | *P* value |
|---|---|---|---|
| Time of surgery (min) | 70.42 (21.77) | 71.35 (19.85) | 0.816 |
| Time of anesthesia (min) | 78.62 (22.99) | 76.49 (20.45) | 0.609 |
| PACU standing time (min) | 39.82 (10.38) | 51.27 (14.51) | <0.001 * |
| Crystalloids (mL) | 600 (600, 1000) | 600 (600,1100) | 0.236 |
| Colloids (mL) | 500 (0,500) | 500 (0,500) | 0.792 |
| Blood loss (mL) | 100 (100,200) | 150 (100,200) | 0.652 |
| *Type of surgery* | | | 0.244 |
| Osteosynthesis | 25/55 (45.5%) | 19/55 (34.5%) | |
| Artificial femoral head replacement | 16/55 (29.1%) | 18/55 (32.7%) | |
| Total hip replacement | 14/55 (25.5%) | 18/55 (32.7%) | |
| Incidence of hypotension | 15/55 (27.3%) | 10/55 (18.2%) | 0.257 |

Statistics are presented as mean (standard deviation), median (interquartile range), or *N*/total number of patients (%) as appropriate. * $P < 0.05$, CLSB group compared with the SA group. CLSB, combined lumbar and sacral plexus block; PACU, postanesthesia care unit; SA, spinal anesthesia.

reported that the improved functional ability achieved by therapy intervention can promote health-related quality of life among elderly with hip fracture [16]. Hence, patients in the CLSB group with a higher rate of declined ADL ability need more therapy intervention to recovery.

No difference in the postoperative pain score was found between groups. This may be associated with the successful block of the both groups and the effective postoperative controlled analgesia. Small dose of sufentanil with propofol (1–1.5 mg/kg) in CLSB was just to reduce or avoid the effect of LMA insertion. This method is consistent with the previous study [10]. It is also consistent with previous findings [17, 18] that patients treated with spinal anesthesia had a shorter stay time in PACU and a lower in-hospital cost. Spinal anesthesia was not widely applied in patients undergoing hip surgery due to the potential hemodynamic compromise and urinary retention. However, the superiority of the unilateral spinal anesthesia was confirmed in outpatients' surgery with fewer complications [19]. Unilateral SA reduced the consumption of general anesthetics and avoided the artificial airway with an exact effect of nerve blocking.

Postoperative delirium is a common complication in elderly patients undergoing hip fracture surgery [20]. However, in the current study, the incidence of postoperative delirium was 5.5% in the SA group and 7.3% in the CLSB group. The lower prevalence of delirium may partially be attributed to strict exclusion of participants and decreased opioids after application of regional anesthesia [10].

The present study had some limitations. Firstly, the number of PCA boluses and the time of the first postoperative requirement of analgesics were not recorded. Secondly, it is limited to assess the chronic pain 30 days after surgery in our study. Thirdly, the follow-up was only to 30 days after surgery. Therefore, the results of long-term ADL need to be investigated in our future study.

In conclusion, the results indicated that the unilateral SA plus MAC can reduce the deterioration of ADL after hip fracture surgery and can provide a better postoperative recovery. In addition, less cost was found in the SA group. Hence, the unilateral SA plus MAC may be more suitable than CLSB for elderly patients undergoing hip fracture surgery.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Authors' Contributions

Lili Tang and Panpan Fang contributed equally to the study.

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*Research Article*

# Effect of Moxibustion on $\beta$-EP and Dyn Levels of Pain-Related Indicators in Patients with Rheumatoid Arthritis

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*Background*. Rheumatoid arthritis (RA) is a systemic immunodeficiency disease characterized by persistent synovial inflammation, pannus formation, and bone and cartilage destruction, resulting in joint malformations and function decline. *Objective*. The purpose of this study is to evaluate the effect of moxibustion on clinical symptoms and levels of pain-related indicators beta-endorphin ($\beta$-EP) and dynorphin (Dyn) in patients with RA and to explore the potential anti-inflammatory and analgesic mechanisms of moxibustion in RA treatment. *Methods*. A total of 64 patients with RA who met the inclusion criteria were randomly and equally classified into the control and treatment groups. The control group received conventional treatment (oral methotrexate, folate, or leflunomide prescribed for a long time). The treatment group was treated with moxibustion at ST36 (Zusanli), BL23 (Shenshu), and Ashi points with respect to the control group. Patients' clinical symptoms and routine inspection indexes (rheumatoid factor [RF], erythrocyte sedimentation rate [ESR], and C-reactive protein [CRP]) were recorded before and after treatment. Serum levels of tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1$\beta$ (IL-1$\beta$), $\beta$-EP, and Dyn were determined by enzyme-linked immunosorbent assay (ELISA). The software SPSS24.0 was used for statistical analysis. *Results*. (1) Compared with the pretreatment result, both of the two groups' clinical symptoms and routine inspection indexes (RF, ESR, and CRP) improved ($P < 0.05$), and the improvement of clinical symptoms in the treatment group outperformed that in the control group ($P < 0.05$). (2) TNF-$\alpha$ and IL-1$\beta$ levels decreased significantly in the treatment group after treatment ($P < 0.01$), while no significant difference was observed in the control group ($P > 0.05$). (3) $\beta$-EP and Dyn levels in the treatment group were significantly increased after treatment ($P < 0.01$, $P < 0.01$), but the control group showed no significant difference ($P > 0.05$, $P > 0.05$). It is worth mentioning that the serum TNF-$\alpha$, IL-1$\beta$, $\beta$-EP, and Dyn levels between the two groups were significantly different after 8 weeks of treatment ($P < 0.05$). (4) Differences in the serum $\beta$-EP and Dyn levels in the patients of the treatment group were correlated with TNF-$\alpha$ and IL-1$\beta$ levels after treatment, and the correlation was mainly negative ($r < 0$). *Conclusion*. Moxibustion can improve joint pain in patients with RA using conventional western medicine. One of the mechanisms may affect the serum $\beta$-EP and Dyn levels by downregulating the inflammatory factors to play an anti-inflammatory and analgesic role.

## 1. Introduction

Rheumatoid arthritis (RA) is a systemic immunodeficiency disease mainly characterized by aggressive arthritis. Cytokines such as interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6), and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) are active in the joints of RA patients. These cytokines play a crucial role in the pathogenesis of RA, causing inflammation, pain, and joint destruction [1]. About 0.5% to 1% of the global population is affected by RA [2], and patients usually show symptoms such as swelling, stiffness, and deformity of multiple joints. Wrist joints, proximal interphalangeal joints, and metacarpophalangeal joints are most commonly involved [3]. Pain caused by an unrelenting inflammatory response can seriously affect people's quality of life.

The opioid family is widely involved in the regulation of nociceptive sensation, which is mainly divided into endogenous opioid peptides and exogenous opioid peptides.

Studies have shown that endogenous opioid peptides are extensively involved in stress regulation and play an important regulatory role between the central nervous system and the immune system [4]. Data from animal and human clinical studies showed the key role of opioid receptors in the regulation of pain and inflammation [5]. Inflammation intensifies the expression, transport, and accumulation of peripheral opioid receptors in sensory nerve endings and triggers the migration of opioid peptides that contain immune cells [6]. Immune cells migrate to inflammatory tissues and release endogenous opioid peptides, which bind to peripheral opioid receptors that produce analgesic effects [7, 8]. Beta-endorphin ($\beta$-EP) and dynorphin (Dyn) are important endogenous opioids that are involved in the body's response to pain. In the early stage of inflammation (within 6 hours), inflammatory cytokines such as noradrenaline, TNF-$\alpha$, and IL-1$\beta$ stimulate the production of $\beta$-EP, enkephalins (ENK), and Dyn in leukocytes to activate the surrounding $\mu$, $\delta$, and $\kappa$ opioid receptors to inhibit nociception [9]. Therefore, the release of endogenous opioid peptides can activate the peripheral opioid receptors to participate in the inhibitory mechanism of pain, which will block further transmission of pain signals, delay the progression of RA disease, and improve the quality of life of RA patients.

At present, western medicine treatments are mostly used for RA. Disease-modifying antirheumatic drugs (DMARDs) are the primary drugs. However, their toxicity and side effects are relatively large, and long-term use of these drugs is likely to harm the functioning of the liver, kidneys, and gastrointestinal system. Studies have shown that moxibustion has a significant impact on the pain of RA patients [10] and can exert immune regulation, anti-inflammatory, and analgesic effects by regulating the expression of inflammatory cytokines, proteins, and related signaling pathways. Moreover, it is easily accepted by patients for the lack of side effects [11–13]. In this study, $\beta$-EP and Dyn were selected to explore the mechanism of moxibustion on RA and provide a more reliable clinical basis for RA treatment.

## 2. Methods

*2.1. Design and Setting.* A total of 64 RA patients, who fulfilled the inclusion criteria, were included in the outpatient and inpatient collection of the Rheumatology Department of Sichuan Hospital of Traditional Chinese Medicine from March 2018 to December 2019. This research conforms to the Hippocratic Declaration and has been approved by the Sichuan Regional Ethics Review Committee of Traditional Chinese Medicine (No. 2015KL-05). Informed consent was obtained from each of the study participants.

All patients were randomly divided into the control group (32 cases) and the treatment group (32 cases). The patients of the control group were given oral methotrexate, folate, or leflunomide at the doctors' recommendation for a long time. The treatment group received moxibustion at ST36 (Zusanli), BL23 (Shenshu), and Ashi points in addition to conventional medicine, twice a week, 4 weeks for a course of treatment, and two consecutive courses of treatment.

*2.2. Study Subjects.* Participants were recruited by advertisement through hospital official accounts and bulletin boards. Baseline assessment was conducted for all participants who met eligibility criteria, and relevant demographic and general medical data were collected.

*2.2.1. Inclusion Criteria.* Participants in the study were required to meet all of the following conditions:

(1) RA was diagnosed in accordance with the 2010 RA diagnostic criteria developed by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) [14]
(2) Age between 25 and 65 years old, visual analogue scale (VAS) $\geq 3$, disease activity score in 28 joints (DAS28) $> 3.2$
(3) Conscious and able to cooperate with the study
(4) Did not participate in other clinical trials
(5) Sign the informed consent to enter the clinical study

*2.2.2. Exclusion Criteria.* Participants were excluded from the study if they had any of the following conditions:

(1) Unconscious and unable to complete the research
(2) With severe joint malformation, stage IV function
(3) With other immune system diseases, such as Sjogren's syndrome
(4) With malignant tumors and serious diseases such as hematopoietic system
(5) Pregnant or lactating women and people with cyclothymic
(6) Allergic constitution or drug allergy
(7) Afraid of moxibustion therapy
(8) Do not take medicine and moxibustion as prescribed

*2.3. Randomization and Blinding.* A random number table generated by the statistical software SPSS24.0 was used to randomly assign eligible participants to the treatment group and the control group in 1 : 1 ratio. Random information was sealed in opaque envelopes, and random operations were supervised by an independent investigator. Because of the particularity of moxibustion therapy, it was easy to know whether moxibustion treatment has been carried out; so it is hardly possible to blind the patients and clinicians. Outcome assessors, data collectors, and statisticians were blinded to the treatment allocation to eliminate potential bias.

*2.4. Interventions.* All patients received oral administration of methotrexate (7.5 mg/dose, 1 dose/week), folate (10 mg/dose, 1 dose/week), or leflunomide (10 mg/dose, 1 dose/day) for a long time. The treatment group was additionally treated by moxibustion at ST36 (Zusanli), BL23 (Shenshu), and Ashi points (Figure 1), which were selected according to the National Standard of the People's Republic of China (GB/T12346-2006). ST36 (Zusanli) and small joints of limbs were

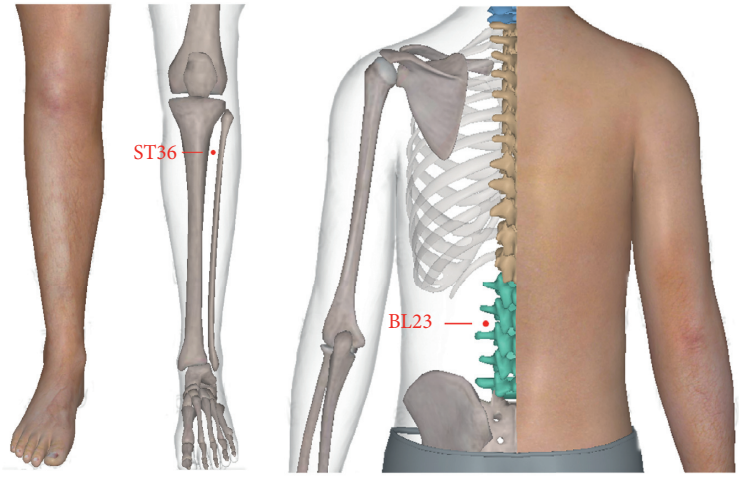



Figure 1: Acupoints. ST36 (Zusanli) is located 3 inches below the outer knee, 1 inch apart from the tibia front margin, BL23 (Shenshu) is located below the second lumbar spine process, 1.5 inches away from the posterior midline, and "Ashi" points are located at where swelling and paining occur.

applied with moxibustion with seed-sized moxa cone, BL23 (Shenshu) on salt, and large joints on ginger, twice a week, 4 weeks for a course of treatment, and two consecutive courses of treatment. Moxibustion was performed by licensed-TCM doctors with over 3 years of experience in clinical practice.

Doctors marked the acupoints with a marker and then made a moxa cone as big as half olive (about 1 cm in diameter and height), put it on a gauze strip filled with a proper amount of salt, and placed it on both sides of BL23 (Shenshu) points (Figure 2(a)). Direct moxibustion was used for the acupoints of ST36 (Zusanli) and the Ashi points of small joints of the limbs. Doctors applied Vaseline on the corresponding acupoints, put the moxa cone (about 0.3 cm in diameter and height) made with mugwort floss on acupoints, and then ignited the top of it (Figures 2(b) and 2(c)). If patients felt burning pain during the treatment, the cone would be lifted quickly and replaced. The Ashi points of the large joints of limbs were applied with moxibustion with ginger (Figure 2(d)), and the moxa cone was burned on the cut ginger and placed on Ashi points. When the patients felt hot, another ginger would be padded under it until the moxa cone was burned out.

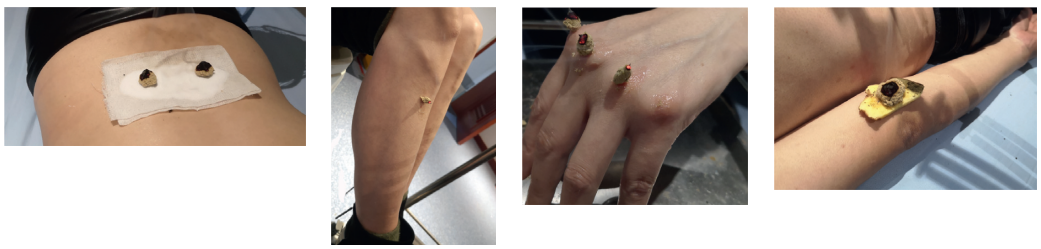
*2.5. Outcome Measures.* Visual analogue score (VAS: measure pain intensity), morning stiffness score, and disease activity score in 28 joints (DAS28: determine disease activity) were used to evaluate the clinical symptoms of the patients before and after treatment. The changes of routine inspection indexes: RF, ESR, and CRP, were compared. The contents of $\beta$-EP, Dyn, TNF-$\alpha$, and IL-1$\beta$ in serum samples of the two groups were determined by enzyme-linked immunosorbent assay (ELISA). The safety of moxibustion was assessed by the occurrence of adverse events, such as burning, scalding, and blisters.

*2.6. Specimen Collection.* About 3–5 ml of elbow venous blood was extracted from the patients before and after treatment, and the serum was isolated and stored at −80°C. After the course of treatment was completed, the serum samples were sent to Chengdu Lilai Biomedical Experimental Center for testing. The ELISA was used to determine the $\beta$-EP, Dyn, TNF-$\alpha$, and IL-1$\beta$ levels in these samples. The methods strictly complied with the kit instructions.

*2.7. Statistical Analysis.* The software SPSS24.0 (SPSS, Inc., Chicago, Illinois, USA) was used for statistical analysis. The chi-square ($\chi^2$) test was used for counting data. The $t$-test was used for measurement of data satisfying normal distribution: paired sample $t$-test was used for in-group analysis, and independent sample $t$-test was used for intergroup analysis. Nonparametric test was used for measurement of data that were not normally distributed; Wilcoxon signed-rank sum test was used for in-group analysis, and Mann–Whitney $U$ test was used for intergroup analysis. All data were expressed in the form of mean ± standard deviation (X ± s). The value of $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered significantly different.

## 3. Results

*3.1. Demographics and Baseline Characteristics.* Sixty-four patients were screened and randomly divided into the control group (32 cases) and the treatment group (32 cases). Two participants in the treatment group and one participant in the control group dropped out during the course of the study because of withdrawal of consent (Figure 3). Therefore, a total of 61 patients completed the study. The treatment group consisted of 30 patients. Out of them, 4 were males and 26 were females (with an average age of 53 ± 8.80 years and mean course of disease of 10.11 ± 9.02 years). There were 31 patients in the control group, including 5 males and 26 females (with an average age of 49.39 ± 7.72 years and a mean course of disease of 9.13 ± 9.07 years).



(a) (b) (c) (d)

Figure 2: The operation diagram of moxibustion. (a) Participant was treated at BL23 (Shenshu) acupoint. (b) Participant was treated at ST36 (Zusanli) acupoint. (c) and (d) participants were treated at Ashi acupoints.

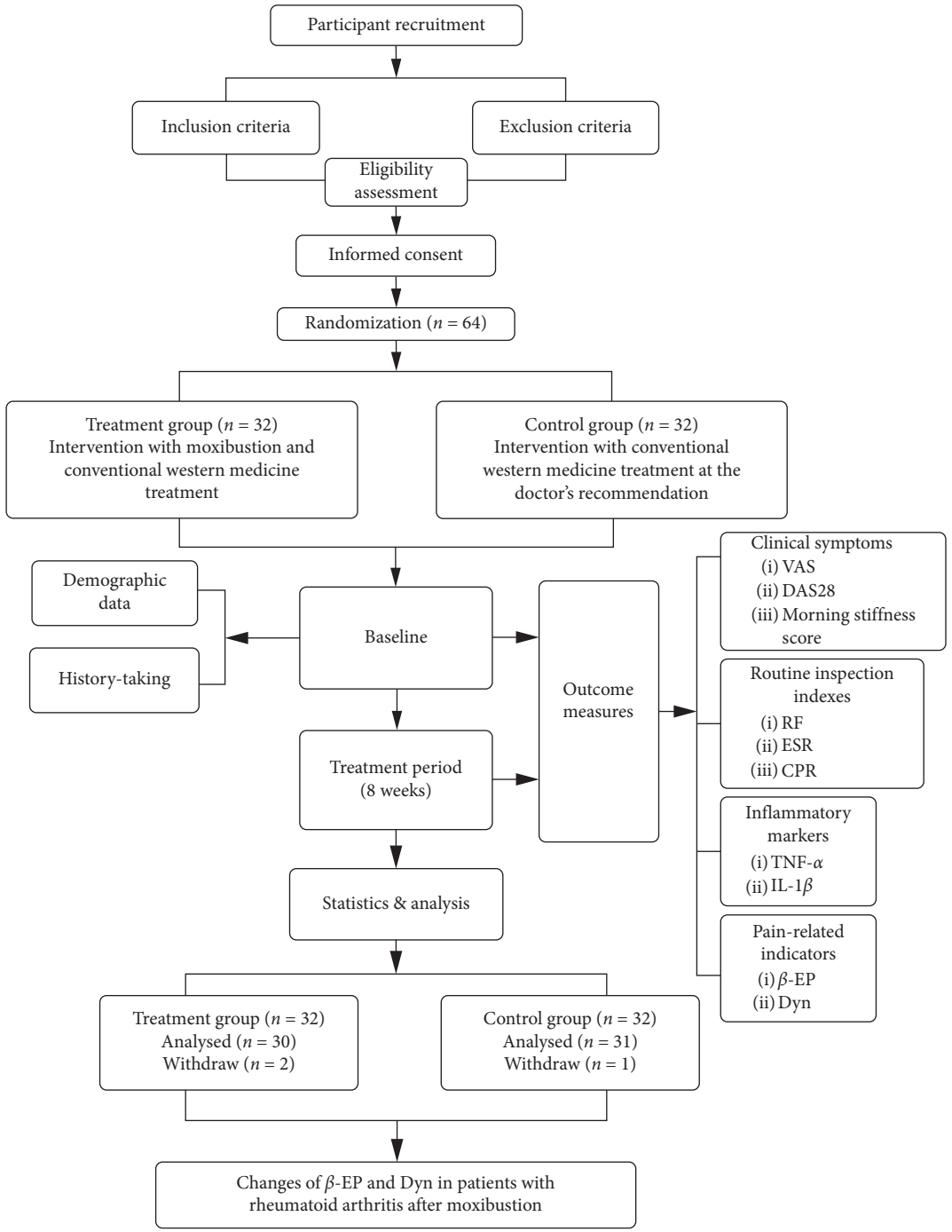



Figure 3: Technology roadmap.

There was no significant difference in general data between the two groups ($P > 0.05$).

The baseline characteristics of demographic data, clinical symptoms, and routine inspection indexes of both groups are shown in Table 1.

*3.2. The Clinical Symptoms and Routine Inspection Indexes.* VAS, morning stiffness score, and DAS28 of the two groups were decreased after treatment as compared to that before treatment, and the difference was statistically significant (for VAS, $P < 0.01$ in both groups; for morning stiffness score and DAS28, $P < 0.01$ in the treatment group and $P < 0.05$ in the control group), indicating that clinical symptoms were improved after treatment. The improvement in the treatment group was more significant than that in the control group after treatment, and the difference was statistically significant ($P < 0.05$) (Table 2).

RF, CRP, and ESR were significantly improved after treatment both in the treatment group ($P < 0.01$) and in the control group (RF and CRF, $P < 0.05$; ESR, $P < 0.01$). However, there was no statistically significant difference in RF, CRP, and ESR content between the two groups after treatment ($P > 0.05$), indicating that the routine inspection indexes of the two groups were not significantly improved after treatment (Table 2).

*3.3. The Contents of TNF-α and IL-1β.* The serum levels of TNF-$\alpha$ and IL-1$\beta$ decreased significantly in the treatment group after treatment ($P < 0.01$), while there was no significant difference in the serum levels of TNF-$\alpha$ and IL-1$\beta$ in the control group ($P > 0.05$). The levels of TNF-$\alpha$ and IL-1$\beta$ in the treatment group were significantly lower than those in the control group, with statistically significant differences between the two groups ($P < 0.05$) (Table 3).

*3.4. The Contents of β-EP and Dyn.* The contents of $\beta$-EP and Dyn in the treatment group were significantly different after treatment ($P < 0.01$, $P < 0.01$), while there were no significant differences in the control group ($P > 0.05$, $P > 0.05$). A significant difference was observed in $\beta$-EP and Dyn levels of both groups after 8 weeks of treatment ($P < 0.05$) (Table 3).

*3.5. Correlation Analysis of β-EP and Dyn with TNF-α and IL-1β in the Treatment Group.* The differences in serum levels of $\beta$-EP and Dyn in the treatment group patients were correlated with the differences in TNF-$\alpha$ and IL-1$\beta$ after treatment, and the correlation was found to be negative ($r < 0$) (Table 4).

*3.6. Adverse Events.* Only one participant experienced an adverse reaction to skin blisters during the study. The skin condition of the patients improved after the timely treatment of the researchers, and the study was continued.

## 4. Discussion

The aim of the research was to assess the clinical effects of moxibustion on $\beta$-EP and Dyn levels in the serum of RA patients and reveal the potential mechanism of moxibustion.

*4.1. The Effect of Moxibustion on Routine Inspection Indexes Related to Clinical Symptoms and Disease Activity in RA Patients.* Subjective scales and routine inspection indexes were used in this study to evaluate the activity of the disease so that the therapeutic effect of this study on RA could be more objectively understood. The results of this study showed that pain and other related symptoms of the two groups of patients were improved after treatment, but the improvement was more obvious in moxibustion combined with conventional drug treatment. However, there was no significant difference between the two groups in terms of routine inspection indexes.

Moxibustion has an obvious effect on pain diseases, such as chronic visceral pain hypersensitivity, rheumatoid arthritis, and other conditions. The analgesic effect of moxibustion may be closely related to its thermal effect, infrared radiation effect, regulation of pain-causing factors, inhibition of synaptic mechanism, and central signal integration [15]. ST36 and BL23 are commonly used acupoints in the treatment of RA. Our studies in the early stage also reported that the application of moxibustion on ST36 and BL23 could relieve pain and significantly improve the clinical symptoms of RA patients [11, 12, 16]. A large number of animal experiments have shown that moxibustion has an exact effect on the treatment of ST36 and BL23 points in RA models. It can effectively reduce synovial tissue and fibrous tissue hyperplasia and improve joint swelling and synovial inflammation in the RA models by affecting related proteins, transcription factors, and signaling pathways [13, 17, 18].

This research suggested that moxibustion therapy based on western medicine can give rise to prominent therapeutic effects, improve the clinical symptoms of patients to a greater extent, and reduce the activity of the disease. It may be related to the fact that RA is part of the bi-syndrome of TCM. Moxibustion can warm channels to remove coldness, activate meridians to stop the pain, and have analgesic and anti-inflammatory effects on the pain and swelling of joints in RA patients. The result that no significant difference was found in routine inspection indexes between the two groups can be speculated to be related to the complexity of the disease. As RA is a chronic disease that reoccurs and delays healing, the main indicators of acute inflammatory response did not significantly improve in a short time. Therefore, more time is needed for observing the changes in serum indicators mentioned earlier.

*4.2. The Effect of Moxibustion on TNF-α, IL-1β, β-EP, and Dyn in Serum of RA Patients.* Our results showed that the levels of TNF-$\alpha$ and IL-1$\beta$ in the treatment group decreased significantly after treatment, while there was no significant difference in the control group. The levels of $\beta$-EP and Dyn were significantly increased after treatment in the treatment group and superior to the control group. The correlation analysis results of the treatment group showed that the differences of $\beta$-EP and Dyn in the serum of patients were correlated with the differences of TNF-$\alpha$ and IL-1$\beta$ after treatment, and the correlation was mainly negative.

Table 1: Baseline characteristics.

| Outcome measures | Treatment group ($n = 30$) | Control group ($n = 31$) | $P$ value |
|---|---|---|---|
| Characteristic | | | |
| Gender, male/female | 4/26 | 5/26 | 0.758* |
| Age (y), mean (SD) | 53.00 (8.80) | 49.39 (7.72) | 0.093# |
| Disease duration (y), mean (SD) | 10.11 (9.02) | 9.13 (9.07) | 0.677# |
| Clinical symptoms | | | |
| VAS score, mean (SD) | 6.47 (1.53) | 6.10 (1.76) | 0.38# |
| Morning stiffness score, mean (SD) | 2.47 (2.01) | 2.58 (1.80) | 0.82# |
| DAS28 score, mean (SD) | 5.33 (0.99) | 5.50 (1.02) | 0.49# |
| Routine inspection indexes | | | |
| RF (IU/ml), mean (SD) | 209.60 (249.12) | 190.95 (210.32) | 0.75▲ |
| ESR (mm/60 min), mean (SD) | 65.40 (34.90) | 64.61 (33.77) | 0.93▲ |
| CRP (mg/L), mean (SD) | 20.21 (22.99) | 18.01 (21.20) | 0.70▲ |

*$P$ value by $\chi^2$ test. #$P$ value by independent samples $t$-test. ▲$P$ value by Mann–Whitney $U$ test.

Table 2: Clinical symptoms and routine inspection indexes after treatment.

| Outcome measures | Treatment group ($n = 30$) | Control group ($n = 31$) | $P$ value |
|---|---|---|---|
| Clinical symptoms | | | |
| VAS score, mean (SD) | 3.57 (1.49) | 4.13 (1.77) | 0.041# |
| Morning stiffness score, mean (SD) | 1.27 (1.23) | 2.06 (1.82) | 0.045# |
| DAS28 score, mean (SD) | 4.17 (0.94) | 4.86 (1.40) | 0.046# |
| Routine inspection indexes | | | |
| RF (IU/ml), mean (SD) | 131.25 (156.01) | 153.72 (178.35) | 0.277▲ |
| ESR (mm/60 min), mean (SD) | 37.17 (27.42) | 49.23 (28.55) | 0.072▲ |
| CRP (mg/L), mean (SD) | 8.65 (9.48) | 9.70 (14.00) | 0.567▲ |

#$P$ value by independent samples $t$-test. ▲$P$ value by Mann–Whitney $U$ test.

Table 3: Changes of contents of TNF-$\alpha$, IL-1$\beta$, $\beta$-EP, and Dyn.

| Outcome measures | Treatment group ($n = 30$) | Control group ($n = 31$) | $P$ value |
|---|---|---|---|
| TNF-$\alpha$ (pg/mL), mean (SD) | | | |
| Baseline | 25.41 (12.01) | 27.29 (14.45) | 0.58 |
| Posttherapy | 20.04 (10.14) | 25.45 (15.60) | 0.045 |
| IL-1$\beta$ (pg/mL), mean (SD) | | | |
| Baseline | 30.54 (13.97) | 29.55 (14.66) | 0.79 |
| Posttherapy | 24.39 (11.76) | 28.26 (15.02) | 0.031 |
| $\beta$-EP (pg/mg), mean (SD) | | | |
| Baseline | 11.42 (6.07) | 12.84 (7.09) | 0.41 |
| Posttherapy | 15.36 (10.56) | 13.60 (8.68) | 0.043 |
| Dyn (pg/mg), mean (SD) | | | |
| Baseline | 63.56 (35.85) | 61.44 (25.20) | 0.79 |
| Posttherapy | 78.30 (43.30) | 65.54 (26.81) | 0.042 |

$P$ value by independent samples $t$-test.

Table 4: Correlation of $\beta$-EP and Dyn values with TNF-$\alpha$ and IL-1$\beta$ values after treatment.

| Outcome measures | Outcome measures | $r$ | $P$ |
|---|---|---|---|
| $\beta$-EP | TNF-$\alpha$ | **−0.119** | **0.531** |
| | IL-1$\beta$ | **−0.361** | **0.050** |
| Dyn | TNF-$\alpha$ | **0.021** | **0.941** |
| | IL-1$\beta$ | **−0.385** | **0.036** |

Many cytokines active in the synovial membrane of RA patients are directly or indirectly involved in inflammatory pain. These cytokines play a crucial role in the pathogenesis of RA and can cause local inflammation, joint pain, and destruction [1]. TNF-$\alpha$ and IL-1$\beta$, as key inflammatory cytokines in the pathogenesis of RA, are highly expressed in the synovial fluid and serum of RA patients [19]. They can not only promote the formation of pannus and lead to the destruction of cartilage and bone but also produce other inflammatory factors such as IL-6 to aggravate joint

inflammation that leads to the further increase of the pain. Hence, these two indicators can indirectly reflect the pain relief of RA. During the initial stage of pain signaling, TNF-$\alpha$ and IL-1$\beta$ are released in large quantities. Opioid peptides can reduce the production of peripheral pain-causing substances such as cytokines (e.g., interleukins) and TNFs while providing peripheral analgesia [20, 21]. Therefore, the increase of peripheral opioid peptides may be closely related to TNF-$\alpha$ and IL-1$\beta$.

$\beta$-EP exerts analgesic effects on both the central and peripheral systems, with a significant effect on inflammatory pain. It can relieve pain by reducing the excitability of pain receptors, the transmission of action potential, and the release of proinflammatory neuropeptides from central and peripheral pain receptors of RA patients. $\beta$-EP is also involved in regulating inflammatory factors such as TNF-$\alpha$ and IL-1$\beta$ by binding to immune cells' receptors [22]. Dyn plays an important role in pain regulation as a member of the endogenous opioid family and has a very complex mechanism. Dyn exerts an analgesic effect in both the central nervous system and the peripheral system. It can not only inhibit the release of cAMP, substance P, norepinephrine, and other neurotransmitters through the G-protein coupling mechanism to prevent the conduction of nerve impulses but also inhibit the activation of $Na^+$ and $K^+$ channels to prolong the action potential and achieve the analgesic effect.

Our study showed that moxibustion could enhance the role of conventional western medicine in improving the mobility of joints and reducing joint pain and swelling. Simultaneously, moxibustion can relieve pain by regulating the expression levels of TNF-$\alpha$, IL-1$\beta$, $\beta$-EP, and Dyn in the serum of RA patients. This may be related to the effect of moxibustion on warming and dispersing cold, activating blood circulation and relieving pain. It can also be seen from the correlation analysis that $\beta$-EP and Dyn were correlated with TNF-$\alpha$ and IL-1$\beta$ in the mechanism of action. With the increase of $\beta$-EP and Dyn, the contents of TNF-$\alpha$ and IL-1$\beta$ decreased. Related studies confirmed that moxibustion could not only improve the RA expression levels of opioid peptides in the hypothalamus of rats and enhance the body's analgesia [23] but also significantly increase the pain threshold of rats with chronic visceral hyperalgesia by increasing the concentration of the Dyn in the spinal cord and inducing inhibition debris (including the postsynaptic inhibition and presynaptic inhibition), thereby blocking the further transmission of pain signals [24]. Meanwhile, moxibustion can stimulate immune cells to secrete more $\beta$-EP, increasing the contents of $\beta$-EP in the pituitary gland and peripheral lymph nodes of adjuvant arthritis rats [25]. These findings are consistent with the results of this study, which suggest that moxibustion therapy for RA can decrease TNF-$\alpha$ and IL-1$\beta$ by increasing the levels of $\beta$-EP and Dyn in the body, thus playing an anti-inflammatory and analgesic role.

## 5. Conclusions

Moxibustion can improve the clinical symptoms of RA patients with conventional western medicine, which may be related to the effect of moxibustion on the levels of TNF-$\alpha$, IL-1$\beta$, $\beta$-EP, and Dyn in the serum of RA patients. One of the effective mechanisms may be that moxibustion can affect the levels of $\beta$-EP and Dyn by downregulating the levels of inflammatory factors in the serum of RA patients, thus can control the degree of joint pain and swelling in these patients.

This study evaluated the effect of moxibustion on $\beta$-EP and Dyn as a pointcut to explore the anti-inflammatory and analgesic mechanism of moxibustion on RA patients. The correlation analysis of $\beta$-EP and Dyn with TNF-$\alpha$ and IL-1$\beta$ showed a weak negative or a very weak positive correlation, which may be related to the small sample size and the slight difference in the degree of improvement in patients. Therefore, the sample size should be expanded in future relevant studies. In addition, this study indicated that moxibustion had anti-inflammatory and analgesic effects on RA. However, the mechanism of moxibustion's anti-inflammatory and analgesic effects on RA could be further studied because of its complexity.

## Data Availability

Data and materials from this trial are available upon reasonable request and approval by the corresponding author.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

YNW and SYT contributed equally to this work. YLi and JT conducted the recruitment and randomization. YNW, SYT, and ZYY were in charge of the treatment of patients. RXS and XYH collected the data. YLuo and GHC analyzed the data. PW designed the study and helped to draft the manuscript. YNW and SYT completed this paper. All authors read and approved the final manuscript. Yingni Wang and Siyu Tao contributed equally to the study.

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*Research Article*

# Acupuncture at the P6 Acupoint to Prevent Postoperative Pain after Craniotomy: A Randomized, Placebo-Controlled Study

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*Objective*. Acute pain management after craniotomy can be challenging. Previous studies have shown inadequate pain control following the procedure. Oral medication can sometimes be delayed by postoperative nausea, and use of anesthetics may impair the assessment of brain function. We conducted this prospective study to evaluate the effect of acupuncture at the P6 acupoint on postoperative pain, nausea, and vomiting in patients undergoing craniotomy. *Methods*. The authors conducted a randomized, placebo-controlled trial among 120 patients scheduled for craniotomy under general anesthesia. 120 patients were randomly assigned into an acupuncture group or a sham acupuncture group. All patients received standardized anesthesia and analgesia treatment. Acupuncture was executed in the recovery room after surgery. For the acupuncture group, the P6 points on each wrist were punctured perpendicularly to a depth of 20 mm. Needles were retained for 30 min and stimulated every 10 min to maintain the De-Qi sensation. For the sham acupuncture group, sham points on each wrist were punctured perpendicularly to a depth of 5 mm. Needles were retained for 30 min with no stimulation during the duration. The postoperative pain scores, PONV, and dose of tramadol were assessed 24 h, 48 h, and 72 h after surgery. *Results*. A total of 117 patients completed the study. There was no statistically significant difference in baseline data between the two groups ($P > 0.05$). The VAS pain score of the acupuncture group was lower than that of the sham acupuncture group, and this difference was statistically significant ($P = 0.002$). There was no difference in pain scores between the two groups during 0–24 h and 48–72 h ($P > 0.05$). The incidence of vomiting in the acupuncture group was lower than that in the sham acupuncture group during the 0–24 h period (13.8% vs. 28.8%, $P = 0.048$). There was no difference in vomiting, however, during the 24–72 h period ($P > 0.05$). No significant differences were found in the degree of nausea and the dose of tramadol between the two groups at either time point in the acupuncture group and sham acupuncture group. *Conclusion*. The use of acupuncture at the P6 acupoint in neurosurgery patients did result in significantly lower pain scores and reduction in the incidence of vomiting after craniotomy. There were no significant side effects. Acupuncture at the P6 acupoint was well tolerated and safe in this patient population.

## 1. Introduction

A review paper has analyzed publications in the international literature regarding the problem of acute postoperative pain in neurosurgical patients who have undergone craniotomy. This review indicated that the problem of acute postoperative pain in patients after craniotomy has been previously underestimated. It had been mistakenly thought that these patients do not experience any pain in the early postoperative period. Results of recent studies have shown that up to 80% of these patients may in fact experience acute mild to severe pain [1]. Postoperative pain after craniotomy is unfavorable for the recovery of patients. Pain can cause postoperative complications including anxiety, nausea, vomiting, arterial hypertension, intracranial hypertension, and postoperative hemorrhage [2, 3].

Pain management after craniotomy is a clinical problem. The main challenge is that analgesic therapy may interfere with nervous system function and postoperative evaluation. Numerous studies have shown that patients in the

neurocritical care (NCC) unit experience inadequate pain control [4, 5]. Optimizing patient comfort after craniotomy is often difficult because the use of narcotic medications can impair the clinical evaluation of neurological function. The sedation and pupillary miosis caused by opioids can directly mask seminal signs of intracranial pathology, and thus, opioids are used judiciously. Additionally, craniotomy is associated with postoperative nausea, which delays the use of oral medications.

Multimodal analgesia is advocated currently for postoperative pain. There are two aspects of multimodal analgesia. (1) Balanced anesthesia affects the local anesthetics of the scalp, opioid, and nonopioid analgesics. (2) Nonpharmacologic treatments include distractibility, massage therapy, transcutaneous electrical nerve stimulation therapy, and acupuncture.

Acupuncture is a traditional Chinese medical technique that has been extensively used as a nonpharmacological analgesic therapy since being developed 2500 years ago. Over the last decade, many clinical studies have focused on acupuncture in the treatment of postoperative pain. For example, a systematic review suggested that acupuncture can relieve acute postoperative pain after back surgery [6]. Another study suggested that acupuncture may help reduce pain during panretinal photocoagulation treatment [7]. Several clinical studies have shown that acupuncture may be effective in improving postoperative analgesia, reducing intraoperative anesthetic requirements and immunosuppression, and reducing the incidence of anesthesia-related side effects [8, 9].

Stimulation of specific points, by using needles or electrodes, releases neurochemical substrates that may block the incoming pain information. Acupuncture can raise the pain threshold. It also reduces pain intensity [10]. The P6 acupoint is one of the most commonly used and well-investigated acupoints for PONV prophylaxis and postoperative pain treatment. Based on the theory of meridian and evidence from previous studies [6, 9], we chose the P6 acupoint for treatment.

The patient's awareness assessment will not be affected because acupuncture is a nonsedating treatment. The aim of this controlled, randomized, single-blind study was to evaluate the effect of acupuncture at the P6 acupoint on postoperative pain following craniotomy. We hypothesized that acupuncture at the P6 acupoint may reduce the postoperative pain experienced by patients.

## 2. Materials and Methods

*2.1. Study Design and Oversight.* Our study protocol was published in Trails in 2013. We followed the methods of Lv et al. [11]. This randomized, blind, controlled trial was approved by the ethics review board of West China Hospital, Sichuan University, and registered with the Chinese Clinical Trial Registry (registration number: ChiCTR-TRC-13003026). All authors assume responsibility for the accuracy and completeness of the data and analyses.

*2.2. Study Population.* All participants gave their written informed consent before being enrolled in this study. In this prospective randomized controlled trial, all cases were from the Department of Neurosurgery, West China Hospital, Sichuan University. Patients were enrolled in the study from October 2014 to September 2017. A total of 120 patients were recruited. SAS 11.0 statistical software was used to design the random number. Patients were randomly assigned into either an acupuncture group or a sham acupuncture group. All the subjects involved were unaware to which group they had been assigned, as was the doctor who observed the postoperative pain and other outcomes.

*2.3. Inclusion Criteria.* Patients who fulfilled the following conditions were included: (1) scheduled for neurosurgery requiring opening of the cranium and dura; (2) aged between 18 and 70 years old; (3) the American Society of Anesthesiologists (ASA) physical status classification of I or II; (4) undergoing general anesthesia; (5) no history of PONV or motion sickness; (6) no use of antiemetics and analgesia 24 hours before surgery; (7) willing to participate; (8) no experience with acupuncture; and (9) having signed an informed consent form.

*2.4. Exclusion Criteria.* Participants that met any of the following criteria were excluded: (1) pain 24 hours before surgery; (2) pregnant or lactating women; (3) drug or alcohol abusers; (4) recipients of chemotherapy or radiation therapy during the previous 7 days; (5) having a cardiac pacemaker fitted; (6) menstruating phase of the menstrual cycle; (7) refusal to accept acupuncture treatment; (8) mental disorder; (9) history of epilepsy and still taking an antiepileptic medicine; (10) unconscious before the surgery; (11) cannot normally communicate; (12) undergoing ventricle or brainstem surgery; (13) cerebral perfusion pressure (CPP) of less than 50 mmHg or greater than 150 mmHg; (14) poorly controlled diabetes mellitus (fasting plasma glucose greater than 12 mmol/L); (15) bleeding disorders (hemophilia or afibrinogenemia); and (16) serious systemic disease (AIDS or sepsis).

*2.5. Dropout Criteria.* Participants who met any of the following criteria were withdrawn from the study: (1) death; (2) waking more than 2 hours after surgery; (3) trachea intubation; (4) persistent coma; (5) cognitive impairment; and (6) further surgery or transfer to the ICU if necessary for the aggravation of the disease. Patients who were withdrawn were not replaced.

*2.6. Anesthesia and Postoperative Analgesia.* All patients underwent general anesthesia with endotracheal intubation. Blood pressure, heart rate, pulse oximetry, and end tidal $CO_2$ were routinely monitored. Induction of anesthesia was achieved with midazolam 0.05 mg/kg, sufentanil 0.3 $\mu$g/kg, atracurium 0.15 mg/kg, and propofol 2 mg/kg. When endotracheal intubation and gastrointestinal decompression with either an orogastric or nasogastric tube were undertaken, the anesthesia was maintained with 50% nitrous oxide

and 3% sevoflurane. The concentration of sevoflurane was adjusted according to BIS and the vital signs; if hypotension occurred and the BIS was low, the sevoflurane dosage was decreased. After the operation had commenced, participants were given sufentanil 0.2 $\mu$g/kg and atracurium 0.1 mg/kg intermittently. 30 minutes prior to the end of the operation, the patients were treated with prophylactic antiemetic drugs: ondansetron injection 8 mg according to the advice of doctors. After surgery, patients were continually monitored in the postanesthesia care unit (PACU) with continued ventilator support. The tracheal tube was removed after the patients woke. The time from the start of anesthesia induction to the time of removal of the tube was recorded. Patients who then met the criteria (Steward Rating Scale $\geq$4 and the blood gas index of special patients being normal as judged by the anesthetist) were sent back to the ward.

After the participants returned to the neurosurgical ward, the doctor decided whether to give pain medication according to the degree of the patient's pain. The patient was given a 10 mg intramuscular injection of tramadol if necessary, and the degree of postoperative pain was recorded when the patient was given the drug.

Randomization and blinding SAS 11.0 statistical software was used to design the random number of patients for the study. The included participants were randomly enrolled by sealed envelope and assigned to the acupuncture group or the sham acupuncture group. Patient allocations were performed by a clinical assistant trained in institutional review board policies. Patients in the two acupuncture groups were unaware to which acupuncture group they were assigned. The outcome assessors, data collectors, and statisticians were also blinded to group allocations during the study.

*2.7. Interventions.* For the acupuncture group, after skin cleaning with 75% alcohol swab, sterile and disposable stainless steel needles (Wuxi Jiajian; 0.25 × 25 mm; made in Jiangsu, China) are quickly and perpendicularly inserted into the skin at P6 acupoints bilaterally to a depth of 20 mm. In this group, downward pressure and upward lifting combined with twirling the needle was used to achieve De-Qi sensation (sensation of soreness, numbness, distention, or radiating, which is considered to indicate effective needling). The needles were kept in place for 30 min and manipulated manually every 10 min to maintain the De-Qi sensation. When the treatment period was over, all needles were carefully removed, and the puncture sites were covered with sterile swabs to avoid bleeding. Acupuncture was performed by licensed acupuncturists with more than 5 years of experience.

For the sham acupuncture group, sham points, which are superficial, nonacupoints at the radial side of each wrist, 15 mm away from each P6 acupoint, were used (Figure 1). After skin cleaning with 75% alcohol swab, sterile and disposable stainless steel needles (Wuxi Jiajian; 0.25 × 25 mm; made in Jiangsu, China) were quickly and perpendicularly inserted into the skin at sham acupoints bilaterally to a depth of 5 mm. The De-Qi sensation was not required in this group. The needles were retained for 30 min as with the acupuncture group, but there was no stimulation or manipulation of the needles. After 30 min, the same method to remove the needles in the acupuncture group was used. Acupuncture was performed by licensed acupuncturists with more than 5 years of experience.

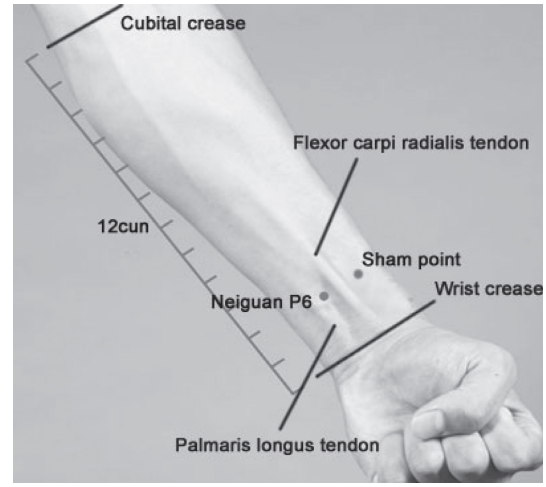


Figure 1: Location of the P6 acupoint and sham acupoint.

Figure 1 is reproduced from Lv et al., P6 acupoint stimulation for prevention of postoperative nausea and vomiting in patients undergoing craniotomy: study protocol for a randomized controlled trial, BMC, 2013 (under the Creative Commons Attribution License/public domain).

*2.8. Measures.* The study period covered 72 h after surgery. A separate research nurse, not involved in the management of patients, recorded anesthesia time, surgery time, endotracheal intubation time, patient demographics, and preoperative data for each patient. Demographic and preoperative data included the following: age, gender, weight, acupuncture experience, and smoking history. Another blinded observer (nurse) recorded the postoperative data, which included assessing the postoperative pain score at rest. Pain scores were collected prospectively during the 72 h postoperative period using the Visual Analog Scale (VAS). Patients were asked to rate their pain on a 0–10 scale, where "0" represented "no pain" and "10" represented the "worst pain I have ever experienced." The data were recorded by the nurse. The physicians recorded the use time and dosage of rescue analgesia (tramadol) for each patient when they requested rescue therapy, and the records were handed over to the observer for assessment. The incidence of postoperative nausea and vomiting was recorded. Assessments were performed at 24, 48, and 72 h. Reasons for withdrawal and acupuncture-associated adverse events (AEs), including bleeding, subcutaneous hemorrhage, hematoma, fainting, serious pain, and local infection, were recorded during the study.

*2.9. Statistical Analysis.* Since there had been no previous studies on acupuncture to prevent postcraniotomy pain, we drew on the results of a similar study that used tramadol. In that study, there was an average VAS score of 3 in the

tramadol group compared with 4.7 in the control group [3]. According to the results of that previous study and our pilot study, we anticipated an average VAS score of 2 after acupuncture treatment between the acupuncture group and sham acupuncture group. The sample size was determined by using PASS 15.0 with $\alpha = 0.05$ (two sides) and $\beta = 0.01$ (power 90%). The resulting sample size is 31 patients per group. Estimating that 20% of patients might be lost means using at least 40 subjects per group. Intending also to observe the occurrence of postoperative nausea and vomiting, we set the sample size of each group at 60 cases in order to ensure statistical viability.

All data input and the statistical analysis were performed in the Department of Epidemiology and Hygienic Statistics of West China Hospital of Sichuan University using SPSS 19.0. Data were checked for normality with the Kolmogorov–Smirnov test. Normally distributed variables were presented as the mean (SD) and were analyzed by a two-sample $t$-test. Nonnormally distributed variables (i.e., pain and PONV) were described as the median (interquartile range (IQR)) and were compared by the Mann–Whitney $U$ test. A $P$ value of <0.05 was considered statistically significant.

## 3. Results

*3.1. Participants and Baseline Characteristics.* A total of 120 patients were enrolled in the study. Three of these (2.5%), two in the acupuncture group and one in the sham acupuncture group, were withdrawn after they subsequently met the withdrawal criteria. Two of these suffered persistent coma, the other cognitive impairment. The data of the remaining 117 patients (46 male and 71 female patients) were analyzed. The characteristics of the patients in the two groups—those receiving acupuncture or sham acupuncture—and their previous medical history were not significantly different ($P > 0.05$) (Figure 2 and Table 1).

Figure 2 is reproduced from Lv et al., P6 acupoint stimulation for prevention of postoperative nausea and vomiting in patients undergoing craniotomy: study protocol for a randomized controlled trial, BMC, 2013 (under the Creative Commons Attribution License/public domain).

*3.2. Effects of Acupuncture at the P6 Acupoint on Postoperative Pain.* There was no statistically significant difference between the two groups regarding their pain scores 0–24 h after surgery ($P = 0.064$). The VAS pain score of the acupuncture group, however, was lower than that of the sham acupuncture group, and this difference is statistically significant 24–48 h after surgery ($P = 0.002$). There was no statistically significant difference in the VAS pain score of the two groups 48–72 h after craniotomy ($P = 0.254$). There was no statistically significant difference between the two groups in the analgesic drug remediation at each period after craniotomy ($P > 0.05$) (Tables 2 and 3).

*3.3. Effects of Acupuncture at the P6 Acupoint on Postoperative Nausea and Vomiting.* In the 0–24 h after surgery, the incidence of vomiting in the acupuncture group was lower than that in the sham acupuncture group. There was a statistically significant difference in the incidence of vomiting between the two groups during 24 h following craniotomy (13.8% vs. 28.8%, $P = 0.048$), though there was no statistically significant difference in the incidence of vomiting between the two groups during 48–72 h following craniotomy ($P > 0.05$). There was no statistical difference in the degree of nausea between the two groups 0–24 h, 24–48 h, and 48–72 h after surgery ($P > 0.05$) (Tables 4 and 5).

*3.4. Safety.* Two patients (one in each group) reported AEs during the testing period. These patients had subcutaneous hemorrhage. All AEs were reported as mild, and none required special medical intervention. The two patients fully recovered from the AEs and did not withdraw from the trial.

## 4. Discussion

It has been accepted in the past that the pain accompanying intracranial surgery was minimal and, when present, dangerous to treat. As a consequence, analgesic therapy for this group of patients has generally been modest [12]. It is now known that pain from intracranial surgery is comparably similar to that caused by other surgical procedures, and it is becoming acceptable to treat this pain. Using traditionally limited analgesic therapy, 69% of patients undergoing craniotomy report some period of pain that is moderate to severe (pain rating: 4/10) on the first postoperative day, and 48% of patients experience this level of pain during some portion of the second postoperative day [13]. Pain can induce nausea and vomiting and aggravate brain edema. The objective of pain control after craniotomy in the present study is to reduce the use of analgesic and sedative drugs that can cause gastrointestinal reactions and interfere with the consciousness of the patients following surgery.

The results of this study indicate that acupuncture at the P6 acupoint can reduce postoperative pain after craniotomy and may also reduce the incidence of vomiting in patients following craniotomy. This study found that patients had moderate-to-severe pain after the procedure, and the pain is more obvious during the first postoperative 24 hours. In both sham acupuncture and acupuncture groups, the VAS scores were gradually decreased in the 24–48 h period after surgery. This result is similar to a previous study indicating that postoperative pain began to decrease from 24–48 h [14].

The acupuncture and sham acupuncture groups displayed no difference in postoperative pain scores 0–24 h after surgery. A possible explanation for this finding is that intraoperative fentanyl may help to alleviate VAS mainly in the early postoperative period. The VAS pain score in the two groups during 24–48 h after surgery, however, did show significant difference. This result is consistent with the conclusion of Mali's study which indicated that multiple electroacupuncture stimulation at different time points can reduce the VAS pain score of postoperative patients with gastrointestinal tumors within 72 h [15]. This is consistent

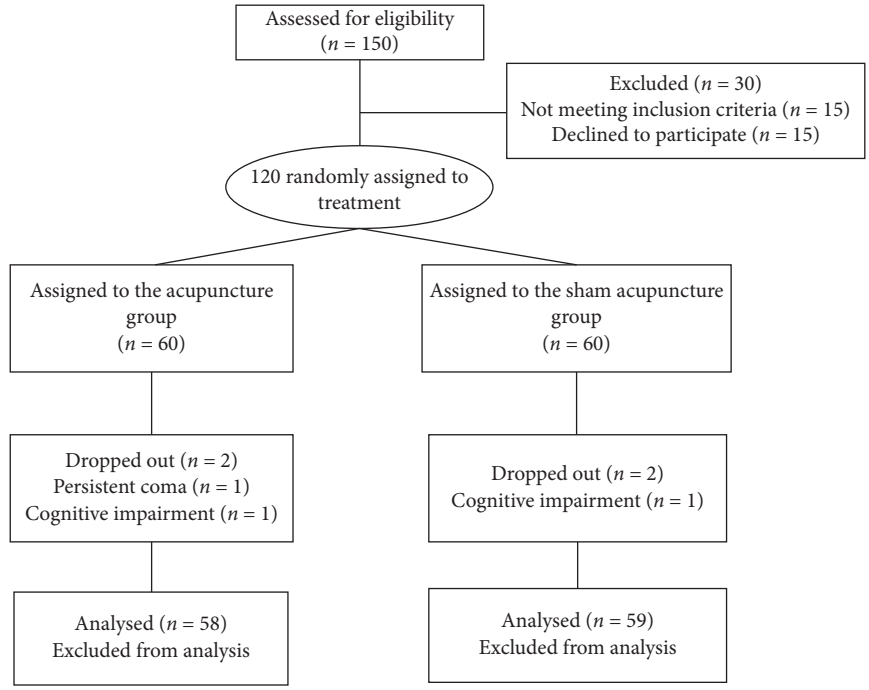


Figure 2: CONSORT flow diagram of the trial.

Table 1: Demographic data and surgical characteristics.

| Characteristic | Acupuncture group ($N = 58$) | Sham acupuncture group ($N = 59$) | $p$ values |
|---|---|---|---|
| Age (years) | 47.97 ± 12.69 | 46.19 ± 14.76 | 0.486 |
| Height (cm) | 162.09 ± 7.88 | 161.69 ± 7.19 | 0.779 |
| Weight (kg) | 59.50 ± 10.13 | 63.12 ± 11.85 | 0.079 |
| Anesthesia time (min) | 257.00 ± 92.41 | 275.75 ± 102.88 | 0.302 |
| Operation time (min) | 166.93 ± 73.34 | 182.42 ± 91.44 | 0.315 |
| Dose of sufentanil (μg) | 30 (25–30) | 30 (25–35) | 0.593 |
| Infusion amount (ml) | 2471.55 ± 1067.26 | 2655.42 ± 961.49 | 0.329 |
| Gender | | | 0.651 |
| Female (%) | 34 (58.6) | 37 (62.7) | |
| Male (%) | 24 (41.4) | 22 (37.3) | |
| Smoking history | | | 0.781 |
| No (%) | 46 (79.3) | 48 (81.4) | |
| Yes (%) | 12 (20.7) | 11 (18.6) | |
| The history of surgery | | | 0.949 |
| No (%) | 39 (67.2) | 40 (67.8) | |
| Yes (%) | 19 (32.8) | 19 (32.2) | |
| Migraine | | | 0.100 |
| No (%) | 51 (87.9) | 45 (76.3) | |
| Yes (%) | 7 (12.1) | 14 (23.7) | |
| History of opioid use | | | 0.896 |
| No (%) | 39 (67.2) | 39 (66.1) | |
| Yes (%) | 19 (32.8) | 20 (33.9) | |
| Motion sickness | | | 0.246 |
| No (%) | 50 (86.2) | 46 (78.0) | |
| Yes (%) | 8 (13.8) | 13 (22.0) | |
| The type of surgery | | | 0.11 |
| Supratentorial (%) | 37 (63.8) | 29 (49.2) | |
| Subtentorial (%) | 21 (36.2) | 30 (50.8) | |

with Chung et al.'s research. This group demonstrated that combined auricular acupressure and TEAS decreased postoperative pain, the use of analgesic morphine, and morphine-related side effects. IAS provides better analgesia when used in conjunction with PCA after lumbar spine surgery and can be regarded as a component of multimodal

Table 2: Patient-reported pain scores over time.

| | Acupuncture group ($N = 58$) | Sham acupuncture group ($N = 59$) | $p$ values |
|---|---|---|---|
| VAS pain score 0–24 h after operation | 3 (2–5) | 3 (3–5) | 0.064 |
| VAS pain score 24–48 h after operation | 3 (0–4) | 4 (3–5) | 0.002 |
| VAS pain score 48–72 h after operation | 2 (0–4) | 3 (2–3) | 0.254 |

Table 3: Postoperative analgesic drug (tramadol) use over time.

| | Acupuncture group ($N = 58$) | Sham acupuncture group ($N = 59$) | $p$ values |
|---|---|---|---|
| The use of tramadol 0–24 h after operation | | | 0.977 |
| No (%) | 53 (91.4) | 54 (91.5) | |
| Yes (%) | 5 (8.6) | 5 (8.5) | |
| The use of tramadol 24–48 h after operation | | | 0.623 |
| No (%) | 57 (98.3) | 56 (94.9) | |
| Yes (%) | 1 (1.7) | 3 (5.1) | |
| The use of tramadol 48–72 h after operation | | | 1.000 |
| No (%) | 58 (100) | 58 (98.3) | |
| Yes (%) | 0 (0) | 1 (1.7) | |

Table 4: The incidence of vomiting over time.

| | Acupuncture group ($N = 58$) | Sham acupuncture group ($N = 59$) | $p$ values |
|---|---|---|---|
| Vomiting in 0–24 h after operation | | | 0.048 |
| No (%) | 50 (86.2) | 42 (71.2) | |
| Yes (%) | 8 (13.8) | 17 (28.8) | |
| Vomiting in 24–48 h after operation | | | 0.969 |
| No (%) | 49 (84.5) | 50 (84.7) | |
| Yes (%) | 9 (15.5) | 9 (15.3) | |
| Vomiting in 48–72 h after operation | | | 1.000 |
| No (%) | 54 (93.1) | 54 (91.5) | |
| Yes (%) | 4 (6.9) | 5 (8.5) | |

Table 5: Postoperative nausea over time.

| | Acupuncture group ($N = 58$) | Sham acupuncture group ($N = 59$) | $p$ values |
|---|---|---|---|
| Score for nausea 0–24 hours after operation | 0 (0-1) | 0 (0-1) | 0.543 |
| Score for nausea 24–48 hours after operation | 0 (0-1) | 0 (0-0) | 0.934 |
| Score for nausea 48–72 hours after operation | 0 (0-0) | 0 (0-0) | 0.822 |

analgesia [16]. This result is contrary to An et al. [17], who found that, during the first six hours following surgery, the VAS score of the electric acupuncture group was much lower than that of the control group, whereas there was no difference during 6–48 h in patients undergoing a supratentorial craniotomy. We hypothesized this difference may be attributable to the patient-controlled intravenous analgesia (PCIA) they used in the first 48 h. This may be the case, in our study, where no PCIA was given. Our results that acupuncture can reduce the postoperative pain of craniotomy in 24–48 h contrast with the research of Liu et al. [18], who found that pain scores after supratentorial craniotomy were significantly lower at postoperative day 1 in the TEAS group than in the sham group. However, the VAS pain score was higher in the TEAS group on postoperative days 2 and 3. They concluded the explanation for this is unknown and may relate to the short-term anesthetic effects of TEAS so that the analgesic effect of TEAS may cease after the operation without further stimulation. We speculate that the difference may be associated with the use of analgesic drugs within 72 h (they also used PCIA) after surgery and different stimulation methods (they used TEAS; we used acupuncture). The effect of acupuncture lasts for a prolonged time after the insertion of the needle. The aftereffects are more significant because they are stronger, broader, more lasting, and can accumulate [19]. There was no significant difference in pain scores during 48–72 h between the two groups. We speculate that this may be related to acupuncture intervention time. It had been 48 hours since the acupuncture intervention, and the aftereffects of acupuncture may have dissipated.

Tramadol is a weak $\mu$-opioid receptor agonist that releases serotonin and inhibits the reuptake of norepinephrine. It is used for the management of postoperative pain in neurosurgical patients in our institution. Tramadol can provide effective pain relief without the side effects associated with opioids or the inhibitory effects on platelets induced by NSAIDs. Analgesics such as paracetamol and

NSAIDs are less frequently used perioperatively to treat postoperative pain in our institution.

This study indicates that acupuncture at the P6 acupoint cannot reduce postoperative analgesic drug (tramadol) use during postoperative 0–72 h. The application rate of analgesic drugs is 8.6% which is much lower than what was found in previous domestic research [20]. The frequently missed diagnosis of postoperative pain and the side effects of analgesic drug use are the main reasons for the low application rate of analgesic drugs [5, 21].

PONV is a common complaint after craniotomy, with an incidence of up to 79% [22]. It can have a highly negative impact on surgical outcomes and is often rated by patients as worse than postoperative pain [23]. Nausea and vomiting after craniotomy also cause electrolyte disorders, intracranial hypertension, and prolonged hospital stays [24].

Our study indicates that acupuncture at the P6 acupoint can reduce postoperative pain, as well as reduce the incidence of vomiting in patients following craniotomy.

Previous studies have shown that nausea and vomiting can also occur within the 24 h postoperative period [17, 25]. This is similar to the results of our study, in which postoperative vomiting occurred in up to 28.8% of patients and vomiting occurred within 24 h. The incidence of vomiting was gradually reduced after 24 h. The results of this study showed that acupuncture can reduce the incidence of vomiting in 24 h following surgery. There was no significant difference between the acupuncture and sham acupuncture groups during 24–72 h after surgery. This agrees with the results of our previous study showing that P6 acupuncture can reduce the degree of postoperative nausea and vomiting [26]. This study also suggested that electrical stimulation at the P6 acupoint can reduce the incidence of nausea and vomiting after craniotomy. It has been suggested that similar effects were observed after treatments involving stimulation at P6 for prevention of postoperative nausea and vomiting [27].

The acupoints and modes used for stimulation may significantly affect the outcome of acupuncture for postoperative nausea and vomiting and postoperative pain relief. In our previous study, we found that P6 acupuncture can reduce the degree of postoperative nausea and postoperative pain [26]. The P6 acupoint is one of the most commonly used and well-investigated acupoints for PONV prophylaxis and postoperative pain treatment. Based on the theory of meridian and evidence from previous studies, we chose the P6 acupoint for treatment.

This is a single-blind, placebo-controlled, randomized trial. The De-Qi response is particularly important. The needle points that readily produce a strong De-Qi sensation are thought to provide better efficacy in patients. Acupuncture stimulation patients were awake during this study, so they were able to confirm the De-Qi sensation. We used shallow skin penetration at nonacupoint caves in the control group so that patients received a sting from the needle, but the possible efficacy of acupuncture was eliminated. The evaluators did not know to which group the patients belonged to. This renders the findings more reliable. However, the degree of pain and nausea evaluation indicators may be dependent on the patient's educational level, and understanding of the degree of pain may affect the results of the study.

Our study has some limitations:

Patients' preconceived expectations of acupuncture treatment may affect their perceptions regarding the effects of the treatment on their pain and nausea. We should, in further studies, conduct questionnaires asking patients about their expectations of acupuncture treatment.

Blinding between the two groups was not evaluated by asking participants to guess to which group they were assigned at the end of the intervention.

We found that there were potential patients unwilling to take part in the study due to their fear of pain caused by acupuncture treatment. Further studies could possibly use more painless treatments such as auricular bean-embedding therapy or intradermal needling.

There have been very few studies of this surgery type, mainly carried out within the same institution. This makes comparing the results with other independent researchers difficult. Our results will hopefully encourage other groups to carry out similar research.

## 5. Conclusions

Compared with the sham acupuncture group, the true acupuncture patients treated using the P6 acupoint experienced reduced levels of pain after craniotomy during the 24–48 h postoperative period. The incidence of vomiting within the 24 hours following craniotomy was also reduced. In addition, the acupuncture treatment also proved safe for patients.

## Data Availability

The datasets used and/or analyzed will be made available from the corresponding author upon reasonable request.

## Ethical Approval

Ethical approval was granted by the Bioethics Subcommittee of West China Hospital, Sichuan University (approval number: 2012249). This study was registered with the Chinese Clinical Trial Registry: ChiCTR-TRC-13003026.

## Disclosure

The funders played no roles in study design, data collection, analysis, interpretation of results, and the manuscript.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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*Review Article*

# Acupoints for Tension-Type Headache: A Literature Study Based on Data Mining Technology

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*Objectives*. This study aimed to explore the characteristics and principles of acupoints, which were applied for treating tension-type headache (TTH). *Methods*. Four databases were searched for the literature studies of treating TTH with acupuncture and moxibustion up to September 1, 2020. Titles, journals, authors, key words, interventions, main acupoints, and outcomes of the included literature studies were extracted and inputted into the self-established Data Excavation Platform of Acupoint Specificity for analysis. *Results*. In total, 128 papers containing 137 prescriptions, 89 meridian acupoints, and 7 extraordinary acupoints of treating TTH with acupuncture and moxibustion were included. The total frequency of acupoints' application was 763 times. Fengchi (GB20), Baihui (GV20), Taiyang (EX-HN5), Hegu (LI4), and Taichong (LR3) were used most frequently. The acupoints in Yang meridians were utilized more than those in Yin meridians (66.1% vs. 17.8%), and the acupoints in the Gallbladder Meridian of Foot Shaoyang were applied most commonly. 59.9% (457/763) of the applied acupoints were on the head, face, and neck, and 31.7% (242/763) were on the four limbs. Additionally, the proportion of specific acupoints' application was 78.2% (597/763). *Conclusions*. The prescription of Fengchi (GB20), Baihui (GV20), Taiyang (EX-HN5), Hegu (LI4), and Taichong (LR3) might be relatively reasonable in clinical practices of treating TTH with acupuncture, which should be verified in further studies.

## 1. Introduction

Headache disorders become one of the most common diseases on the nervous system worldwide. The fact sheet of the World Health Organization (WHO) in April 2016 reported that half to three-quarters of adults aged 18–65 years have suffered from headache within one year. Tension-type headache (TTH), the most prevalent type of headache disorders, is characterized by a typically bilateral pain of pressing or tightening in quality, mild to moderate in intensity, and not becoming worse with routine physical activity [1]. According to the Global Burden of Disease study, TTH has become the second most common chronic disease worldwide, affecting more than 10% of the world population [2]. The unclear pathogenesis makes TTH difficult to be cured. Despite simple analgesics and NSAIDs which are the mainstays in the acute therapy of TTH, there are some unpleasant gastrointestinal side effects associated with these acute drugs, including ulcers, hemorrhage, and perforation of the gastrointestinal tract [3, 4]. Moreover, frequent and excessive use of analgesics in acute attacks may lead to a risk of medication-overuse headache [5].

According to the guideline developed by the European Federation of Neurological Societies (EFNS), nondrug management should be considered in TTH treatment [6]. As a traditional treatment technique inherited thousands of years, acupuncture has been used for various diseases around the world. Previous meta-analyses and systematic reviews revealed that acupuncture was an effective therapy in treating pain disorders, such as tension-type headache [1],

lateral elbow pain [7], labour pain [8], low back pain [9], migraine prophylaxis [10], shoulder pain [11], and peripheral joint osteoarthritis [12]. The systematic reviews from the Cochrane Library conclude that acupuncture is a valuable nonpharmacological tool in patients with frequent episodic or chronic TTH [1, 13], and it appears to be effective and safe for TTH [14, 15].

In traditional Chinese medicine (TCM) theories, the proper selection of acupoints contributes to a significant therapeutic effect. However, the selection methods of acupoints for the same disease are various from different ideas and experiences of the clinical acupuncturists. Nowadays, using modern data mining techniques to find out the principle of acupoint selections and ascertain the optimal combination of acupoints to specific diseases is not only necessary in acupuncture clinical practice but also beneficial to the development of acupuncture from traditional empirical medicine to evidence-based medicine (EBM).

This literature study aimed to explore the characteristics and association rules of acupoints used for treating TTH and to provide a relatively standard prescription for the acupuncture treatment.

## 2. Methods

*2.1. Search Strategy.* Three Chinese databases and an English database (i.e., China National Knowledge Infrastructure, Chinese Biomedicine Database, Wanfang Data, and PubMed) were searched for the literature studies of treating TTH with acupuncture and moxibustion up to September 1, 2020. Language was restricted to English and Chinese. The search terms were as follows: tension-type headache, acupuncture, moxibustion, and their variations. The search strategy is included in Table 1.

*2.2. Selection Criteria*

*2.2.1. Types of Studies.* Clinical trials aimed to evaluate the effectiveness of treating TTH with acupuncture or moxibustion, with or without randomization, and/or control could be included, whereas the following types such as animal experiments, case reports, reviews, meta-analyses, and systematic reviews should be excluded.

*2.2.2. Participants.* Participants in the included studies must be diagnosed with TTH, and the minimum sample size should be ten in each group. However, the studies enrolling the participants who were not diagnosed as TTH according to the International Classification of Headache Disorders should be ruled out.

*2.2.3. Intervention.* Acupuncture and/or moxibustion must be involved in the treatments of TTH with or without additional interventions (e.g., Chinese herb, Western medicine, or other physical therapies) in the included studies. The studies without using needle insertion (e.g., laser stimulating) should be excluded.

*2.2.4. Outcomes.* At least one clinical outcome measurement related to TTH must be reported in the included studies, such as duration, frequency, and pain intensity of headache. The controlled trials could be included if the patients treated with acupuncture gained more benefits than those who did not get acupuncture therapy. The studies should be excluded if data were published duplicately or only laboratory parameters were reported.

*2.3. Screening Process.* Firstly, potential literature studies were preidentified by LL to exclude those which were obviously irrelevant (e.g., animal experiments, case reports, reviews, meta-analyses, and systematic reviews). Secondly, full texts of remained studies were obtained and screened again by LL and QW. Thirdly, eligible studies were double-checked based on the above selection criteria by XH and QZ separately. Disagreements were solved by discussion.

*2.4. Data Extraction.* Information of the study, such as titles, journals, authors, key words, interventions, main acupoints, and outcomes, was extracted and inputted into the self-established Data Excavation Platform of Acupoint Specificity (Copyright Registration number: 2009SR014647) [16]. If there were several prescriptions in the study, all of the prescriptions of main acupoints should be extracted in different items. The premise is that the therapeutic effect of the acupuncture treatments must be better compared with nonacupuncture treatments.

*2.5. Data Processing.* In the Data Excavation Platform of Acupoint Specificity (Copyright Registration number: 2009SR014647), data related to selection and combination of acupoints could be calculated and analyzed according to the multilevel association rules and the frequent pattern growth (FP-growth) algorithm [17]. FP-growth is one of the most classic and efficient algorithm of frequent itemset mining. In our platform, it was proceeded in two steps: (1) establishing a FP-tree, which was an extended prefix-tree structure for storing acupoint prescriptions about frequent patterns (i.e., acupoint combinations); (2) mining the complete set of acupoint combinations by pattern fragment growth [17].

In the analysis of acupoint combinations, support and confidence were applied. Mathematically, support was the fraction of the total number of transactions in which the itemset occurred, measuring the statistical significance of association rules in the whole dataset. Confidence was the conditional probability of the occurrence of consequent, given the antecedent, reflecting the credibility degree of association rules [18]. In brief, the support of $A \longrightarrow B$ indicated that the prescriptions containing both acupoint $A$ and acupoint $B$ account for the total ones; and the

Table 1: Search strategy of the literature study.

| | |
|---|---|
| A. Search strategy to locate "tension-type headache" | #1. tension-type headache [MeSH]<br>#2. TTH [tw]<br>#3. primary headache [MeSH]<br>#4. or/#1-#3 |
| B. Search strategy to locate acupuncture interventions | #5. acupuncture [MeSH]<br>#6. acupuncture therapy [MeSH]<br>#7. acupuncture points [MeSH]<br>#8. body acupuncture [tw]<br>#9. electroacupuncture [MeSH]<br>#10. electro-acupuncture [tw]<br>#11. electrical acupuncture [tw]<br>#12. scalp acupuncture<br>#13. dry needling<br>#14. triggers point [tw]<br>#15. moxibustion [MeSH]<br>#16. acupoint [tw]<br>#17. or/#5-#16 |
| C. Search strategy to locate literature studies for this study | #4 and #17 |

confidence of $A \longrightarrow B$ displayed that the prescriptions containing both acupoint $A$ and acupoint $B$ account for the ones containing acupoint $A$.

## 3. Results

*3.1. Search Results and Profile of Prescriptions.* In total, 868 records were identified. After screening and evaluation, 137 prescriptions of the main acupoints from 128 records were included in this study. The process of filtering the literature studies and extracting the prescriptions is outlined in Figure 1.

*3.2. Application of Acupoints.* In 137 prescriptions, 89 meridian acupoints and 7 extraordinary acupoints were recorded for 763 times in TTH acupuncture and/or moxibustion treatments. Twenty acupoints applied most frequently are listed in Table 2 in the descending order, and Fengchi (GB20), Baihui (GV20), Taiyang (EX-HN5), Hegu (LI4), and Taichong (LR3) are the top 5 used acupoints.

*3.3. Association of Meridians and Acupoints.* Acupoints could be divided into two categories: meridian acupoints and extraordinary acupoints. In this part, we only discuss the meridian acupoints belonging to the fourteen meridians (the twelve regular meridians, Conception Vessel, and Governor Vessel). Eighty-nine acupoints were distributed in the fourteen meridians. The information of meridian applications is presented in Table 3, including the frequencies and proportions of meridians and the numbers and proportions of the acupoints used in each meridian. From the results of meridians' application, the acupoints in the Gall Bladder Meridian of Foot Shaoyang were applied most frequently. According to the Yin and Yang classification of TCM, 66.1% (504/763) Yang meridians and 17.8% (136/763) Yin meridians were utilized in these studies, respectively.

*3.4. Correlation between Acupoints and Body Parts.* The analysis of acupoints' application on different body parts displays the frequencies and proportions of body distributions, the numbers and percentages of the acupoints used on each body part, and frequency of each used acupoint. Acupoints on the head, face, and neck were used most frequently, with 35 acupoints applied 457 times totally. Detailed information is listed in Table 4.

*3.5. Application of Specific Acupoints.* The utilization rate of specific acupoints needs calculating indirectly through the frequency of nonspecific acupoints, which can eliminate the duplication caused by the direct computation of specific acupoint frequency, because some specific acupoints belong to two or more categories. For example, LR3 belongs to both Five-Shu point and Yuan-primary point, and the record of this point will add once in the frequencies of Five-Shu point and Yuan-primary point, respectively. The computational formula is as follows: utilization rate of specific acupoints = ((total frequency − nonspecific acupoints' application frequency)/total frequency) × 100%. We found that the utilization rate of specific acupoints was as high as 78.2% (597/763) and the nonspecific ones was 21.8% (166/763). Convergent acupoints were most widely used among the nine categories of specific ones. The frequencies of different specific acupoint categories are exhibited in Table 5.

*3.6. Acupoint Combinations.* In this part, the support and confidence were applied to measure the effect of acupoint combinations in those prescriptions, which contained two or more acupoints. The results of acupoint combinations are listed in Table 6. The pairwise combinations of Fengchi (GB20), Baihui (GV20), and Taiyang (EX-HN5) had the top three supports, among which GB20 combined with GV20 (support: 40.9%) was the core for TTH treatment. In addition, LI4 combined with GB20 (support: 28.5%;

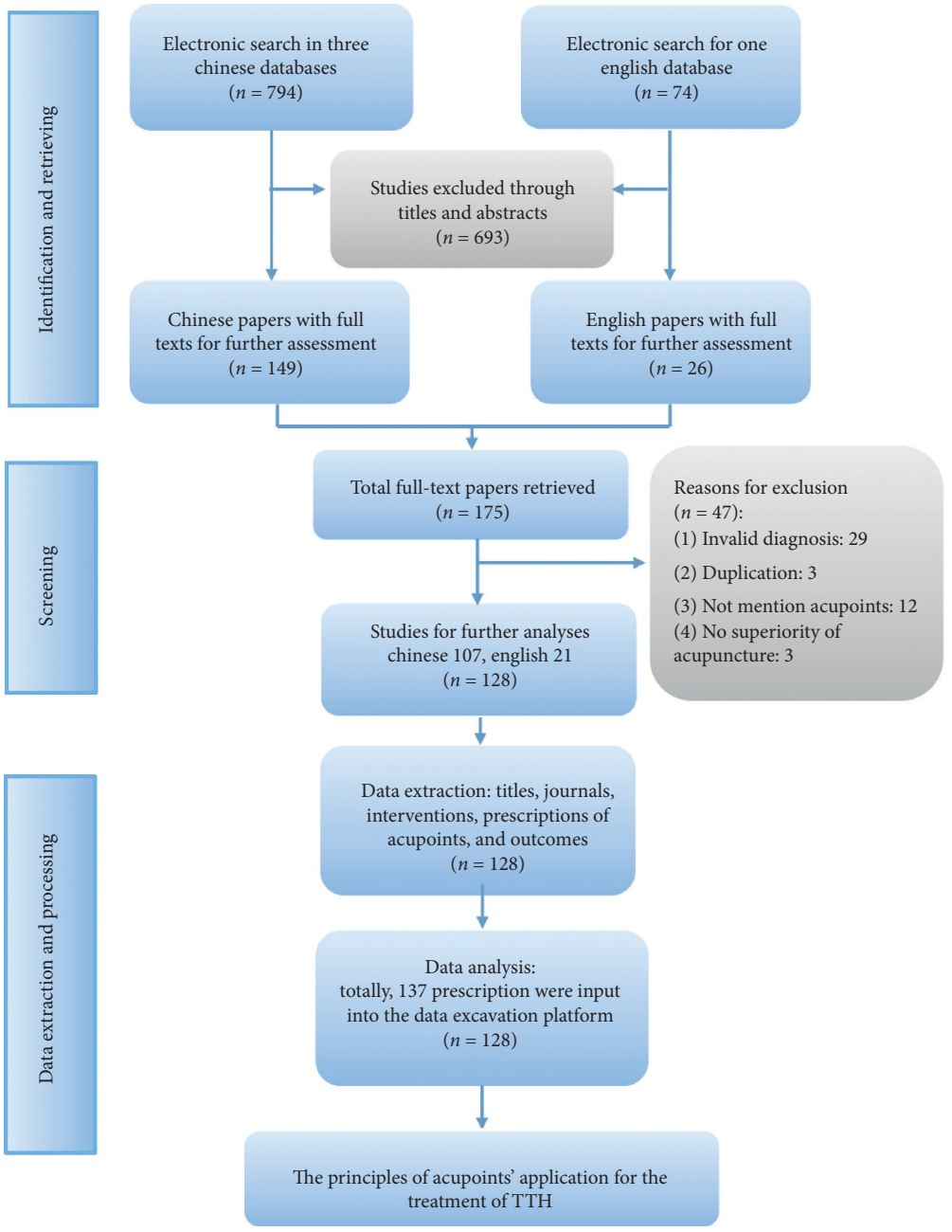


Figure 1: Process of filtering the literature studies and extracting the prescriptions.

confidence: 79.6%) and LR3 combined with GB20 (support: 23.4%; confidence: 68.1%) reflected the important role of the distant-local combination principle in TTH treatment.

## 4. Discussion

Nowadays, the acupoint prescriptions for TTH are manifold and lack the uniform standard. A total of four main categories of acupoints' selection can be summarized from the previous studies: (1) the unified acupoint prescription (the unified acupoints applied in all acupuncture treatment sessions) [19]; (2) the semiunified acupoints' prescription (a combination of unified main points and additional points chosen via syndromes or symptoms) [20–23]; (3) the quantitative prescription (choosing a certain number of acupoints from a unified acupoints' pool) [24]; and (4) the nonunified prescription (selecting acupoints completely based on syndrome differentiation without unified main acupoints and developing an individual prescription) [25]. Formulating a standard and effective prescription for TTH

Table 2: The top twenty acupoints for TTH treatment.

| Number | Acupoint | Frequency | *Proportion (%) |
|---|---|---|---|
| 1 | Fengchi (GB20) | 100 | 73.0 |
| 2 | Baihui (GV20) | 74 | 54.0 |
| 3 | Taiyang (EX-HN5) | 68 | 49.6 |
| 4 | Hegu (LI04) | 49 | 35.8 |
| 5 | Taichong (LR03) | 47 | 34.3 |
| 6 | Touwei (ST08) | 35 | 25.6 |
| 7 | Shuaigu (GB08) | 29 | 21.2 |
| 8 | Sanyinjiao (SP06) | 22 | 16.1 |
| 9 | Zusanli (ST36) | 19 | 13.9 |
| 10 | Sishencong (EX-HN1) | 18 | 13.1 |
| 11 | Shenting (GV24) | 16 | 11.7 |
| 12 | Yintang (EX-HN3) | 15 | 11.0 |
| 13 | Tianzhu (BL10) | 15 | 11.0 |
| 14 | Shenmen (HT07) | 13 | 9.5 |
| 15 | Lieque (LU07) | 11 | 8.0 |
| 16 | Fengfu (GV16) | 11 | 8.0 |
| 17 | Neiguan (PC06) | 10 | 7.3 |
| 18 | Kunlun (BL60) | 9 | 6.6 |
| 19 | Waiguan (TE05) | 9 | 6.6 |
| 20 | Shangxing (GV23) | 8 | 5.8 |

*Proportion refers to the percentage that an acupoint frequency accounts for the total frequency of all acupoints.

seems not only beneficial for diminishing confounding factors in explanatory studies from the perspective of EBM but also extremely essential for optimizing treatment regimens and achieving good effects for the acupuncture clinical practice.

Fortunately, the emergence of data mining techniques makes it feasible to extract effective hidden information and correlation of acupoints from the massive data. In recent years, the acupoints applied for poststroke disorder [26], migraine [27], functional diarrhea [28], vertigo [29], perimenopausal syndrome [30], and primary dysmenorrhea [31] have been explored based on data mining technology, and most of the results have been translated into applications of clinical practice and trials. Our study utilized data mining for secondary analysis of literature studies to explore the characteristics of acupoints for TTH treatment, which may contribute to the development of the optimal standard acupoint prescription.

Our results suggested that acupoints in the Yang meridian and distributed on the head, face, and neck might play some important roles in the TTH treatment. Acupoints of the Gall Bladder Meridian of Foot Shaoyang (GB) and Governor Vessel (GV) were applied with high proportions, a majority of which were on the head. In the TCM theory, head is the confluence of all the Yang meridians, and the hyperactivity of Yang and the stasis of Qi are considered to be the main pathogenesis of headache [32]. Yang and Yin meridians provide layers of energy to protect the integrity of the body. Yang distributes outside, while Yin hides inside. Generally, the acute headache is caused by the abnormal distribution of superficial Qi controlled by Yang meridians initially and disrupts Yin meridians as symptoms become chronic [33]. Therefore, stimulating acupoints of Yang meridians can restore the harmony of Yang and Qi to prevent disturbing Yin. Acupoints on the head, face, and neck were applied more frequently than other parts of the body, which reflects the principle of local and nearby acupoints' selection in line with the traditional saying that where there is an acupoint, there is a corresponding indication. In the modern medicine, although the mechanisms of TTH still remain unclear, muscle nociceptors, myofascial tenderness, and muscle contraction have been demonstrated to play roles in the pathophysiology of TTH [34]. Increased pericranial myofascial tissue tenderness and pressure pain hypersensitivity with the prevalent neck pain are the prominent manifestations in TTH patients [35–38]. The locations of tenderness points, also called myofascial trigger points (MTrPs), are consistent with the distribution range of the GB and GV meridians on the head and neck [37]. Therefore, stimulating these acupoints on the head and neck is a direct and symptomatic treatment for TTH.

Fengchi (GB20) and Baihui (GV20) proved to be the core acupoint combination for TTH in our study, which showed pleasant effects in some previous studies around the world [22, 39]. Pericranial muscle abnormal metabolism, caused by inflammation, decreased skeletal muscle blood flow and muscle atrophy and demonstrated a potential peripheral mechanism of TTH [40–44]. Stimulating GB20 can regulate blood flow velocity positively [45, 46] and inhibit hyperalgesia by increasing the number of mast cells and macrophages and eliminating the serum proinflammatory factors (IL-1$\beta$, IL-6, TNF-$\alpha$, and COX-2) [47]. Moreover, GB20 can improve the electromyographic activity of sternocleidomastoid and trapezius muscles and increase the tolerance to chronic pain [48]. GV20 shows similar properties of anti-inflammatory and blood flow regulation. Acupuncturing GV20 can relieve cerebral vasospasm [49, 50], increase anti-inflammatory cytokine production, and provide pronounced analgesic relief via the release of mesenchymal stem cells [51]. The above underlying mechanism may be the reason why the combination of GB20 and GV20 can treat TTH collaboratively. According to the local and adjacent therapeutic property of acupoints, EX-HE05 can be taken as the key acupoint for TTH treatment theoretically because it is located in the temporalis muscles, where many MTrPs are distributed [37].

In addition to peripheral mechanisms, central sensitization, mediated by the spinal cord and the trigeminal nerve nucleus caudalis [52], may take an important part in the pathogenesis of TTH, provoked by the continuous peripheral nociception inputs from pericranial myofascial tissues [53]. Hegu (LI4) and Taichong (LR3), known as Siguan, are the classical acupoints for relieving pain and inducing resuscitation. Previous studies have found that LI4 and LR3 exerted a neuroprotective role for the central system, manifested as repairing neurons, alleviating nerve inflammation, and inhibiting cell apoptosis [54–56]. Functional magnetic resonance imaging (fMRI) studies also found that stimulating LI4 and LR3 could transform the pain-activated brain regions to the inhibitory state, exert analgesic effects, and relieve physical pain caused by central sensitization [57, 58]. Moreover, psychological factor is also known as an important inducement for TTH [59, 60].

Table 3: Association analysis of meridians and acupoints used in TTH treatment.

| Number | Meridian | Frequency | *Proportion (%) | Acupoints in each meridian | | |
|---|---|---|---|---|---|---|
| | | | | Number | #Proportion (%) | Acupoints and their frequency |
| 1 | GB | 175 | 23.6 | 17 | 20.5 | Fengchi (GB20) 100, Shuaigu (GB08) 29, Jianjing (GB21) 7, Yangbai (GB14) 6, Yanglingquan (GB34) 5, Xuanlu (GB05) 4, Wangu (GB12) 4, Zhengying (GB17) 4, Zulinqi (GB41) 4, Hanyan (GB04) 2, Toulinqi (GB15) 2, Naokong (GB19) 2, Xiaxi (GB43) 2, Benshen (GB13) 1, Huantiao (GB30) 1, Xuanzhong (GB39) 1, and Zuqiaoyin (GB44) 1 |
| 2 | GV | 126 | 17.0 | 10 | 12.1 | Baihui (GV20) 74, Shenting (GV24) 16, Fengfu (GV16), Shangxing (GV23) 8, Dazhui (GV14) 5, Houding (GV19) 4, Qianding (GV21) 3, Yamen (GV15) 2, Naohu (GV17) 2, and Shuigou (GV26) |
| 3 | ST | 64 | 8.6 | 7 | 8.4 | Touwei (ST08) 35, Zusanli (ST36) 19, Neiting (ST44) 4, Huaroumen (ST24) 2, Fenglong (ST40) 2, Tianshu (ST25) 1, and Wailing (ST26) 1 |
| 4 | BL | 54 | 7.3 | 15 | 18.1 | Tianzhu (BL10) 15, Kunlun (BL60) 9, Cuanzhu (BL02) 7, Ganshu (BL18) 4, Tongtian (BL07) 3, Shenmai (BL62) 3, Quchai (BL04) 2, Wuchu (BL05) 2, Chengguang (BL06) 2, Luoque (BL08) 2, Yuzhen (BL09) 1, Fengmen (BL12) 1, Feishu (BL13) 1, Xinshu (BL15) 1, and Pishu (BL20) 1 |
| 5 | LR | 52 | 7.0 | 2 | 2.4 | Taichong (LR03) 47 and Xingjian (LR02) 5 |
| 6 | LI | 51 | 6.9 | 3 | 3.6 | Hegu (LI04) 49, Shousanli (LI10) 1, and Quchi (LI11) 1 |
| 7 | SP | 25 | 3.4 | 2 | 2.4 | Sanyinjiao (SP06) 22 and Xuehai (SP10) 3 |
| 8 | TE | 25 | 3.4 | 8 | 9.6 | Waiguan (TE05) 9, Yifeng (TE17) 4, Jiaosun (TE20) 3, Sizhukong (TE23) 3, Zhigou (TE06) 2, Luxi (TE19) 2, Zhongzhu (TE03) 1, and Tianliao (TE15) 1 |
| 9 | CV | 14 | 1.9 | 6 | 7.2 | Zhongwan (CV12) 4, Guanyuan (CV04) 3, Qihai (CV06) 3, Juque (CV14) 2, Shuifen (CV09) 1, and Danzhong (CV17) 1 |
| 10 | HT | 13 | 1.8 | 1 | 1.2 | Shenmen (HT07) 13 |
| 11 | PC | 12 | 1.6 | 2 | 2.4 | Neiguan (PC06) 10 and Daling (PC07) 2 |
| 12 | LU | 11 | 1.5 | 1 | 1.2 | Lieque (LU07) 11 |
| 13 | SI | 9 | 1.2 | 3 | 3.6 | Houxi (SI03) 7, Jianwaishu (SI14) 1, and Jianzhongshu (SI15) 1 |
| 14 | KI | 9 | 1.2 | 3 | 3.6 | Taixi (KI03) 7, Yongquan (KI01) 1, and Yindu (KI19) 1 |

GB refers to the Gallbladder Meridian of Foot Shaoyang. GV refers to the Governor Vessel. ST stands for the Stomach Meridian of Foot Yangming. BL refers to the Bladder Meridian of Foot Taiyang. LR refers to the Liver Meridian of Foot Jueyin. LI refers to the Large Intestine Meridian of Hand Yangming. SP refers to the Spleen Meridian of Foot Taiyin. TE refers to Triple Energizer of Hand Shaoyang. CV refers to the Conception Vessel. HT refers to the Heart Meridian of Hand Shaoyin. PC refers to the Pericardium Meridian of Hand Jueyin. LU refers to the Lung Meridian of Hand Taiyin. SI refers to the Small Intestine Meridian of Hand Taiyang. KI refers to the Kidney Meridian of Foot Shaoyin. *Proportion means the percentage that a specific meridian frequency accounts for the total frequency of all meridians. #Proportion refers to the percentage that the number of acupoints in a meridian accounts for the total number of meridian acupoints.

Table 4: Association analysis of body parts and acupoints used in TTH treatment.

| Number | Body part | Frequency | Proportion (%) | Acupoints in each body part | | |
|---|---|---|---|---|---|---|
| | | | | Number | Proportion (%) | Selected acupoints and their frequency |
| 1 | Head, face, and neck | 457 | 59.9 | 35 | 36.5 | Fengchi (GB20) 100, Baihui (GV20) 74, Taiyang (EX-HN5) 68, Touwei (ST08) 35, and Shuaigu (GB08) 29 |
| 2 | Lower limbs | 136 | 17.8 | 17 | 17.7 | Taichong (LR03) 47, Sanyinjiao (SP06) 22, Zusanli (ST36) 19, Kunlun (BL60) 9, and Taixi (KI03) 7 |
| 3 | Upper limbs | 106 | 13.9 | 11 | 11.5 | Hegu (LI04) 49, Shenmen (HT07) 13, Lieque (LU07) 11, Neiguan (PC06) 10, and Waiguan (TE05) 9 |
| 4 | Back and lumbar | 33 | 4.3 | 18 | 18.8 | Jianjing (GB21) 7, Dazhui (GV14) 5, Ganshu (BL18) 4, Jianwaishu (SI14) 2, and Jianzhongshu (SI15) 2 |
| 5 | Chest and abdomen | 31 | 4.1 | 15 | 15.6 | Zhongwan (CV12) 4, Guanyuan (CV04) 3, Qihai (CV06) 3, Juque (CV14) 2, and Huaroumen (ST24) 2 |

Table 5: Different types of specific acupoints applied in TTH treatment.

| Number | Specific acupoint | Frequency | Amount of acupoints | Selected acupoints and their frequency |
|---|---|---|---|---|
| 1 | Crossing point | 353 | 30 | Fengchi (GB20) 100, Baihui (GV20) 74, Touwei (ST08) 35, Shuaigu (GB08) 29, and Sanyinjiao (SP06) 22 |
| 2 | Five-Shu point | 130 | 17 | Taichong (LR03) 47, Zusanli (ST36) 19, Shenmen (HT07) 13, Kunlun (BL60) 9, Houxi (KI03) 7, and Taixi (KI03) 7 |
| 3 | Yuan-primary point | 118 | 5 | Hegu (LI04) 49, Taichong (LR03) 47, Shenmen (HT07) 13, Taixi (KI03), and Daling (PC07) 2 |
| 4 | Eight confluent points | 44 | 6 | Lieque (LU07) 11, Neiguan (PC06) 10, Waiguan (TE05) 9, Houxi (SI03) 7, and Zulingqi (GB41) 4 |
| 5 | Luo-connecting point | 32 | 4 | Lieque (LU07) 11, Neiguan (PC06) 10, Waiguan (TE05) 9, and Fenglong (ST40) 2 |
| 6 | Lower He-sea point | 24 | 2 | Zusanli (ST36) 19 and Yanglingquan (GB34) 5 |
| 7 | Eight converging points | 11 | 4 | Yanglingquan (GB34) 5, Zhongwan (CV12) 4, Tanzhong (CV17) 1, and Xuanzhong (GB39) 1 |
| 8 | Front-Mu point | 11 | 5 | Zhongwan (CV12) 4, Guanyuan (CV04) 3, Juque (CV14) 2, Tanzhong (CV17) 1, and Tianshu (ST25) 1 |
| 9 | Back-Shu point | 7 | 4 | Ganshu (BL18) 4, Feishu (BL13) 1, Xinshu (BL15) 1, and Pishu (BL20) 1 |

Table 6: The top ten acupoint combinations in TTH treatment.

| Number | Combination of acupoints | Support (%) | Confidence (%) |
|---|---|---|---|
| 1 | Baihui (GV20) ⟶ Fengchi (GB20) | 40.9 | 75.7 |
| 2 | Fengchi (GB20) ⟶ Taiyang (EX-HN5) | 40.1 | 80.9 |
| 3 | Taiyang (EX-HN5) ⟶ Baihui (GV20) | 32.8 | 66.2 |
| 4 | Hegu (LI04) ⟶ Fengchi (GB20) | 28.5 | 79.6 |
| 5 | Fengchi (GB20) ⟶ Baihui (GV20), Taiyang (EX-HN5) | 27.0 | 82.2 |
| 6 | Taichong (LR03) ⟶ Fengchi (GB20) | 23.4 | 68.1 |
| 7 | Taichong (LR03) ⟶ Baihui (GV20) | 23.4 | 68.1 |
| 8 | Touwei (ST08) ⟶ Fengchi (GB20) | 21.9 | 85.7 |
| 9 | Taichong (LR03) ⟶ Taiyang (EX-HN5) | 21.9 | 63.8 |
| 10 | Shuaigu (GB08) ⟶ Fengchi (GB20) | 19.7 | 93.1 |

Support of $A \longrightarrow B$ indicated that the prescriptions containing both acupoint $A$ and acupoint $B$ account for the total ones; confidence of $A \longrightarrow B$ displayed that the prescriptions containing both acupoint $A$ and acupoint $B$ account for the ones containing acupoint $A$.

Acupuncture at Siguan acupoints can relieve depression [61], partly via upregulating hippocampal AMPA receptors [62]. Thus, the therapeutic effects of LI4 and LR3 on TTH may be achieved through both physiological and psychological approaches, indicating the remote therapeutic property of acupoints.

Combining the local or nearby acupoints on the head (i.g., GB20, GV20, and EX-HN05) with the distal acupoint on the limbs (i.g., LI04 and LR03) reflects principles of distant-local and upper-lower acupoints' combinations in the acupuncture theory, which has been adopted in several previous trials of good quality [20–22, 39]. Analysis of treatment details applied in a multicentre randomised trial of acupuncture for tension-type headache (ARTTTH) from Germany has shown that GB20, LR3, and LI4 were the high-frequency acupoints, treated in 96%, 97%, and 67% of sessions, respectively [63]. Not only that, the combination of the above five acupoints also shows therapeutic advantages in the treatment of other primary headaches, such as migraine [64], and the application proportions of GB20, GV20, EX-HN5, LI4, and LR3 calculated from 11 studies of migraine are 82%, 55%, 55%, 46%, and 64%, respectively [65].

However, our results should be interpreted with caution due to the following limitations. Firstly, the outcome measurements and acupuncture stimulation parameters of the included studies were not unified, which might make it confused to evaluate the therapeutic effects contributed by acupoints. Secondly, owing to the lack of the studies accorded with EBM methodologies, some self-controlled studies were included in the analyses, which were difficult to evaluate the qualities and might affect the objectivity of the results.

## 5. Conclusions

Our findings provided a reasonable reference of acupoints' selection and combination for TTH. Fengchi (GB20), Baihui (GV20), Taiyang (EX-HN05), Hegu (LI04), and Taichong (LR03) were recommended as the main acupoints for TTH, and the prescription might be conducive to the standardization of treating TTH with acupuncture, which needed to be further verified through clinical trials and mechanism research.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Authors' Contributions

LL and QW contributed equally to this article, participated in the design of the study, and drafted the manuscript. XH and QZ participated in the retrieval of the literature and extracted information. NL and YL helped revise the manuscript.

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*Research Article*

# Anti-Inflammatory Investigations of Extracts of *Zanthoxylum rhetsa*

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*Zanthoxylum rhetsa* has been consumed in the diet in northern Thailand and also used as a medicament in ancient scripture for arthropathies. Thus, this study aimed to evaluate the activity of various extracts from differential parts of *Z. rhetsa* via inhibition of inflammatory mediators (NO, TNF-$\alpha$, and $PGE_2$) in RAW264.7 macrophages. The chemical composition in active extracts was also analyzed by GC/MS. The parts of this plant studied were whole fruits (F), pericarp (P), and seed (O). The methods of extraction included maceration in hexane, 95% ethanol and 50% ethanol, boiling in water, and water distillation. The results demonstrated that the hexane and 95% ethanolic extract from pericarp (PH and P95) and seed essential oil (SO) were the most active extracts. PH and P95 gave the highest inhibition of NO production with $IC_{50}$ as $11.99 \pm 1.66\,\mu$g/ml and $15.33 \pm 1.05\,\mu$g/ml, respectively, and they also showed the highest anti-inflammatory effect on TNF-$\alpha$ with $IC_{50}$ as $36.08 \pm 0.55\,\mu$g/ml and $34.90 \pm 2.58\,\mu$g/ml, respectively. PH and P95 also showed the highest inhibitory effect on $PGE_2$ but less than SO with $IC_{50}$ as $13.72 \pm 0.81\,\mu$g/ml, $12.26 \pm 0.71\,\mu$g/ml, and $8.61 \pm 2.23\,\mu$g/ml, respectively. 2,3-Pinanediol was the major anti-inflammatory compound analyzed in PH (11.28%) and P95 (19.82%) while terpinen-4-ol constituted a major anti-inflammatory compound in SO at 35.13%. These findings are the first supportive data for ethnomedical use for analgesic and anti-inflammatory activity in acute (SO) and chronic (PH and P95) inflammation.

## 1. Introduction

Pain is a common symptom and sign of inflammation and tissue damage [1–3]. Etiology including physical, biological, and chemical factors such as trauma, overuse, chemical, toxins, and pathogens can activate inflammatory response [1]. Inflammation is a response to protect and restore cells and tissues to a normal state [4]. The stimulus activates leukocytes to produce inflammatory cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) [1]. In a site of tissue injury, prostaglandin $E_2$ ($PGE_2$) plays an important role in acute inflammation and causes vasodilation edema, acute pain, and fever [5]. TNF-$\alpha$ is an inflammatory cytokine that is intertwined with $PGE_2$ as it stimulates phospholipase $A_2$ and releases eicosanoids from the cyclooxygenase and lipoxygenase pathways in arachidonic acid metabolism [5]. The important product from cyclooxygenase is $PGE_2$ [5]. Additionally, high levels of TNF-$\alpha$ can trigger fever and activate endothelial cells to express adhesion molecules resulting in leukocytes adherence and prolonged inflammation [6].

Macrophages trigger production of TNF-$\alpha$ cytokines causing pain and fever, loss of cell function, or loss of mobility in joints [2]. TNF-$\alpha$ can also activate macrophages to produce nitric oxide (NO) [7]. NO is a free radical derived from L-arginine and oxygen by inducible nitric oxide synthase (iNOS) enzyme from macrophages [8]. NO induces toxicity by interaction with superoxide and produces peroxynitrite which is highly toxic to microorganisms and normal neighboring cells [8]. Cells and tissues are gradually destroyed by excessive NO production, and as a result, the perception of pain remains.

Although the outcome of inflammatory responses involves physiological functions to protect and restore cells and tissues to a normal state, excessive inflammatory response is the cause of persistent inflammation and leads to chronic inflammation and pain [9, 10]. The impact of chronic inflammation involvement in chronic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, osteoarthritis, cancer, and cardiovascular diseases is well known [10].

Herbal remedies have been used for their anti-inflammatory and pain-relieving properties according to folk wisdom and in traditional ethnomedicine for centuries. According to Thai traditional medicine principles, herbs which have a spicy taste and pungent aroma such as capsicum, ginger, and plai (*Zingiber cassumunar*) are often used for pain relief [11].

The chemotaxonomy study of some *Zanthoxylum* species such as *Z. acanthopodium*, *Z. nitidum*, and *Z. myriacanthum* are found in Northern Thailand [12, 13] or *Z. budrunga*, *Z. bungeanum*, and *Z. schinifolium*, all have shown anti-inflammatory and antinociceptive action [14–17]. *Zanthoxylum rhetsa* is a pungent plant and a member of the Rutaceae family. Its whole fruit consists of pericarp and seed and is used in the diet in the Northern part of Thailand. Both pericarp and fruit are described in Pra-O-Sod-Pra-Narai scripture and Thai Traditional Household Remedy for muscle spasm, a pain relief from swelling of muscle and tendons and also as pain relief from abscesses and hemorrhoids [11, 18]. *Z. rhetsa* fruit is also extensively used as an anti-inflammatory agent and antiseptic in India [19]. *Z. rhetsa* fruit and seed are also used as a pain relief treatment from toothache, digestion problems, inflammation, and infection in Southeast Asia [19]. *Z. rhetsa* activity is a mosquito repellent, and its larvicidal, antimicrobial, antioxidant, and antitumor activities have been characterized [20]. Additionally, major chemical compounds in pericarp, fruit, and seed of *Z. rhetsa* as monoterpenes such as limonene, terpinen-4-ol, sabinene, and $\alpha$-pinene [21–30] have been reported for their anti-inflammatory activity [31–33].

Therefore, the present study compared and investigated the anti-inflammatory activity of various anatomical parts such as whole fruits, pericarp, and seed of *Z. rhetsa* extracts through the inhibition of lipopolysaccharide- (LPS-) induced NO, TNF-$\alpha$, and $PGE_2$ in RAW264.7 macrophages. Additionally, chemical compositions of the active extracts were also delineated as anti-inflammatory, and pain relief activity of *Z. rhetsa* has been poorly studied [21–23]. Furthermore, the analysis of chemical constituents in pericarp, fruit, and seed of *Z. rhetsa* of various extractions and characterizing the anti-inflammatory activity has not been undertaken [31–33].

## 2. Materials and Methods

*2.1. Plant Materials.* *Z. rhetsa* was collected from its natural habitat in Ban Mae Khaw Tom Thasud village, Muang district, Chiang Rai province, Thailand. The voucher specimen was identified by using important characteristic of the morphology of both flower and fruit. After that, the scientific name of plant material was identified by botanists in the Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand. The voucher specimen BKF number 193835 was preserved in the office of the Forest Herbarium, Bangkok, Thailand.

*2.2. Chemicals and Reagents.* Ethanol 95% (EtOH) (commercial grade) was purchased from C.M.J. Anchor Company (Thailand). Analytical grade dimethyl sulfoxide (DMSO), hexane, hydrochloric acid (HCl), and isopropanol were purchased from RCI Labscan (Thailand). Distilled water was produced by Milli-Q water purification system from Millipore (USA). Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid), thiazolyl blue tetrazolium bromide (MTT), lipopolysaccharide (LPS) from *E.coli* (O55:B5), and prednisolone were purchased from Sigma-Aldrich (USA). Fetal bovine serum (FBS), penicillin-streptomycin (P/S), RPMI 1640 medium, and Dulbecco's modified eagle medium (DMEM) were purchased from Gibco (USA). The prostaglandin $E_2$ ELISA kit was purchased from Cayman Chemical (USA), and Mouse TNF-$\alpha$ Quantikine ELISA test kit was purchased from R&D System Inc (USA).

*2.3. Preparation of Extracts.* After plant materials were sun-dried, they were separated into pericarp, fruit, and seed. Each part was ground to coarse powder and then was extracted by 3 methods consisting of maceration with hexane, 95% EtOH and 50% EtOH, water distillation, and decoction.

For maceration: each part powder (1 kg) was extracted by maceration with different solvent for three days (solvent: powder ratio = 2 : 1) and filtered through Whatman no.1 filter paper. The marc was remacerated twice, and the combined filtrate was evaporated by rotary evaporator to give the hexane extract, 95% ethanolic extract, and 50% ethanolic extract of pericarp (PH, P95, and P50), fruit (FH, F95, and F50), and seed (SH, S95, and S50), respectively.

For water distillation: each part powder (500 g) was distilled in a Clevenger apparatus for 100 minutes and the essential oil was collected and gave the essential oil from pericarp (PO), fruit (FO), and seed (SO).

For decoction: each part of powder (500 g) was boiled in distilled water for 15 minutes and filtered. The residue had twice repeated decoction, and the combined filtrate was reduced to 1/3 by boiling then freeze dried to give the water extract from pericarp (PW), fruit (FW), and seed (SW). All crude extracts showed percentage of yield on Figure 1. The

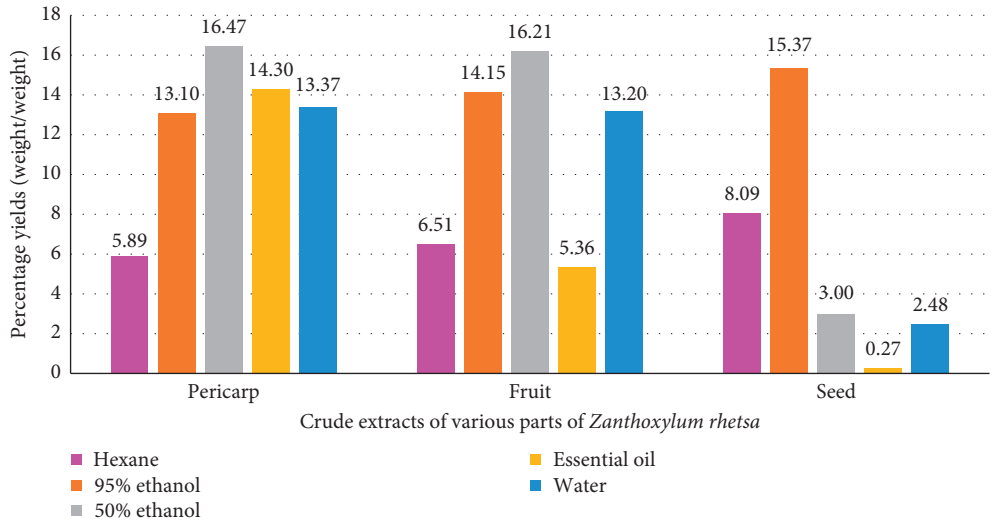


Figure 1: The percentage yields of the crude extracts of various parts of *Z. rhetsa*.

crude extracts were kept at −20°C, and the essential oils were kept at 4°C before use.

*2.4. Cell Culture and Culture Media.* RAW 264.7 macrophages from mouse (*Mus musculus*) were purchased from American Type Culture Collection (ATCC®TIB-71) (USA). Cells were cultured in two types of media according to assays: (1) RPMI 1640 medium for the assays of inhibition of LPS-induced nitric oxide (NO) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) production following the established method [34] and the procedure in the manufacturer's manual [35], respectively, and (2) DMEM medium for the assay of inhibition of LPS-induced prostaglandin $E_2$ ($PGE_2$) production following the method of the procedure in the manufacturer's manual [36]. Each medium was supplemented with 10% FBS and 1% P/S (100 unit/ml) and incubated in an incubator at 37°C, 5% $CO_2$, and 95% humidity.

*2.5. Determination of Cell Viability.* Cell viability was done in triplicate by using MTT assay [34]. Briefly, after $1 \times 10^5$ cells/well of RAW 264.7 macrophages were seeded in sterilized 96 well-plate (100 $\mu$l/well) and incubated for 24 h, the medium was removed and replaced with 100 $\mu$l/well of fresh medium. Various dilutions of samples were added (100 $\mu$l/well) and incubated for another 24 h. Subsequently, the supernatants (100 $\mu$l/well) were removed, and the viable cells were determined by adding 10 $\mu$l/well of the MTT solution (5 mg/ml) and further incubated for 2 h. The medium was then removed and replaced with 100 $\mu$l/well of isopropanol containing 0.04 M HCl to dissolve formazan in the cells. The absorbance was measured by microplate reader at 570 nm. Cell viability that was higher than 70% compared with control (control medium for water extracts and control solvent: 0.2% DMSO of final concentration for crude extracts, essential oils, and prednisolone) indicated that the activity of the tested samples was not due to cytotoxicity [34]. The percentage of cell viability was calculated by using the following equation:

$$\% \text{ cell viability} = \left[\frac{\text{OD sample}}{\text{OD control}}\right] \times 100, \quad (1)$$

where OD = optical density; OD sample = mean of sample ODs; OD control = mean of control ODs.

*2.6. Anti-Inflammatory Activities*

*2.6.1. Determination of Inhibition of LPS-Induced NO Production.* The determination of inhibitory effect of LPS-induced NO production was done in triplicate following the protocol of an established method [34]. Briefly, 100 $\mu$l/well of RAW 264.7 macrophages ($1 \times 10^5$ cells/well) were seeded in sterilized 96 well-plate and incubated for 24 h, and then the medium was removed and replaced with 100 $\mu$l/well of fresh medium containing LPS (2 ng/ml of final concentration). Various dilutions of samples were added (100 $\mu$l/well) and incubated for another 24 h. Subsequently, a 100 $\mu$l/well of supernatant was transferred into a nonsterilized 96 well-plate and added with Griess reagent (100 $\mu$l/well). The absorbance of the mixed solution was measured by microplate reader at 570 nm. The result of the tested sample was compared with that of prednisolone, a positive control. The percentage of the inhibition of LPS-induced NO production was calculated by using the following equation, and $IC_{50}$ values were calculated by using GraphPad Prism software (CA, USA):

$$\% \text{ inhibition} = \left[\frac{\text{OD control} - \text{OD sample}}{\text{OD control}}\right] \times 100, \quad (2)$$

where OD = optical density; OD control = mean of control ODs (+LPS) − mean of control ODs (−LPS); OD sample = mean of sample ODs (+LPS) − mean of sample ODs (−LPS).

*2.6.2. Determination of Inhibition of LPS-Induced TNF-α Production.* The inhibition of LPS-induced TNF-α production was determined by using Mouse TNF-α Quantikine ELISA test kit following the procedure in the manufacturer's manual [35]. Briefly, RAW 264.7 macrophages ($1 \times 10^5$ cells/well) were seeded in sterilized 96 well-plate (100 μl/well) and incubated for 24 h; then, the medium was removed and replaced with 100 μl/well of fresh medium containing LPS at 2 ng/ml final concentration. Various dilutions of samples were added (100 μl/well) and incubated for another 24 h. After incubation, the supernatant (50 μl/well) was transferred into 96 well-plate of ELISA kit and it was carried out according to the method in the manufacturer's manual [35]. The absorbance was measured at 450 nm by using the microplate reader. The result of the tested sample was compared with that of prednisolone, a positive control. The experiment was conducted in triplicate. The percentage of the inhibition of LPS-induced TNF-α production was calculated by using the following equation, and $IC_{50}$ values were calculated by using GraphPad Prism software (CA, USA):

$$\%\ \text{inhibition} = \left[\frac{\text{OD control} - \text{OD sample}}{\text{OD control}}\right] \times 100, \quad (3)$$

where OD = optical density; OD control = mean of control ODs (+LPS) − mean of control ODs (-LPS); OD sample = mean of sample ODs (+LPS) − mean of sample ODs (−LPS).

*2.6.3. Determination of Inhibition of LPS-Induced $PGE_2$ Production.* The inhibition of LPS-induced $PGE_2$ production was determined by using prostaglandin $E_2$ ELISA Kit-Monoclonal following the procedure in the manufacturer's manual [36]. Briefly, RAW 264.7 macrophages ($1 \times 10^5$ cells/well) were seeded in sterilized 96 well-plate (100 μl/well) and incubated for 24 h, and then the medium was removed and replaced with 100 μl/well of fresh medium containing LPS at 5 μg/ml final concentration. Various dilutions of samples were added (100 μl/well) and incubated for another 24 h. After incubation, the supernatant (50 μl/well) was transferred into 96 well-plate of ELISA kit and the procedure carried out according to the method in the manufacturer's manual [36]. The absorbance was measured at 412 nm by using the microplate reader. The result of tested sample was compared with that of prednisolone, a positive control. The experiment was conducted in triplicate. The percentage of the inhibition of LPS-induced $PGE_2$ production was calculated by using the following equation, and $IC_{50}$ values were calculated by using GraphPad Prism software (CA, USA):

$$\%\ \text{inhibition} = \left[\frac{\text{mean OD sample (+LPS)} - \text{mean OD control (+LPS)}}{\text{mean OD control (−LPS)} - \text{mean OD control (+LPS)}}\right] \times 100, \quad (4)$$

where OD = optical density.

*2.6.4. Chemical Composition Analysis by Gas Chromatography/Mass Spectrometry (GC/MS).* The chemical compositions of the active extracts were analyzed by using a Thermo Focus GC, Polaris *Q* with an autoinjector and a capillary column TG-5 slims (30 m × 0.25 mm × 0.25 μm) (Thermo Fisher Scientific). Column oven temperature was programmed using the initial temperature at 60°C and 5 min initial time and then heated at the rate of 5°C/min to 300°C and held for 5 min. The injector temperature was 200°C, helium (He) was used as the carrier gas with constant flow rate of 1.0 ml/min, and the injection volume was 2 μl (splitting ratio 1 : 50). The ionization energy was 70 eV. Mass spectrum of the GC/MS peak was detected by mass spectrometry and compared with library database of the National Institute of Standards and Technology (NIST 08, MD, USA) which matches the score for all compounds analyzed more than 870 would be selected [37]. Chemical composition analysis was carried out by the Herb and Thai Traditional Medicine Division, Thailand Science Park.

*2.6.5. Statistical Analysis.* Cell viability, percentage of the inhibition of LPS-induced NO, TNF-α and $PGE_2$ production, and $IC_{50}$ were presented as mean ± standard error of means (SEM). Comparison of means between control and treatment groups was done by one-way analysis of variance followed by Dunnett's multiple comparison test. Comparison of means in between independent treatment groups (2 groups) was analyzed by using unpaired *t* test. Comparison of means in multiple treatment groups (≥3 groups) was analyzed by using one-way analysis of variance followed by one-way ANOVA. The level of significant difference was $p < 0.05$.

## 3. Results

*3.1. Preparation of Extract.* The percentage yields of extracts and essential oils are shown in Figure 1. The pericarp showed the highest yield of extraction by three methods such as 50% ethanol, oil part, and water extract (16.47%, 14.30%, and 13.37%, respectively). The seed showed the highest yield of extraction by hexane and 95% ethanol.

*3.2. Determination of Cell Viability.* Cell viability after exposure to the various extracts of *Z. rhetsa* and prednisolone (Pred) (positive control) is presented in Figure 2(a) for inhibition of LPS-induced NO and TNF-α production and in Figure 2(b) for inhibition of LPS-induced $PGE_2$ production. The various extracts of *Z. rhetsa* and prednisolone

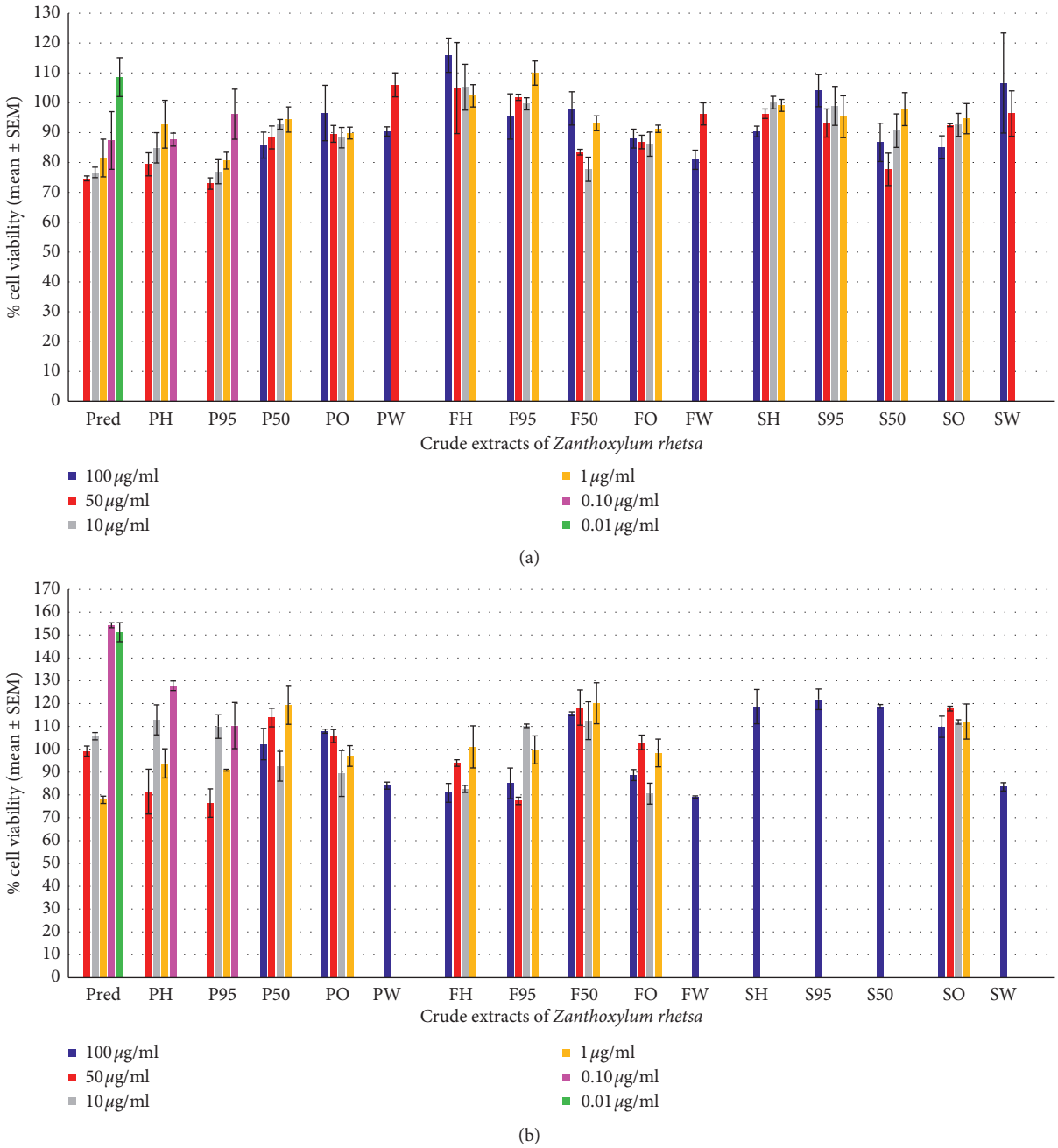


Figure 2: Cell viability of the various extracts of *Z. rhetsa* and prednisolone (Pred) at various concentrations ($n = 3$). (a) Viable cells for inhibition of LPS-induced NO and TNF-$\alpha$ production and (b) viable cells for inhibition of LPS-induced $PGE_2$ production.

(Pred) showed greater than 70% cell viability at all concentrations when they were tested.

*3.3. Determination of Inhibition of LPS-Induced NO Production.* Anti-inflammatory activity of the various extracts of *Z. rhetsa* via the inhibition of NO production by the induction of LPS in RAW 264.7 macrophages compared with prednisolone (positive control) is shown in Table 1.

PH and P95 at 50 $\mu$g/ml gave the highest %inhibition of NO production (97.15% ± 0.37 and 97.66% ± 1.12, respectively) while FH, F95, F50, and S50 at 100 $\mu$g/ml gave the highest %inhibition of NO production (91.55% ± 3.04, 93.36% ± 3.23, 82.62% ± 1.26, and 81.94% ± 2.79, respectively). These results were not significantly different from prednisolone at 50 $\mu$g/ml (96.82% ± 0.34) (Figure 3).

The extract results showed that PH and P95 had an inhibitory effect on NO production with $IC_{50}$ values as 11.99 ± 1.66 $\mu$g/ml and 15.33 ± 1.05 $\mu$g/ml, respectively. They were significantly different ($p$ value < 0.01 and $p$ value < 0.001, respectively) from prednisolone ($IC_{50} = 0.07 \pm 0.001$ $\mu$g/ml or 0.19 ± 0.001 $\mu$M). However, the pericarp

Table 1: Inhibitory effect and IC50 values of LPS-induced nitric oxide (NO) production in RAW264.7 macrophages of various *Zanthoxylum rhetsa* extracts.

| Part of plant | Extract and positive control | Code | Percentage of inhibition at various concentrations | | | | | | $IC_{50}$ ($\mu$g/ml) |
|---|---|---|---|---|---|---|---|---|---|
| | | | 100 $\mu$g/ml | 50 $\mu$g/ml | 10 $\mu$g/ml | 1 $\mu$g/ml | 0.10 $\mu$g/ml | 0.01 $\mu$g/ml | |
| Pericarp | Hexane | PH | — | 97.15 ± 0.37† | 38.47 ± 8.69 | −11.70 ± 3.94 | −12.38 ± 3.61 | — | 11.99 ± 1.66$^{**,a}$ |
| | 95% ethanol | P95 | — | 97.66 ± 1.12† | 24.46 ± 2.71 | −15.60 ± 3.58 | −15.65 ± 2.27 | — | 15.33 ± 1.05$^{***,a}$ |
| | 50% ethanol | P50 | 72.96 ± 1.04$^{**}$ | 35.78 ± 1.83 | 3.10 ± 2.50 | −1.68 ± 4.25 | — | — | 67.55 ± 2.22$^{***}$ |
| | Essential oil | PO | 47.75 ± 6.07$^{***}$ | 15.29 ± 3.43 | −14.02 ± 1.79 | −15.51 ± 2.41 | — | — | >100$^{***}$ |
| | Water | PW | 16.23 ± 4.25$^{***}$ | 8.75 ± 1.53 | — | — | — | — | >100$^{***}$ |
| Fruit | Hexane | FH | 91.55 ± 3.04† | 60.95 ± 0.84 | 8.69 ± 3.68 | −5.20 ± 4.00 | — | — | 39.81 ± 0.53$^{***,c}$ |
| | 95% ethanol | F95 | 93.36 ± 3.23† | 72.35 ± 4.53 | 11.88 ± 1.01 | −14.05 ± 6.36 | — | — | 29.42 ± 3.05$^{***,b}$ |
| | 50% ethanol | F50 | 82.62 ± 1.26† | 48.32 ± 0.51 | 4.02 ± 1.80 | −2.95 ± 0.87 | — | — | 51.63 ± 0.43$^{***}$ |
| | Essential oil | FO | 42.23 ± 10.48$^{***}$ | 15.29 ± 7.11 | −13.02 ± 3.56 | −11.27 ± 3.42 | — | — | >100$^{***}$ |
| | Water | FW | 20.97 ± 2.36$^{***}$ | 8.77 ± 1.86 | — | — | — | — | >100$^{***}$ |
| Seed | Hexane | SH | 45.92 ± 1.91$^{***}$ | 22.75 ± 1.03 | −1.17 ± 5.23 | −11.93 ± 8.02 | — | — | >100$^{***}$ |
| | 95% ethanol | S95 | 62.56 ± 0.98$^{***}$ | 35.63 ± 1.36 | 2.52 ± 7.24 | −3.69 ± 8.17 | — | — | 73.10 ± 1.55$^{***}$ |
| | 50% ethanol | S50 | 81.94 ± 2.79† | 46.88 ± 1.10 | 5.86 ± 3.64 | −10.45 ± 2.58 | — | — | 54.36 ± 1.21$^{***}$ |
| | Essential oil | SO | 76.57 ± 1.91$^{**}$ | 35.40 ± 3.07 | −6.95 ± 2.64 | −13.79 ± 2.95 | — | — | 65.34 ± 3.18$^{***}$ |
| | Water | SW | 18.83 ± 4.06$^{***}$ | 10.88 ± 3.05 | — | — | — | — | >100$^{***}$ |
| | Prednisolone | Pred | — | 96.82 ± 0.34† | 89.32 ± 0.31 | 81.49 ± 6.98 | 72.90 ± 2.26 | 5.16 ± 1.25 | 0.07 ± 0.001 (0.19 ± 0.001 $\mu$M) |

The results are shown as mean ± standard error of mean (SEM) ($n = 3$). LPS: lipopolysaccharide; $IC_{50}$: the half maximal inhibitory concentration. †: the %inhibition was not different significantly from prednisolone; $^{**}p$ value < 0.01, $^{***}p$ value < 0.001 compared with prednisolone as a positive control; [a]not significantly different between PH and P95; [b]significantly different ($p$ value < 0.01) between PH, P95, and F95; [c]significantly different ($p$ value < 0.05) between F95 and FH; (–) not tested.

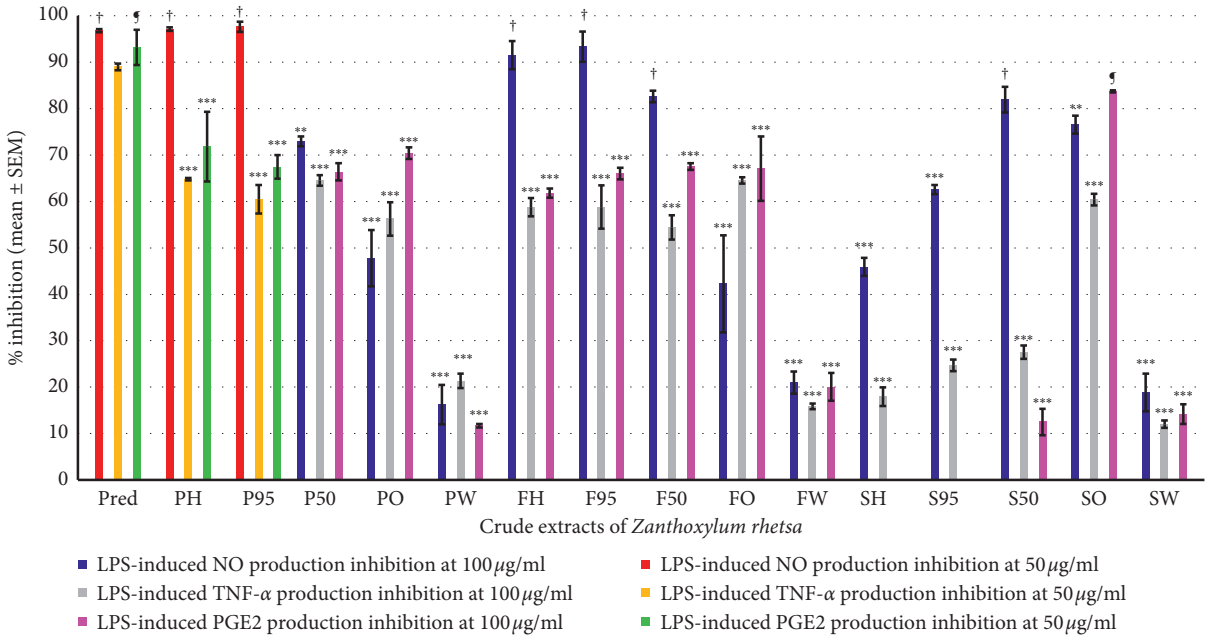


Figure 3: Percentage of the inhibition on LPS-induced NO, TNF-$\alpha$ and $PGE_2$ production in RAW264.7 macrophages of crude extracts of *Z. rhetsa* and prednisolone at 100 $\mu$g/ml and 50 $\mu$g/ml ($n = 3$). † and ¶: the %inhibition on LPS-induced NO and $PGE_2$ production, respectively, which were not different significantly from prednisolone. $^{**}p$ value < 0.01, $^{***}p$ value < 0.001 compared with prednisolone.

was macerated in hexane and 95% ethanol. The results demonstrated with the whole fruits macerated in hexane and 95% ethanol showed higher activity than decoction in water and maceration in 50% ethanol. For seeds which underwent water distillation, significant anti-inflamatory activity on NO production was demonstrated compared to other extraction means. The method of extraction revealed the most activity in the pericarp on the inhibition of NO production which was demonstrated with maceration in 95% ethanol and hexane. All water extracts (PW, FW, and SW), the essential oil of both percarp (PO) and fruits (FO), and the hexane extract of seed (SH) were not active ($IC_{50} > 100$ $\mu$g/ml).

*3.4. Determination of Inhibition of LPS-Induced TNF-$\alpha$ Production.* PH and P95 at 50 $\mu$g/ml gave the highest % inhibition of TNF-$\alpha$ production (64.79% ± 0.26 and 60.46% ± 3.07, respectively) which were significantly different ($p$-value < 0.001) from prednisolone at 50 $\mu$g/ml (89.00% ± 0.70) as the same as other extracts at 100 $\mu$g/ml which gave the highest %inhibition of TNF-$\alpha$ production which were significantly different ($p$ value < 0.001) from prednisolone at 50 $\mu$g/ml (Figure 3).

The results of $IC_{50}$ on inhibitory effect of TNF-$\alpha$ production are shown in Table 2. The pericarp which was macerated in hexane and 95% ethanol maintained inhibitory effects of NO production. PH and P95 were 36.08 ± 0.55 $\mu$g/ml and 34.90 ± 2.58 $\mu$g/ml, respectively, but were significantly different ($p$ value < 0.001) from prednisolone ($IC_{50} = 0.08 \pm 0.003$ $\mu$g/ml or 0.22 ± 0.003 $\mu$M). The $IC_{50}$ of SO (49.85 ± 4.29 $\mu$g/ml) was significantly different ($p$ value < 0.05) from PH and P95. All water extracts (PW, FW and SW) and all extracts of the seed (except for the essential oil of the seed: SO) did not have the activity on LPS-induced TNF-$\alpha$ production inhibition ($IC_{50} > 100$ $\mu$g/ml).

*3.5. Determination of Inhibition of LPS-Induced $PGE_2$ Production.* SO at 100 $\mu$g/ml gave the highest %inhibition of $PGE_2$ production (83.70% ± 0.22) which were not significantly different from prednisolone at 50 $\mu$g/ml (93.20% ± 3.80), while PH and P95 at 50 $\mu$g/ml gave the highest %inhibition of $PGE_2$ production (71.83% ± 7.51 and 67.44% ± 2.53, respectively) which were significantly different ($p$ value < 0.001) from prednisolone (Figure 3).

The results on inhibitory effect on $PGE_2$ production are shown in $IC_{50}$ values (Table 3); SO exhibited the highest anti-inflammatory effect on $PGE_2$ with $IC_{50}$ as 8.61 ± 2.23 $\mu$g/ml and was significantly different ($p$ value < 0.05) from prednisolone ($IC_{50} = 0.07 \pm 0.003$ $\mu$g/ml or 0.19 ± 0.003 $\mu$M). The inhibitory effect on PGE2 production of PH and P95 (IC50 = 13.72 ± 0.8 and 12.26 ± 0.71 $\mu$g/ml) were not significantly different with SO but they were significantly different with prednisolone. However, its pericarp demonstrated higher anti-inflammatory activity on the inhibitory effect of $PGE_2$ production than whole fruit and seed accept only seed oil (SO). All water extracts (PW, FW, and SW) and all extracts of the seed (except the essential oil of the seed: SO) did not have the activity on LPS-induced TNF-$\alpha$ production inhibition ($IC_{50} > 100$ $\mu$g/ml).

*3.6. Chemical Composition Analysis by Gas Chromatography/Mass Spectrometry (GC/MS).* PH and P95 showed the highest production inhibition of LPS-induced NO, TNF-$\alpha$, and $PGE_2$ while SO showed the highest production

Table 2: Inhibitory effect and IC50 values of LPS-induced tumor necrosis factor-$\alpha$ (TNF-$\alpha$) production in RAW264.7 macrophages of various *Zanthoxylum rhetsa* extracts.

| Part of plant | Extract and positive control | Code | Percentage of inhibition at various concentrations | | | | | | $IC_{50}$ ($\mu$g/ml) |
|---|---|---|---|---|---|---|---|---|---|
| | | | 100 $\mu$g/ml | 50 $\mu$g/ml | 10 $\mu$g/ml | 1 $\mu$g/ml | 0.10 $\mu$g/ml | 0.01 $\mu$g/ml | |
| Pericarp | Hexane | PH | — | $64.79 \pm 0.26^{***}$ | $22.26 \pm 1.19$ | $4.24 \pm 9.04$ | $-34.86 \pm 12.07$ | — | $36.08 \pm 0.55^{***,a}$ |
| | 95% ethanol | P95 | — | $60.46 \pm 3.07^{***}$ | $30.49 \pm 10.19$ | $15.66 \pm 9.88$ | $-16.05 \pm 6.31$ | — | $34.90 \pm 2.58^{***,a}$ |
| | 50% ethanol | P50 | $64.53 \pm 1.14^{***}$ | $45.57 \pm 2.06$ | $26.86 \pm 0.76$ | $17.22 \pm 1.13$ | — | — | $63.15 \pm 3.82^{***}$ |
| | Essential oil | PO | $56.24 \pm 3.61^{***}$ | $30.24 \pm 4.30$ | $1.03 \pm 0.48$ | $-7.37 \pm 7.59$ | — | — | $85.05 \pm 3.24^{***}$ |
| | Water | PW | $21.33 \pm 1.57^{***}$ | — | — | — | — | — | $>100^{***}$ |
| Fruit | Hexane | FH | $58.77 \pm 2.00^{***}$ | $26.36 \pm 0.55$ | $0.59 \pm 4.06$ | $-11.31 \pm 8.34$ | — | — | $88.11 \pm 1.85^{***}$ |
| | 95% ethanol | F95 | $58.81 \pm 4.68^{***}$ | $21.74 \pm 7.55$ | $-1.13 \pm 15.71$ | $-16.78 \pm 3.36$ | — | — | $91.12 \pm 3.42^{***}$ |
| | 50% ethanol | F50 | $54.42 \pm 2.59^{***}$ | $30.00 \pm 4.20$ | $16.52 \pm 4.33$ | $0.53 \pm 3.97$ | — | — | $93.54 \pm 4.02^{***}$ |
| | Essential oil | FO | $64.54 \pm 0.70^{***}$ | $43.48 \pm 3.46$ | $12.06 \pm 2.08$ | $-16.44 \pm 1.98$ | — | — | $73.22 \pm 3.85^{***}$ |
| | Water | FW | $15.88 \pm 0.60^{***}$ | — | — | — | — | — | $>100^{***}$ |
| Seed | Hexane | SH | $17.92 \pm 2.03^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | 95% ethanol | S95 | $24.69 \pm 1.25^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | 50% ethanol | S50 | $27.53 \pm 1.47^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | Essential oil | SO | $60.41 \pm 1.24^{***}$ | $50.23 \pm 1.13$ | $29.93 \pm 0.19$ | $20.31 \pm 0.80$ | — | — | $49.85 \pm 4.29^{***,b}$ |
| | Water | SW | $12.02 \pm 0.83^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | Prednisolone | Pred | — | $89.00 \pm 0.70$ | $77.18 \pm 0.69$ | $71.01 \pm 2.74$ | $56.05 \pm 0.08$ | $28.59 \pm 2.59$ | $0.08 \pm 0.003$ ($0.22 \pm 0.003$ $\mu$M) |

The results are shown as mean ± standard error of mean (SEM) ($n = 3$). LPS: lipopolysaccharide; $IC_{50}$: the half maximal inhibitory concentration. $^{***}p$ value < 0.001 compared with prednisolone as a positive control; [a]not different significantly statistic between PH and P95; [b]different significantly statistic ($p$ value < 0.05) between PH, P95, and SO; (–) not tested.

Table 3: Inhibitory effect and IC50 values of LPS-induced prostaglandin *E*2 (PGE2) production in RAW264.7 macrophages of various *Zanthoxylum rhetsa* extracts.

| Part of plant | Extract and positive control | Code | Percentage of inhibition at various concentrations | | | | | | $IC_{50}$ ($\mu$g/ml) |
|---|---|---|---|---|---|---|---|---|---|
| | | | 100 $\mu$g/ml | 50 $\mu$g/ml | 10 $\mu$g/ml | 1 $\mu$g/ml | 0.10 $\mu$g/ml | 0.01 $\mu$g/ml | |
| Pericarp | Hexane | PH | — | $71.83 \pm 7.51^{***}$ | $36.28 \pm 1.19$ | $3.28 \pm 0.52$ | $2.27 \pm 0.07$ | — | $13.72 \pm 0.81^{**,a}$ |
| | 95% ethanol | P95 | — | $67.44 \pm 2.53^{***}$ | $40.34 \pm 1.77$ | $2.79 \pm 1.62$ | $1.66 \pm 1.37$ | — | $12.26 \pm 0.71^{**,a}$ |
| | 50% ethanol | P50 | $66.39 \pm 1.83^{***}$ | $52.66 \pm 1.82$ | $36.74 \pm 0.55$ | $31.50 \pm 3.95$ | — | — | $42.30 \pm 1.20^{***,c}$ |
| | Essential oil | PO | $70.42 \pm 1.26^{***}$ | $63.32 \pm 1.44$ | $33.74 \pm 0.62$ | $18.84 \pm 0.77$ | — | — | $24.13 \pm 2.03^{***,b}$ |
| | Water | PW | $11.67 \pm 0.39^{***}$ | — | — | — | — | — | $>100^{***}$ |
| Fruit | Hexane | FH | $61.81 \pm 1.00^{***}$ | $20.93 \pm 2.47$ | $6.24 \pm 0.10$ | $4.68 \pm 0.002$ | — | — | $87.15 \pm 0.55^{***}$ |
| | 95% ethanol | F95 | $66.00 \pm 1.26^{***}$ | $52.35 \pm 0.28$ | $39.33 \pm 1.31$ | $37.64 \pm 1.55$ | — | — | $43.24 \pm 1.04^{***,c}$ |
| | 50% ethanol | F50 | $67.51 \pm 0.72^{***}$ | $40.90 \pm 2.76$ | $22.51 \pm 1.75$ | $13.23 \pm 0.95$ | — | — | $69.97 \pm 3.37^{***}$ |
| | Essential oil | FO | $67.07 \pm 6.94^{***}$ | $54.22 \pm 0.03$ | $31.35 \pm 4.12$ | $26.08 \pm 2.77$ | — | — | $40.85 \pm 1.99^{***,c}$ |
| | Water | FW | $20.06 \pm 3.00^{***}$ | — | — | — | — | — | $>100^{***}$ |
| Seed | Hexane | SH | $-9.09 \pm 0.28^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | 95% ethanol | S95 | $-15.24 \pm 4.16^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | 50% ethanol | S50 | $12.48 \pm 2.82^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | Essential oil | SO | $83.70 \pm 0.22$¶ | $69.15 \pm 2.48$ | $52.50 \pm 4.25$ | $31.86 \pm 6.71$ | — | — | $8.61 \pm 2.23^{*,a}$ |
| | Water | SW | $14.19 \pm 2.12^{***}$ | — | — | — | — | — | $>100^{***}$ |
| | Prednisolone | Pred | — | $93.20 \pm 3.80$¶ | $87.74 \pm 1.51$ | $89.16 \pm 1.82$ | $81.57 \pm 0.87$ | $52.93 \pm 1.35$ | $0.07 \pm 0.003$ ($0.19 \pm 0.003$ $\mu$M) |

The results are shown as mean ± standard error of mean (SEM) ($n = 3$). LPS: lipopolysaccharide; $IC_{50}$: the half maximal inhibitory concentration. ¶the %inhibition was not different significantly from prednisolone; $^{*}p$ value < 0.05, $^{**}p$ value < 0.01, $^{***}p$ value < 0.001 compared with prednisolone as a positive control; [a]not different significantly statistic between PH, P95, and SO; [b]different significantly statistic ($p$ value < 0.01) between PH, P95, and PO; [c]not different significantly statistic between P50, F95, and FO; (–) not tested.

inhibition of LPS-induced $PGE_2$. Therefore, PH, P95, and SO compositions were analyzed by GC/MS (Table 4) and presented GC/MS chromatogram of PH (Figure 4(a)), P95 (Figure 4(b)), and SO (Figure 4(c)). PH and P95 contained some chemical compounds as in SO; these were $\gamma$-terpinene (0.68%, 0.79%, and 4.91%, respectively), terpinen-4-ol (1.07%, 3.38%, and 35.13%, respectively), and terpinenyl acetate (1.57%, 1.62%, and 6.65%, respectively). PH and P95 shared similar composition but different in percentages. Bicyclo(3.1.1)heptane-2,3-diol,2,6,6-trimethyl or 2,3-pinanediol (11.28%), neryl acetate (7.65%), caryophyllene oxide (7.50%), spathulenol (6.65%), and cetanol (3.78%) are constituents in top 5 of PH. Bicyclo(3.1.1)heptane-2,3-diol,2,6,6-trimethyl or 2,3-pinanediol (19.82%), 2,3-camphanediol (5.87%), durenol (4.53%), piperitone oxide (4.46%), and spathulenol (4.39%) are in top 5 constituents of P95. Terpinen-4-ol was the major compound (35.13%) in SO; the next top 5 compounds were *p*-cymene (10.95%), terpinenyl acetate (6.65%), cuminol (5.60%), and limonene (5.48%).

## 4. Discussion

Pain may be acute or chronic depending on the duration of inflammatory response in the body [38, 39]. Inflammatory mechanisms assist in eliminating pathogens or stimulating wound healing in order to protect and restore cells and tissues into normal physiological functions [4]. Inflammatory responses, resulting in excessive release of inflammatory mediators and cytokines, can lead to tissue damage, chronic disease, and pain [9, 10]. Although medication can be effective for pain relief from inflammation, side effects from medication (i.e., steroid, NSAIDs, opioids, acetaminophen, etc) are significant. Herbal medicine is considered and utilized as a natural alternative for treatment of pain relief with potential to avoid some side-effects [40].

After cell and tissue damage, the body perceives pain. An acute inflammatory mechanism is induced by inflammatory mediators. $PGE_2$ is the one of chemical mediators: histamine, substance P, bradykinin, acetylcholine, leukotrienes, and prostaglandins, resulting in heat, redness, swelling, and nociception. $PGE_2$-induced vasodilation in the first step of acute inflammatory mechanism leads to increase microvascular permeability and induces pain by acting on peripheral sensory neurons [41]. The inhibition of $PGE_2$ production can be effective to reduce heat, redness, edema, and pain. In the present study, PH, P95, and SO of *Z. rhetsa* were the most potent groups ($IC_{50} < 20\,\mu$g/ml) which showed the greatest potency of LPS-induced $PGE_2$ production in RAW264.7 macrophages, while PO was the second most potent group ($IC_{50} < 30\,\mu$g/ml); P50, FO, and F95 was in the third group for potency ($IC_{50} < 50\,\mu$g/ml), and other extracts of *Z. rhetsa* were weak to inactive ($IC_{50} > 50\,\mu$g/ml). These results indicate that whole *Z. rhetsa* fruit should be separated into pericarp and seed, and the inhibitory effect of $PGE_2$ production is higher as a consequence. A previous study reported that an ethanolic extract from *Z. rhetsa* fruit (consisting of pericarp and seed) could inhibit COX-1 (90.80%) and COX-2 (94.40%) [21]. $PGE_2$ is one of the products derived from cyclooxygenase pathway [5]; therefore, PH, P95, and SO may reduce acute pain from an acute inflammatory mechanism through inhibition of COX-1 and COX-2 as well as the eicosanoid product $PGE_2$. Additionally, an *in vivo* study on a bioadhesive gel containing essential oil from the fruit could inhibit licking behavior, edema, and redness of the buccal cavity in rats [22] which was also due to reduced $PGE_2$ in acute inflammation. In clinical trials, a massage oil containing essential oil from fruit relieved pain in the calf muscle compared with carrier oil (placebo) in healthy volunteers after induction by standing and heel raise [23].

Though the previous study was done on whole fruit, our study has shown that *Z. rhetsa* pericarp and seed could perform the same pharmacological functions. This is an important finding since it would be the preparation of this herbal medicine from pericarp or seed not only whole fruit. Although the percentage yields of SO was less (0.27%) (Figure 1), the preparation of the distillation of the seed should be studied further in order to increase its yield. The present study also showed the highest %inhibition of $PGE_2$ production of SO at $100\,\mu$g/ml ($83.70\% \pm 0.22$) which was not significantly different from prednisolone at $50\,\mu$g/ml ($93.20\% \pm 3.80$) while PH and P95 at $50\,\mu$g/ml showed the highest %inhibition of $PGE_2$ production ($71.83\% \pm 7.51$ and $67.44\% \pm 2.53$, respectively) which was significantly different ($p$ value $< 0.001$) from prednisolone (Figure 3), whereas $IC_{50}$ values of PH and P95 were not significantly different from SO (Table 3). Our result was indicated; the preparation of analgesic and anti-inflammatory agents in acute inflammation from PH, P95, and SO was apparent. Whereas percentage yields of PH (5.89%) and P95 (13.10%) were higher than SO (0.27%) (Figure 1). Our study is also the first report on anti-inflammatory activity of PH, P95, and SO from *Z. rhetsa* by the inhibition of $PGE_2$ production in RAW 264.7 macrophages.

TNF-$\alpha$ is an inflammatory cytokine which releases in both acute and chronic inflammation; TNF-$\alpha$ induces pain and fever and plays a role in rheumatoid arthritis, osteoarthritis, and systemic lupus erythematosus [42]. Thai ethnomedicine use of *Z. rhetsa* was able to demonstrate the anti-inflammatory action in joints. *Z. rhetsa* fruit is used as an oil (named Pa-Ra-Ti-Tri) and ointment (named Bee-Pra-Sen) for treatment of muscle and joint inflammation in Thai ancient scripture (named Pra-O-Sod-Pra-Na-Rai) [18]. Additionally, our extracts were effective on TNF-$\alpha$ production by PH and P95, whereas SO was less active. Therefore, PH and P95 may relieve pain and inflammation via inhibition of TNF-$\alpha$ production. Our findings could be utilized to improve ethnomedicine use by developing a topical analgesic remedy from PH or P95 which demonstrates clinical utility.

PH and P95 also demonstrated the highest potency in the inhibition of NO production, which is a free radical synthesized by inducible nitric oxide synthase (iNOS) from macrophages with L-arginine as a precursor [8]. Increasing concentrations of nitrite in synovial fluid of joints are related to rheumatoid arthritis and osteoarthritis [43]. Therefore, PH and P95 could protect cells and tissues from injury due to

Table 4: Chemical profiles in the active extracts of *Zanthoxylum rhetsa* by GC/MS.

| No. | Chemical composition | The active extracts | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hexane extract from pericarp (PH) | | | 95% ethanolic extract from pericarp (P95) | | | Essential oil from seed (SO) | | |
| | | RT (min) | % Area | Match score | RT (min) | % Area | Match score | RT (min) | % Area | Match score |
| 1 | Sabinene | ND | ND | ND | ND | ND | ND | 8.56 | 1.91 | 889 |
| 2 | Alpha-phellandrene | ND | ND | ND | ND | ND | ND | 9.69 | 1.49 | 887 |
| 3 | Alpha-terpinene | ND | ND | ND | ND | ND | ND | 10.07 | 4.07 | 879 |
| 4 | *p*-Cymene | ND | ND | ND | ND | ND | ND | 10.32 | 10.95 | 882 |
| 5 | Limonene | ND | ND | ND | ND | ND | ND | 10.49 | 5.48 | 884 |
| 6 | Gamma-terpinene | 11.47 | 0.68 | 885 | 11.42 | 0.79 | 885 | 11.47 | 4.91 | 878 |
| 7 | Linalool oxide | ND | ND | ND | ND | ND | ND | 11.92 | 0.57 | 880 |
| 8 | Terpinolen | ND | ND | ND | ND | ND | ND | 12.38 | 1.43 | 885 |
| 9 | 2-Methyl-1-phenylpropene | ND | ND | ND | ND | ND | ND | 12.52 | 1.26 | 871 |
| 10 | Linalool | ND | ND | ND | ND | ND | ND | 12.84 | 1.63 | 877 |
| 11 | Terpinen-4-ol | 15.37 | 1.07 | 898 | 15.36 | 3.38 | 898 | 15.38 | 35.13 | 905 |
| 12 | Terpinenyl acetate | 15.81 | 1.57 | 892 | 15.80 | 1.62 | 892 | 15.82 | 6.65 | 901 |
| 13 | L-carvone | 17.24 | 1.07 | 874 | 17.22 | 1.01 | 874 | ND | ND | ND |
| 14 | Cuminal | ND | ND | ND | ND | ND | ND | 17.24 | 5.60 | 885 |
| 15 | *p*-Cymen-3-ol | ND | ND | ND | ND | ND | ND | 18.81 | 2.43 | 871 |
| 16 | Durenol | 18.82 | 3.71 | 880 | 18.79 | 4.53 | 880 | ND | ND | ND |
| 17 | Bicyclo(3.1.1)heptane-2,3-Diol, 2,6,6-trimethyl | 19.51 | 11.28 | 896 | 19.48 | 19.82 | 896 | ND | ND | ND |
| 18 | Limonene oxide | 20.11 | 2.07 | 873 | 20.09 | 4.03 | 873 | ND | ND | ND |
| 19 | Nerol | ND | ND | ND | ND | ND | ND | 20.93 | 0.87 | 878 |
| 20 | Neryl acetate | 20.95 | 7.65 | 872 | 20.92 | 4.28 | 872 | ND | ND | ND |
| 21 | 2,3-Camphanediol | 21.46 | 2.57 | 880 | 21.44 | 5.87 | 880 | ND | ND | ND |
| 22 | 7-Tetradecene | 21.76 | 1.81 | 873 | 21.73 | 0.91 | 873 | ND | ND | ND |
| 23 | Linoleic acid | ND | ND | ND | ND | ND | ND | 21.76 | 0.15 | 881 |
| 24 | Piperitone oxide | 22.04 | 3.16 | 872 | 22.00 | 4.46 | 872 | ND | ND | ND |
| 25 | Lauric acid | 25.60 | 0.87 | 879 | 25.53 | 1.01 | 879 | ND | ND | ND |
| 26 | Spathulenol | 25.97 | 6.65 | 890 | 25.94 | 4.39 | 890 | ND | ND | ND |
| 27 | Caryophyllene oxide | 26.09 | 7.50 | 889 | 26.07 | 3.66 | 889 | ND | ND | ND |
| 28 | Cetanol | 26.60 | 3.78 | 877 | 26.58 | 2.34 | 877 | ND | ND | ND |
| 29 | Ethyl linoleolate | 31.92 | 1.97 | 871 | 31.91 | 1.12 | 871 | ND | ND | ND |

GC/MS: gas chromatography/mass spectrometry; RT: retention time; min: minutes; ND: not detected.

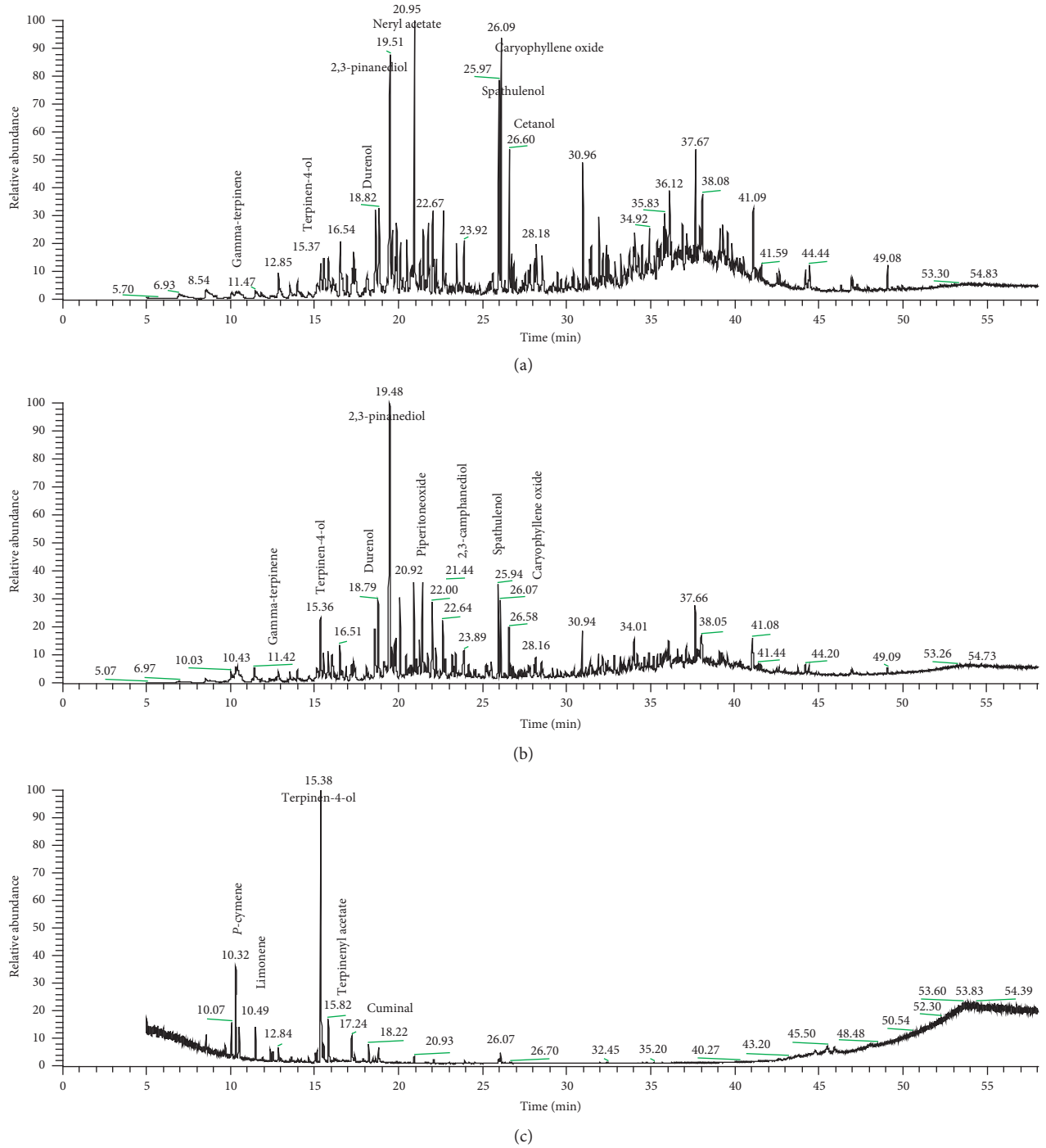


Figure 4: GC/MS chromatogram of active extracts of *Z. rhetsa*. (a) The hexane extract from pericarp (PH), (b) the 95% ethanolic extract from pericarp (P95), and (c) the essential oil from seed (SO).

NO. Our results also demonstrated the highest potency on the % inhibition of NO production by PH (97.15% ± 0.37) and P95 (97.66% ± 1.12) at 50 $\mu$g/ml which were not significantly different from prednisolone (96.82% ± 0.34) at 50 $\mu$g/ml (Figure 3); therefore, PH and P95 may relieve pain from inflammation. FH and F95 at 100 $\mu$g/ml gave the highest %inhibition of NO production (91.55% ± 3.04 and 93.36% ± 3.23, respectively) which were not significantly different from prednisolone (96.82% ± 0.34) at 50 $\mu$g/ml (Figure 3). These results indicate the potency of *Z. rhetsa* pericarp is higher than *Z. rhetsa* fruit for use as anti-inflammatory agent due to infection. Ethnomedicine use of *Z. rhetsa* fruit was able to demonstrate the anti-inflammatory action due to infection by a component in the Ma-Ha-Wat-Ta-Na remedy for the treatment of abscesses in Pra-O-Sod-Pra-Na-Rai ancient scripture [18].

Additionally, both NO and TNF-$\alpha$ have important roles in progressive osteoarthritis and rheumatoid arthritis [44–46]. TNF-$\alpha$ stimulates chondrocytes in cartilages to produce high levels of NO [44]. PH and P95 may reduce pain, swelling, and tissue damage through inhibiting NO and TNF-$\alpha$ production.

All extracts and essential oils from *Z. rhetsa* and prednisolone (positive control) showed greater than 70% cell viability at all concentrations (Figure 2) when tested, indicating that compounds were not cytotoxic to the cells, and their anti-inflammatory activity via the inhibition of LPS-induced NO, TNF-$\alpha$, and $PGE_2$ production in RAW 264.7 macrophages was not due to cytotoxicity [34].

Additionally, our extraction methods and results were supportive data for the Thai traditional preparation of drugs as the extraction by hexane is similar to preparation of the folk method called Hung-Nam-Mun (hot oil extract) [47]. These methods extensively use coconut oil for frying plant materials; however, a rancid odor because of coconut oil is apparent. Whereas maceration in hexane has no odor and a high extraction yield.

Some compounds analyzed in the active extracts, PH, P95, and SO (Table 4), had previously been reported to inhibit inflammatory mediators. Terpinen-4-ol was found in both PH (1.07%), P95 (3.38%), and SO (35.13%), and previous studies reported that terpinen-4-ol could inhibit TNF-$\alpha$, IL-1$\beta$, and $PGE_2$ production by LPS-activated human blood monocytes [31]. The second most abundant compounds in SO, *p*-cymene (10.95%), has previously been demonstrated to exhibit analgesic and anti-inflammatory properties in mice [48, 49]. Cuminaldehyde (5.60%) competitively inhibited the activity of 15-lipoxygenase, an enzyme involved in the production of inflammatory mediators such as leukotrienes, using lipoxygenase inhibition assay [50]. Limonene (5.48%) was found to be in the top five compounds of SO and also previously shown to suppress the production of LPS-induced NO, $PGE_2$, TNF-$\alpha$, IL-1$\beta$, and IL-6 [33]. The occurrence of these compounds in SO was the reasons for its in vitro activities. The compounds in PH and P95, spathulenol (6.65% and 4.39%, respectively) and caryophyllene oxide (7.50% and 3.66%, respectively), previously showed that they could inhibit the production of NO, IL-1$\beta$, and IL-6 [32]. The major component in PH and P95 was found to be bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl or 2,3-pinanediol (11.28% and 19.82%). This compound was earlier reported as an agent that increased microcirculation when applied topically [51]; thus, 2,3-pinanediol could contribute to pain relief when applied in PH and P95 on inflamed areas [52].

## 5. Conclusions

PH, P95, and SO of *Z. rhetsa* exerted pain-relieving and anti-inflammatory activity through inhibition of inflammatory mediators via LPS-induced NO, TNF-$\alpha$, and $PGE_2$ in RAW264.7 macrophages. Our study suggests that the PH and P95 extract fractions analyzed could provide constituents suitable for pain relief in chronic inflammation due to their activity on NO and TNF-$\alpha$ and SO inhibitory effect on $PGE_2$ production. Moreover, PH, P95, and SO contained terpinen-4-ol that was previously reported as an inhibitor of LPS-induced $PGE_2$ and TNF-$\alpha$. Other components in SO, *p*-cymene, and limonene have previously been reported for their in vitro and in vivo anti-inflammatory activity. Therefore, SO may have potential for the development into an analgesic and anti-inflammatory product for inflammation, and its active constituents should be further refine or studied further with additional reference standards where possible. A main active constituent determined in PH and P95 which enables inhibition of NO, TNF-$\alpha$, and $PGE_2$ appears to be 2,3-pinanediol which comprises almost 20% of P95. These findings are the first foundational supportive data for ethnomedical use as anti-inflammatory and analgesic herbal medicine treatment. *Z. rhetsa* pericarp that is macerated with hexane and 95% ethanol and seed essential oil are now being studied for analgesic product development in ongoing studies in our laboratories.

## Data Availability

The data used to support the findings of this study are available within the article.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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*Review Article*

# Clinical Evidence for the Effects of Manual Therapy on Cancer Pain: A Systematic Review and Meta-Analysis

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*Objective*. This meta-analysis aimed to evaluate the effects of manual therapy (MT) on cancer pain, so as to provide clinical evidence for application. *Methods*. Five English and Chinese databases were searched until February 29, 2020, for randomized controlled trials (RCTs) of MT for cancer pain. Articles published in the English or Chinese language were included. Two authors independently reviewed all articles and extracted the data, and any disagreements in the above process were discussed with other reviewers until the authors reached consensus. Review Manager 5.3 was used to calculate the effect size and 95% confidence intervals. This review was registered in PROSPERO, number CRD42020172053. *Results*. The intensity of cancer pain is our primary outcome measure, and compared with standard care, MT can significantly relieve the pain of patients with cancer (SMD, 0.63; 95% CI [0.18, 1.08]; $P = 0.006 < 0.01$); the effects of MT plus active activity were significantly different from AT alone (SMD, 0.79; 95% CI [0.28, 1.30]; $P = 0.002 < 0.01$); there was no statistical difference in the efficacy of MT and AT alone (SMD, -0.24; 95% CI [-1.09, 0.62]; $P = 0.53 > 0.05$). In other related symptoms, the above evidence cannot support that MT had a good effect on fatigue (SMD, 0.77; 95% CI [-0.09, 1.63]; $P = 0.08 > 0.05$), nausea (SMD, 0.24; 95% CI [-0.00, 0.48]; $P = 0.05$), anxiety (SMD, 0.76; 95 % CI [-0.32, 1.84]; $P = 0.17 > 0.05$), and depression (SMD, 0.67; 95 % CI [-0.28, 1.62]; $P = 0.17 > 0.05$); however, MT intervention can improve physical function ($n = 271$; SMD, 0.35; 95 % CI [-0.04, 0.74]; $P = 0.04 < 0.05$) and global well-being (SMD, 0.50; 95 % CI [0.02, 0.98]; $P = 0.04 < 0.05$). In addition, MT had a significant effect on pain relief (SMD, 0.52; 95% CI [0.03, 1.01]; $P = 0.04 < 0.05$) and improvement of physical function (SMD, 0.28; 95% CI [0.02, 0.53]; $P = 0.03 < 0.05$) even after a period of time after treatment. *Conclusion*. MT was an effective intervention, which may have immediate effect on cancer pain and may improve physical function and global well-being. In the view of follow-up effects, MT had good effects for the reduction of pain and the recovery of physical function. However, because of limitations, the seemingly promising results should be interpreted with caution.

## 1. Introduction

With the rapid development of modern medicine, the cure rate of many diseases has increased considerably, but tumor is still the main killer affecting human health [1]. Constantly updated anticancer methods and pharmacologic agents significantly increased the survival rate of patients with malignant tumors, but their quality of life (QoL) was not obviously improved [2]. Continuous pain not only affects the physical health of cancer patients but also leads to severe anxiety, depression, insomnia, fatigue, and other symptoms [3]. Though the three-step analgesic ladder for managing cancer pain provided by the World Health Organization has been widely used in clinical practice [4], insufficient ability of pain assessment [5, 6], adverse reactions of analgesic drugs, and rising health costs make government organizations have to seek nonpharmacologic treatment [7]. In the 2019 version of adult cancer pain guidelines [8], the National

Comprehensive Cancer Network (NCCN) has integrated a large number of nonpharmacologic therapies for cancer pain including massage, acupressure, acupuncture, psychological support, and exercise.

Manual therapy (MT), as complementary and alternative therapy, is skilled hand manipulations, including massage, chiropractic, osteopathic medicine, and others. MT was widely applied in many countries intended to improve soft tissue movement restriction, relieve pain, and promoting psychological well-being. Several studies reported that MT showed beneficial improvements in cancer-related pain and emotional problems [9, 10]. However, some reviews indicated that there was insufficient evidence on the effect of MT in relieving cancer pain [11]. The effects of MT for cancer pain and related symptoms are controversial.

In the past decade, many cancer sufferers used MT as a complementary therapy, to not only relieve pain but also promote psychological well-being [12]. And some high-quality randomized controlled trials (RCTs) were published [13–15], which paid more attention to the follow-up effects of MT for cancer patients. In China, MT, named Tuina, was widely applied to relieving pain in patients [16, 17], but the related studies did not get sufficient attentions in the previous reviews. In this study, more rigorous RCTs published in recent years and Chinese studies were included.

The current systematic review was aimed to examine the evidence on the effect of MT for cancer pain and related psychological well-being. The following questions are focused: (1) the effectiveness of MT in relieving cancer pain compared with standard care or other nonpharmacologic treatments; (2) the effects of MT in promoting psychological well-being by improving depression, anxiety, nausea, and others; (3) the follow-up effects of MT after the final treatment.

## 2. Methods

This study followed the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). This review was registered in PROSPERO (registration number: CRD42020172053).

*2.1. Search Strategy.* Five databases and reference lists were searched for RCTs published until February 29, 2020. English databases included PubMed and EMBASE, and Chinese databases included China National Knowledge Infrastructure, VIP Database for Chinese Technical Periodicals, and Wanfang Data. The search strategy consisted of four components: disease diagnosis ("neoplasia" OR "tumor" OR "cancer" OR "malignancy" OR "carcinoma"), clinical condition ("pain" OR "analgesia" OR "symptom relief"), intervention method ("massage" OR "Tuina" OR "zone therapy" OR "reflexology" OR "Rolfing" OR "bodywork" OR "manipulation" OR "chiropractic" OR "osteopathic" OR "physical therapy" OR "motion therapy"), and study type (randomized clinical trial). Appropriate keywords from MeSH headings were used in combination to develop searches by titles or abstracts to establish the eligibility of the studies. In addition, reference lists from all relevant articles were reviewed to make sure no RCTs were missed.

*2.2. Study Selection.* Two authors (C. Y. and L. K.) independently reviewed all articles by titles and abstracts, or full text if necessary, to evaluate their eligibility for inclusion. Articles published in the English or Chinese language were included if they were RCTs (excluding crossover design) investigating the association of MT with cancer pain. Patients with various types (breast cancer, lung cancer, colon cancer, etc.) of cancers were included without any restrictions on the age, gender, race, clinical status, and duration of cancer, but the baseline data must show no significant statistical difference between the experiment group (EG) and control group (CG) in each independent RCT. Eligible EG interventions were any technique of MT, including massage, osteotomy, chiropractic, acupressure, reflexology, trigger point therapy, and other physical therapies operated only by hands, compared to placebo, standard care, and any active treatments not related to MT as the CG. In addition, cancer pain in this article included pain directly caused by the development of cancer, chronic pain associated with cancer treatment, and acute pain after surgery.

The primary outcome of interest was pain, which can be measured by the Numerical Rating Scale (NRS), the Visual Analog Scale (VAS), the Brief Pain Inventory (BPI), and any other validated instrument. The secondary outcomes were QoL, functional improvement, negative emotions, and other cancer-related symptoms, for which no restriction set on the type of tool used in the studies as there were no universally accepted tools available. These symptoms had clear diagnostic criteria in related RCTs and were assessed by different scales, such as Short Form-36 questionnaire (SF-36), Functional Assessment of Cancer Therapy-Breast (FACT-B), Brief Fatigue Inventory (BFI), and others.

Studies were excluded if any of the following were identified: (1) the study only reported improvement rates and no other specific data to refer to; (2) the use of MT was not the single variable between intervention of the EG and CG, because other factors in the experiment may affect the results (e.g., music and acupuncture); (3) the intervention of CG contained MT, because the effects of MT could not be assessed; (4) the language of articles was neither English nor Chinese.

*2.3. Data Abstraction and Methodological Quality Assessment.* All the data were independently extracted by two reviewers (C. Y. and L. K.) in the mentioned databases according to predefined criteria, including first author, country of the study, year of the study, clinical situation, sample size, mean age of participants, duration of treatments, follow-up time, interventions of the EG and CG, outcome measures, and results. However, some less frequent outcome measures were not analyzed to better integrate the data ($n < 3$). Studies were excluded if they did not provide complete data needed to calculate the effect size. If there were multiple assessment time points, the time point of the last postintervention was chosen. To ensure rigor in the data abstraction process, the

two reviewers also independently checked all the records to minimize bias.

All RCTs included in the study were assessed independently by two reviewers according to the physiotherapy evidence database (PEDro) scale, which is reported to have excellent reliability for RCTs of the physiotherapy [18]. The risk of bias of each study was assessed through the generation of a score, which was calculated by 11 items in the PEDro scale. Each item is scored as either 1 or 0 according to whether the item is met or not. However, the first item is not used to calculate the final score, so the total score ranges from 0 to 10. A higher score indicates better methodological quality, but it has been reported a score of at least 6 is considered a high-quality study [19]. We contacted the study authors if more information is needed, and any disagreements in the above process were discussed with another reviewer (Y. C.) until the authors reached consensus.

*2.4. Data Synthesis and Statistical Analysis.* The meta-analysis was performed by calculating the effect size and 95% confidence intervals (CI) in the Review Manager 5.3. To assess the effects of MT on each outcome measure in the meta-analyses, we used the mean changes in outcomes between the end of final intervention and the baseline, which showed the difference between the EG and CG. The standardized mean difference (SMD) was used because different scales were applied to evaluate the outcomes, including NRS, VAS, and BPI. For studies with more than one CG, the results were split into comparisons between the EG and each CG. Statistical heterogeneity was assessed using $I^2$, and the value of which more than 50% was determined as a high level of heterogeneity. If $I^2 < 50\%$ in the results, we used a fixed effect model, otherwise a random effects model was used. A funnel plot was used to analyze bias.

## 3. Results

1662 records were searched from 5 databases, and reference lists were included. After removing duplicates and screening eligibility by title and abstract, fifty-one articles were included to be fully assessed. Thirty-four studies were excluded because they did not meet the inclusion criteria, and we selected 17 eligible articles. In the process of exclusion, the studies were excluded due to inappropriate intervention ($n = 2$) [20, 21], insufficient data ($n = 1$) [22], and inappropriate control method ($n = 1$) [23]. In the end, a total of 13 studies were included in our meta-analysis, including 11 English articles and 2 Chinese. The study selection process is summarized in Figure 1.

*3.1. Study Characteristics.* A total of 13 eligible studies, ranging from 2000 to 2019, evaluated the effects of MT on cancer pain. Eleven hundred participants, including 556 in the EG and 544 in the CG, with the mean age of 55.23, were conducted, respectively, in the USA, Germany, Italy, the UK, China, and other countries. Six studies focused on a specific kind of cancer (5 breast cancer [13–15, 24, 25] and 1 gastric or liver cancer [26]), and the remaining 7 RCTs involved any type of cancer in any stage [27–33].

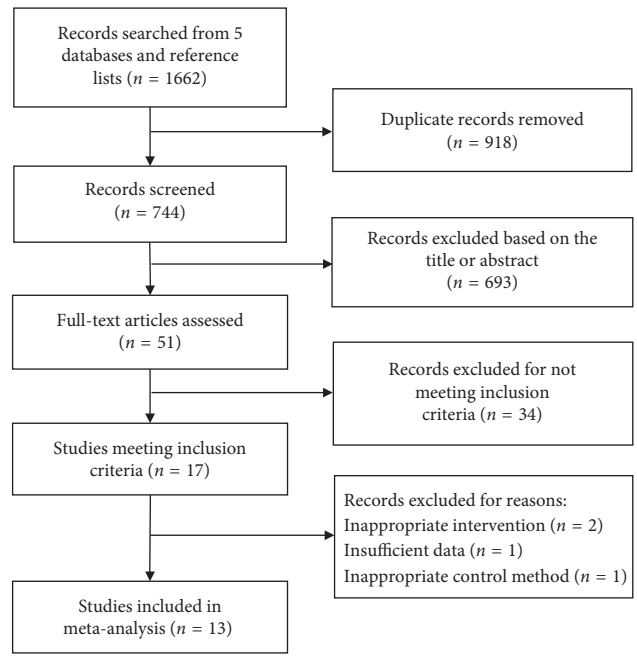


Figure 1: Study selection process.

Two studies [26, 33] observed the short-term effects of MT on cancer pain, and the treatment duration was 2 days and 3 days, respectively, so no follow-up was conducted. The other 11 studies [13–15, 24, 25, 27–32] lasted from 2 weeks to 3 months, 5 [13–15, 25, 29] of which involved follow-up for 6 weeks to 3 months. One study [29] claimed that the results of follow-up would be reported separately, but we did not find them. Of the 13 RCTs, one study [33] only observed cancer pain and other studies involved cancer-related side effects such as anxiety, depression, and fatigue.

MT in the studies mainly included massage therapy [15, 25, 27–29, 32], myofascial therapy [14, 24, 30], foot reflexology [13, 26], osteopathic manipulative treatment [31], and acupressure [33]. The control therapies contained standard care [13, 15, 26, 27, 30, 32, 33] and active therapies (AT) including physical therapy [14, 31], kinesiotherapy [24, 25], reading therapy [29], and psychological support [28]. The frequency of intervention was from twice a day to once a week, and each intervention method lasted from 10 to 50 minutes. When assessing the effects of MT, seven studies [13, 15, 26, 27, 30, 32, 33] compared the efficacy differences between MT and standard care. In addition, four studies [14, 24, 25, 31] compared MT plus AT and AT alone, and two studies [28, 29] compared MT with AT. The details of all studies are summarized in Tables 1 and 2.

*3.2. Methodological Quality.* The methodological quality of the studies was accessed in Table 3. According to the PEDro scale, all the studies received a score of 6 or more, indicating they were considered to be of high quality. However, five studies [15, 27, 31–33] were at the limit of the cutoff with scores of 6, and the reason for which was that although

Table 1: Characteristics of studies of manual therapy on cancer pain.

| First author, year, country | Clinical situation | Sample size | Mean age (year) | Duration | Followup | Outcome measures | |
|---|---|---|---|---|---|---|---|
| De Groef, 2017, Belgium [14] | Breast cancer (postoperation) | EG: 25<br>CG: 25 | EG: 55.3<br>CG: 53.1 | 3 months | 3 months | Primary | Pain (VAS↓) |
| | | | | | | Secondary | Physical function<br>QoL (SF-36↑)<br>Mental function |
| Listing, 2009, Germany [15] | Breast cancer (I-II, postoperation) | EG: 50<br>CG: 36 | EG: 57.6<br>CG: 61.4 | 5 weeks | 6 weeks | Primary | Pain (SF-8↑) |
| | | | | | | Secondary | Breast symptoms<br>QoL (EORTC QLQ-BR23↓)<br>Arm symptoms |
| Rangon, 2017, Brazil [24] | Breast cancer (postoperation) | EG: 10<br>CG: 10 | EG: 55.4<br>CG: 54.4 | 5 weeks | No follow-up | Primary | Pain (NRS↓) |
| | | | | | | Secondary | QoL (FACT-B↑)<br>QoL (FACT-B↑) |
| Wyatt, 2012, USA [13] | Breast cancer (III-IV, or I-II with metastasis or recurrence) | EG: 95<br>CG: 96 | EG: 55.3<br>CG: 57.3 | 5 weeks | 6 weeks | Primary | Physical function (SF-36↑) |
| | | | | | | Secondary | Pain (BPI↓)<br>Fatigue (BFI↓)<br>Depression (CES-D↓) |
| Tsay, 2008, Taiwan [26] | Gastric or liver cancer (postoperation within 24 hours) | EG: 30<br>CG: 30 | 59.8 ± 14.7 | 3 days | No follow-up | Primary | Pain (VAS↓) |
| | | | | | | Secondary | Anxiety (HADS↓) |
| Beurskens, 2007, Netherlands [25] | Breast cancer (postoperation) | EG: 15<br>CG: 15 | EG: 53.7<br>CG: 55.4 | 3 months | 3 months | Primary | Pain (VAS↓) |
| | | | | | | Secondary | Physical function (DASH↓)<br>QoL (SIP↓) |
| Wilkie, 2000, USA [27] | Any type of cancers (advanced) | EG: 15<br>CG: 14 | 64 | 2 weeks | No follow-up | Primary | Pain (VAS↓) |
| | | | | | | Secondary | QoL (Graham's QoL↑)<br>Global well-being |
| | | | | | | Primary | Anxiety (SAI↓) |
| Wilkinson, 2007, UK [28] | Any type of cancers (any stage) | EG: 144<br>CG: 144 | EG: 51.5<br>CG: 52.8 | 10 weeks | No follow-up | Primary | Depression (CES-D↓) |
| | | | | | | Secondary | Pain<br>Fatigue<br>QoL (EORTC QLQ-C30↓)<br>Nausea<br>Global well-being |
| Collinge, 2013, USA [29] | Any type of cancers (any stage) | EG: 47<br>CG: 50 | 54.7 | 4 weeks | 16 weeks | Primary | Anxiety (NRS↓) |
| | | | | | | Secondary | QoL (NRS↓)<br>Pain<br>Fatigue<br>Depression<br>Nausea |

Table 1: Continued.

| First author, year, country | Clinical situation | Sample size | Mean age (year) | Duration | Followup | Outcome measures | | |
|---|---|---|---|---|---|---|---|---|
| Pyszora, 2017, Poland [30] | Any type of cancers (advanced) | EG: 30<br>CG: 30 | EG: 72.4<br>CG: 69.3 | 2 weeks | No follow-up | Primary<br>Secondary | Fatigue (BFI↓)<br>Symptoms (ESAS↓) | Pain<br>Nausea<br>Depression<br>Anxiety<br>Global well-being |
| Arienti, 2018, Italy [31] | Any type of cancers (postoperation) | EG: 12<br>CG: 11 | EG: 76.5<br>CG: 76.5 | 4 weeks | No follow-up | Primary<br>Secondary | Pain (NRS↓)<br>QoL (EORTC QLQ-C30↓) | Global well-being<br>Financial difficulties<br>Summary score |
| Qian, 2018, China [32] | Any type of cancers (advanced) | EG: 68<br>CG: 68 | EG: 43.7<br>CG: 45.1 | 2 weeks | No follow-up | Primary<br>Secondary | Pain (NRS↓)<br>Anxiety (SAS↓)<br>Depression (SDS↓) | |
| Chen, 2019, China [33] | Any type of cancers (advanced) | EG: 15<br>CG: 15 | EG: 55.3<br>CG: 54.2 | 2 days | No follow-up | Primary | Pain (NRS↓) | |

EG: experiment group; CG: control group; VAS: Visual Analog Scale; QoL: quality of life; SF-36 : Short Form-36 Questionnaire; SF-8 : Short Form-8 Health Survey; EORTC QLQ: European Organization of Research and Treatment of Cancer QoL Questionnaire; NRS: Numerical Rating Scale; FACT-B: Functional Assessment of Cancer Therapy-Breast; BPI: Brief Pain Inventory; BFI: Brief Fatigue Inventory; CES-D: Center of Epidemiologic Studies-Depression Scale; HADS: Hospital Anxiety and Depression Scale; DASH: Disability of the Arm, Shoulder, and Hand Questionnaire; SIP: Sickness Impact Profile; SAI: State Anxiety Inventory; ESAS: Edmonton Symptom Assessment Scale; SAS: Self-Rating Anxiety Scale; SDS: Self-Rating Depression Scale. "↑" indicates that the higher the score was, the better the symptoms, and "↓" indicates that the lower the score was, the better the symptoms.

random assignment of patients was adopted, they did not use the appropriate method of assignment concealment. The most common defect was the lack of blinded therapists and blinded subjects, but this situation cannot be considered as a defect because it was difficult to implement in the study, and all the studies used blinded assessors. The highest score among the included studies was 9, which was for the only study that blinded the participants [14]. Most studies did not use intention-to-treat analysis because they cancelled the dropout data in the last results. In other items on a PEDro scale, the studies showed high methodological quality, including similarity between groups at baseline, less than 15% dropouts, between-group statistical comparisons, and point measures and variability data.

*3.3. Quantitative Data Synthesis.* Pain is the primary outcome to be analyzed, and we further analyzed the subgroups according to the different intervention methods of the CG. In addition, we also studied the effects of MT on other cancer-related side effects mentioned in RCTs included.

*3.3.1. The Effects of MT on Cancer Pain*

*(1) MT versus Standard Care.* As shown in Figure 2, 7 (54%) studies [13, 15, 26, 27, 30, 32, 33] took standard care as the CG to observe the effects of MT on cancer pain. In these RCTs, only one study [27] showed that there was no significant difference between the EG and CG. Our analysis demonstrated that, compared with standard care, MT can significantly relieve the pain of patients with cancer ($n = 592$, SMD, 0.63; 95% CI [0.18, 1.08]; $P = 0.006 < 0.01$). From the existing evidence, acupressure may have a good effect on cancer pain. Chen et al. [33] and Qian et al. [32] significantly reduced pain after pressing on acupoints related to symptoms.

*(2) MT plus AT versus AT.* In Figure 2, 4 (31%) RCTs [14, 24, 25, 31] observed whether the addition of MT would increase the efficacy of AT alone. One study [31] suggested that MT combined with passive mobility, active exercises, and walk could not significantly increase the efficacy. However, through integrating the results of 4 studies, we believed that the effects of MT plus AT can be significantly

Table 2: Intervention process of studies of manual therapy on cancer pain.

| First author, year, country | Intervention EG | Procedure EG | Intervention CG | Procedure CG |
|---|---|---|---|---|
| De Groef, 2017, Belgium [14] | 18 sessions of a standard physical therapy program of 30 min (week 1–8 twice a week, week 9–12 once a week). 12 sessions of myofascial therapy of 30 min (once a week) | Physical therapy: shoulder mobilization; pectoral muscle stretching; exercise therapy. Myofascial therapy: myofascial release on active myofascial trigger points at the upper body, on myofascial adhesions in the pectoral, axilla, cervical region, diaphragm, and scars | 18 sessions of a standard physical therapy program of 30 min (week 1–8 twice a week, week 9–12 once a week). 12 sessions of placebo treatment of 30 min (once a week) | Physical therapy: shoulder mobilization; pectoral muscle stretching; exercise therapy. Placebo: placements of hands up and down the upper body and arm on the affected side and lasted for 10–15 sec at one location |
| Listing, 2009, Germany [15] | 10 sessions of classical massage of 30 min (twice a week) | Classical massage: massage of the back, neck, and head, consisted of Swedish techniques such as stroking, kneading, frictions, pressing on the trigger points, stretching the neck and the lumbar spine area, and depressing the shoulders and the hip area | Medical routine | No intervention, standard care |
| Rangon, 2017, Brazil [24] | 10 sessions of kinesiotherapy of 50 min (twice a week). 10 sessions of ischemic compression of 90 sec (twice a week) | Kinesiotherapy: walk; neck active stretching, anterior and posterior chain of higher trunk; active mobilization of the cervical spine, upper limbs; relaxation exercises. Ischemic compression: pressing bilaterally on the myofascial trigger point centrally located in the upper trapezius muscle | 10 sessions of kinesiotherapy of 50 min (twice a week) | Kinesiotherapy: walk; neck active stretching, anterior and posterior chain of higher trunk; active mobilization of the cervical spine, upper limbs; relaxation exercises |
| Wyatt, 2012, USA [13] | 20 sessions of foot reflexology of 30 min (4 times a week) | Foot reflexology: stimulation of the nine essential breast cancer-specific reflexes with reflexology-specific deep thumb-walking pressure | Medical routine | No intervention, standard care |
| Tsay, 2008, Taiwan [26] | 3 sessions of foot reflexotherapy of 20 min (once a day) | Foot reflexology: massage of digestive reflex zones of upper and lower abdomen, liver, spleen, gall bladder, duodenal, intestine, and colon | Medical routine | No intervention, standard care |
| Beurskens, 2007, Netherlands [25] | 9 sessions of physiotherapy (once or twice a week for the first 3 weeks, and thereafter once a fortnight or less). 90 sessions of home exercises of 10 min (once a day) | Physiotherapy: soft tissue massage of the surgical scar; exercise for arm/shoulder, muscular strength, coordination, and improvement of general physical condition. Home exercises: exercises for the arm/shoulder | 90 sessions of home exercises of 10 min (once a day) | Home exercises: exercises for the arm/shoulder |
| Wilkie, 2000, USA [27] | 4 sessions of massage therapy of 30–45 min (twice a week) | Massage therapy: massage of head/back/gluteus muscles/four extremities, including effleurage, light petrissage, naive stroke, light compression, vibration, and tapotement | Medical routine | No intervention, standard care |

Table 2: Continued.

| First author, year, country | Intervention EG | Procedure EG | Intervention CG | Procedure CG |
|---|---|---|---|---|
| Wilkinson, 2007, UK [28] | 40 sessions of aromatherapy massage of 60 min (4 times a week) | Aromatherapy massage: massage with essential oils, massage strokes, timings, and overall style | Usual supportive care | Usual supportive care: psychological support services |
| Collinge, 2013, USA [29] | 12 sessions of massage therapy of 20 min (3 times a week) | Massage therapy: manual techniques for comfort and relaxation of head/neck/shoulders/back/feet/hands, including touching and acupressure | 12 sessions of reading therapy of 20 min (3 times a week) | Reading therapy: reading any literature such as poetry, fiction, nonfiction, and religious |
| Pyszora, 2017, Poland [30] | 6 sessions of physiotherapy programme of 30 min (3 times a week) | Physiotherapy programme: techniques of myofascial release and proprioceptive neuromuscular facilitation | Medical routine | No intervention, standard care |
| Arienti, 2018, Italy [31] | 4 sessions of osteopathic manipulative treatment of 45 min (once a week). 28 sessions of physiotherapy of 30 min (once a day) | Osteopathic manipulative treatment: dorsal/lumbar soft tissue/rib raising; back/abdominal myofascial release; cervical spine soft tissue/suboccipital decompression; sacroiliac myofascial release; strain-counterstain; and muscle energy technique. Physiotherapy: passive mobilization, active exercises, and walk | 28 sessions of physiotherapy of 30 min (once a day) | Physiotherapy: passive mobilization, active exercises, and walk |
| Qian, 2018, China [32] | 28 sessions of massage therapy of 10 min (twice a day) | Massage therapy: pressing and rubbing with oils on Baihui (DU20)/Shenmen (HT7) and other acupoints related to symptoms | Medical routine | No intervention, standard care |
| Chen, 2019, China [33] | 28 sessions of acupressure of 20 min (twice a day) | Acupressure: pressing on Neiguan (PC6) and Zusanli (ST36) | Medical routine | No intervention, standard care |

EG: experiment group; CG: control group.

different from AT alone ($n = 123$, SMD, 0.79; 95% CI [0.28, 1.30]; $P = 0.002 < 0.01$).

*(3) MT versus AT*. In Figure 2, the last 2 (15%) studies compared the effects of MT alone and AT on cancer pain. In one study, 288 patients were observed, and it was found that the effects of MT were worse than psychological support, although there was no statistical difference. Another study with a smaller sample size [29] suggested that MT was more effective in relieving pain than reading therapy. Through comprehensive analysis, we believed that the existing evidence cannot demonstrate the efficacy of MT is better than AT alone ($n = 385$, SMD, −0.24; 95% CI [−1.09, 0.62]; $P = 0.53 > 0.05$).

### 3.3.2. The Effects of MT on Other Related Symptoms

*(1) Fatigue*. As shown in Figure 3, fatigue, another major physical symptom related to cancer besides pain, was proved in 3 studies [13, 28, 29] that there was no significant difference between the EG and CG on relieving symptom. Pyszora et al. [30] suggested that compared with standard care, MT can significantly improve the fatigue symptom of patients, which was different from other RCTs. In addition, one study [15] which was not included in the analysis due to the lack of definite data indicated that the results were also in favor of EG but did not reach statistical significance. Therefore, we cannot support statistically that MT intervention can have a better effect ($n = 636$, SMD, 0.77; 95% CI [−0.09, 1.63]; $P = 0.08 > 0.05$), although MT may have a positive result from the clinical evidence.

*(2) Nausea*. In Figure 3, 3 studies [28–30] observed the improvement of MT on nausea. Only one study [29] reported that MT could improve symptoms statistically better than reading therapy. In another study [30], the intervention of MT was less effective than standard care, although the difference was not statistically significant. Therefore, based on the existing evidence, we can only consider that MT

Table 3: PEDro scale of methodological quality assessment for the studies.

| First author | Eligibility criteria | Random allocation | Concealed allocation | Similar at baseline | Subjects blinded | Therapists blinded | Assessors blinded | <15% dropouts | Intention-to-treat analysis | Between-group comparisons | Point measures and variability data | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| De Groef [14] | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 9 |
| Listing [15] | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 6 |
| Rangon [24] | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 7 |
| Wyatt [13] | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 7 |
| Tsay [26] | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 7 |
| Beurskens [25] | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 7 |
| Wilkie [27] | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 6 |
| Collinge [29] | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 7 |
| Wilkinson [28] | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 7 |
| Pyszora [30] | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 7 |
| Arienti [31] | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 6 |
| Qian [32] | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 6 |
| Chen [33] | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 6 |

PEDro: physiotherapy evidence database. Criteria (2–11) were used to calculate the total PEDro score. Each criterion was scored as either 1 or 0 according to whether the criteria was met or not, respectively.

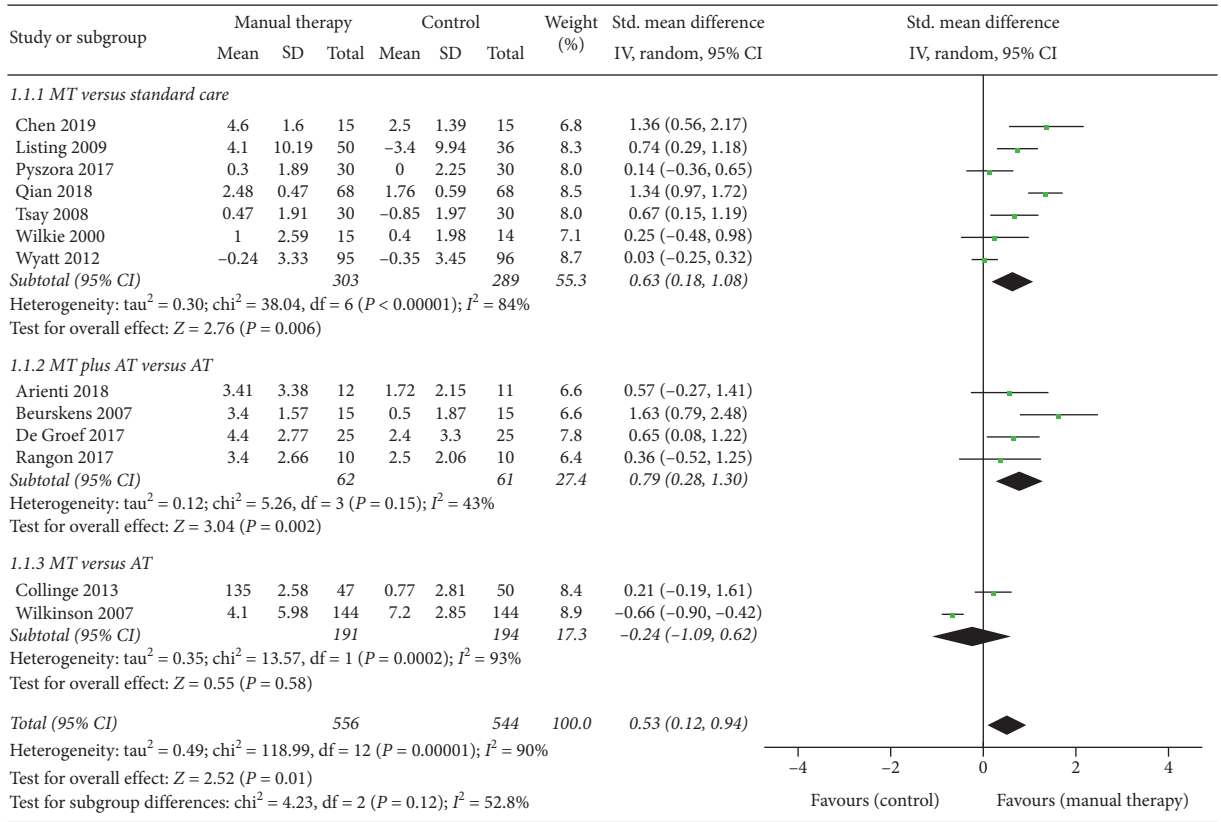


Figure 2: Forest plot of the effects of manual therapy on cancer pain.

intervention may have a weak advantage in nausea ($n = 445$, SMD, 0.24; 95% CI [−0.00, 0.48]; $P = 0.05$).

*(3) Anxiety*. In Figure 3, one study [32] suggested that MT can significantly improve the anxiety of cancer patients. However, in most (80%) studies [26, 28–30], although MT may relieve anxiety, the difference was not statistically significant. Therefore, the above evidence cannot support that MT had a good effect on anxiety ($n = 641$, SMD, 0.76; 95% CI [−0.32, 1.84]; $P = 0.17 > 0.05$).

*(4) Depression*. In Figure 3, it was demonstrated that MT had no effect on depression ($n = 772$, SMD, 0.67; 95% CI [−0.28, 1.62]; $P = 0.17 > 0.05$), although all the studies reported that MT intervention may have a positive effect on symptoms. Only Qian et al. [32] demonstrated that acupressure could significantly relieve depression of patients compared with standard care.

*(5) Global Well-Being*. In Figure 3, 3 studies [27, 28, 30] suggested that MT could improve global well-being compared with the CG, although 2 of them [27, 28] did not have statistical difference. However, in the study of Pyszora et al. [30], the researchers achieved significant effects through MT intervention. In addition, another study [31] added MT on the basis of exercise therapy, making the curative effect worse, although the difference was not statistically significant. Therefore, our evidence supported that MT intervention can increase the global well-being of cancer patients ($n = 400$, SMD, 0.50; 95% CI [0.02, 0.98]; $P = 0.04 < 0.05$).

*3.3.3. The Effects of MT on Physical Function.* As shown in Figure 4, the effects of MT on the recovery of physical function were observed in 3 studies [13, 14, 25]. Although the results of De Groef et al. [14] supported the positive effects of MT, there was no statistical difference. Wyatt et al. [13] studied the effects of foot reflexology on physical function, demonstrated that MT can improve the function, while standard care can reduce the original function, and the difference was statistically significant. In another study, Beurskens et al. [25] added massage to home exercise, which significantly improved the efficacy. Therefore, our evidence supported that MT got better effect in improving physical function ($n = 271$, SMD, 0.35; 95% CI [−0.04, 0.74]; $P = 0.04 < 0.05$).

*3.3.4. The Follow-Up Effects of MT.* As shown in Figure 5, our study was the first time to analyze the follow-up effects of MT. During the follow-up, the main observation was pain and physical function, and the time was 6 weeks to 3 months. The changes of pain after the end of intervention were observed in four studies [13–15, 25]. The results suggested that the effects of MT on cancer pain were still beneficial in the follow-up, although the results of 2 studies [13, 14] were

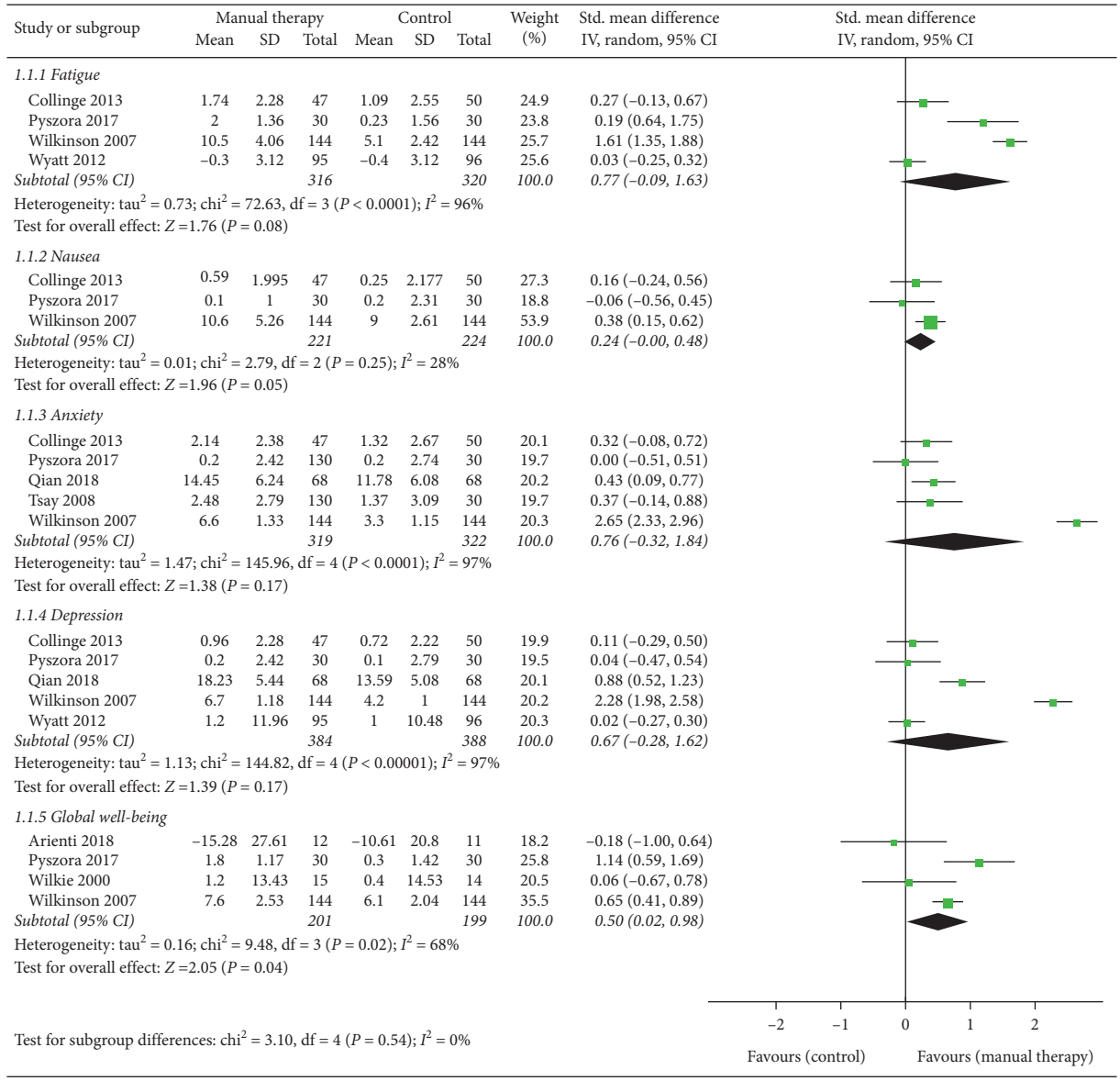


Figure 3: Forest plot of the effects of manual therapy on other related symptoms.

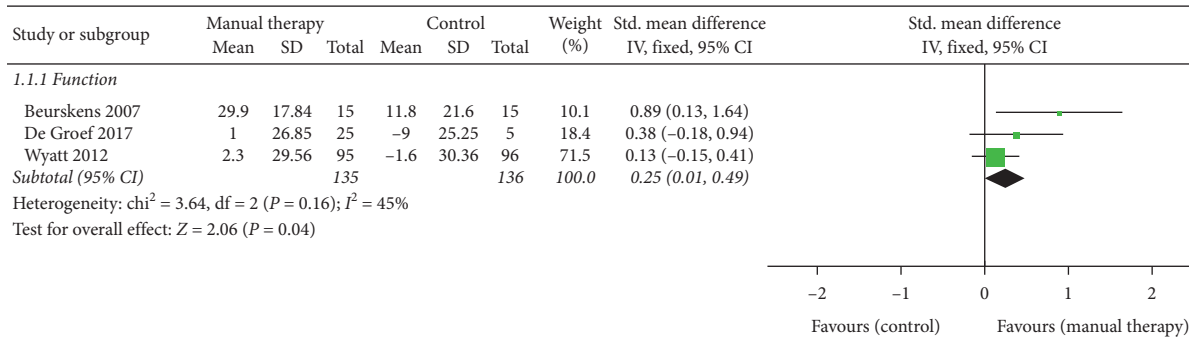


Figure 4: Forest plot of the effects of manual therapy on physical function.

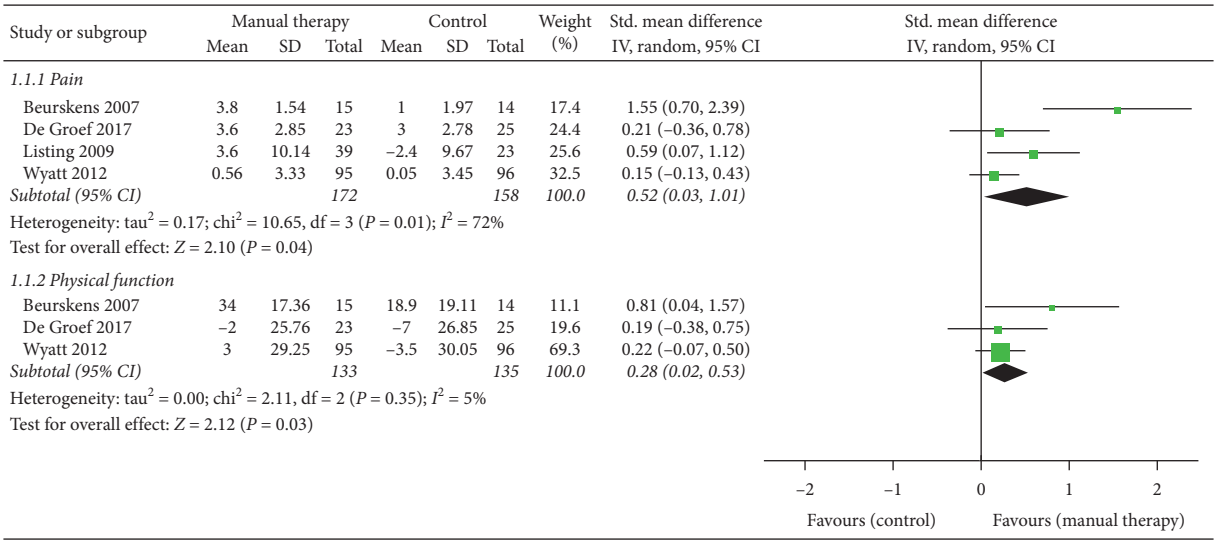


Figure 5: Forest plot of the follow-up effects of manual therapy.

not statistically significant. In the study of Listing et al. [15], standard care alone exacerbated pain during follow-up, while MT significantly improved the situation. Therefore, current evidence demonstrated that MT had a significant effect on pain relief even after a period of time after treatment ($n = 330$, SMD, 0.52; 95% CI [0.03, 1.01]; $P = 0.04 < 0.05$).

The follow-up effects of MT on physical function were studied in 3 studies [13, 14, 25]. MT had a positive effect on functional recovery, but the result of one study [14] was not statistically significant. In the other 2 studies [13, 25], MT had significantly improved the function in the evaluation after the intervention, and the improvement was maintained until the follow-up. Therefore, based on the above research studies, we can consider that MT was conducive to the future recovery of physical function, and the results were statistically significant ($n = 268$, SMD, 0.28; 95% CI [0.02, 0.53]; $P = 0.03 < 0.05$).

*3.3.5. Risk of Bias.* In Figure 6, no obvious asymmetric distribution of the trials was observed in a funnel plot, but the possibility of publication bias cannot be ruled out. The small sample size may be a major reason for this possible bias.

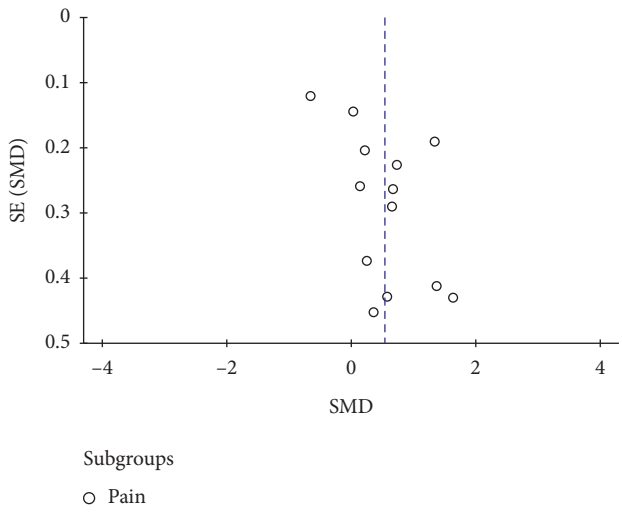


Figure 6: Funnel plot of the risk of bias.

## 4. Discussion

The meta-analysis included 13 RCTs with 1100 patients, to provide an updated synthesis of the current evidence for the effects of MT on cancer pain and fill some research gaps remained to be addressed in the past. We not only brought into more studies and pay attention to follow-up after intervention, but also searched Chinese RCTs, which were not analyzed in the past.

*4.1. Analysis of Research Results.* Consistent with results of a few systematic reviews and meta-analyses in the past [34, 35], evidence was found of an association between MT and reduction in pain, especially when compared with standard care or MT is added on the basis of AT. However, more previous studies supported that MT had no beneficial effects on cancer pain relief. In the Cochrane Database of Systematic Reviews, Shin et al. [36] held the view that there was a lack of evidence for effectiveness of massage on symptom relief of cancer, and most studies were unreliable and did not report key outcomes. In addition, Boyd et al. [12] and Pan et al. [37] suggested that the evidence was not enough to demonstrate that massage can reduce cancer pain compared to no treatment or sham control. There were also a few studies suggested the seemingly promising results should be interpreted with caution because of limitations [11].

Based on the discussion of pain, we further analyzed the other cancer-related side effects involved in RCTs included, which are often accompanied by pain. In other symptoms, the effects of MT were mainly reflected in improving global

well-being, which was never been mentioned before. Consistent with the results of previous studies [36, 37], MT showed no significant difference compared with the CG in anxiety and depression, but we found that MT had a weak advantage in relieving nausea. In addition, fatigue, which was supported to be improved by MT in previous studies [36], did not reflex beneficial effect in our study, but it was reported positive results in most RCTs [15, 28–30].

We believed that the above differences were mainly due to the following reasons. First of all, this study analyzed massage, osteology, chiropractic, acupressure, reflexology, trigger point therapy, and any other physical therapy operated only by hands as MT, instead of studying them separately as in the past [9, 11], because it was impossible to completely separate them in practical application. Secondly, we included a part of Chinese RCTs [32, 33], although few Chinese studies in this field were of high quality. In addition, we did not include crossover design experiments because it may affect the final results, especially when we need to consider follow-up results. Finally, studies that did not involve pain were not included in the analysis of MT on other related symptoms, because pain relief was the main purpose of this study.

The current review, to our knowledge, was the first to evaluate the follow-up effects of MT for cancer pain. According to our results, it can be demonstrated that MT could significantly reduce cancer pain and improve physical function at the end of the intervention, and the significant effects can even last until the follow-up. Therefore, the intervention of MT not only had immediate and sustained analgesic effect but also brought great benefits for the future physical function recovery.

In addition, QoL is a frequent outcome in the RCTs, which we mentioned in Table 3. However, the results of QoL cannot be analyzed comprehensively because there was no unified standard for it. De Groef et al. [14] took QoL as a comprehensive index to evaluate physical function and mental function, and we made another analysis on the former. In the study of Listing et al. [15], QoL was divided into breast symptoms and arm symptoms, and the former was significantly improved after MT intervention. Rangon et al. [24] and Wyatt et al. [13] used FACT-B scale to evaluate QoL, so their research perspective was similar. European Organization of Research and Treatment of Cancer QoL Questionnaire (EORTC QLQ) was also applied to access QoL, but researchers chose different subprojects according to different research purposes [28, 31]. Beurskens et al. [25] used Sickness Impact Profile (SIP) to evaluate the physical disability of patients to demonstrate the significant improvement of QoL. In addition, Collinge and his colleagues [29] used the simple NRS with pain, fatigue, depression, and nausea as the subprojects of QoL, which were analyzed in our study. Therefore, the direct analysis of QoL in the past research was worth further discussion [38].

*4.2. Limitations of the Review.* There were several limitations in our study. First of all, substantial heterogeneity was observed which mainly owed to the application of different kinds of measurement methods. As our primary outcome measure, pain was evaluated by VAS or NRS in 9 (69%) studies [14, 24–27, 29, 31–33]. The two scales were similar because they measured pain on a score of 1 to 10, and the higher the score, the more severe the pain. Although the VAS score in some studies [14, 26] was 1–100, it was also converted to the maximum of 10 in our review, which did not affect the final result. However, some special scales were used in other research studies. In the study of Listing et al. [15], Short Form-8 Health (SF-8) survey was applied to evaluate pain, which contained one item for each of the eight concepts of the SF-36, and the pain of patients decreased with increasing scores. Other scales were not described in details here, but obviously different measurement methods were the main reason for high heterogeneity. Cancer, on the other hand, is so complex that various measures may describe different dimensions of symptoms, and pain types and treatments can also affect outcomes. In addition, although we thought it was difficult to distinguish different MT methods completely, the difference of MT technology, frequency, duration, and treatment courses may affect the heterogeneity. Therefore, more studies were needed to fully assess how these factors play a role in heterogeneity.

Secondly, the RCTs we included may have possible selection bias. The results did not change when we restricted the analysis to the methodological quality through the PEDro scale. Almost in all studies, both performance and response biases were possible since the lack of blinded therapists and blinded subjects, which were hard to avoid in practical treatment. However, the small sample size and low methodological quality of some of the included studies was worthy of our attention. In addition, although our funnel plot did not show obvious asymmetric distribution, it is difficult to interpret the results of publication bias due to such a small subset of studies. Thus, larger sample sizes and carefully planned designs are required for future analysis, as well as better monitoring of selected parameters.

Finally, the analysis of cancer-related side effects in this study was based on patients suffering from pain. Because pain was our primary outcome measure, a large number of RCTs that did not involve pain were not included in our study, which meant that many studies on fatigue, depression, anxiety, and other symptoms were ignored. On one hand, we mainly focused on cancer pain, so other studies that had nothing to do with pain were excluded; on the other hand, almost all studies of cancer pain involved other accompanying symptoms, which we cannot ignore. Moreover, there were few studies involving each symptom, because of many kinds of other cancer-related symptoms in different RCTs, which may have an impact on the results. In addition, only a few RCTs including follow-up studies were found, and the follow-up results in which were often similar to those after the intervention. Therefore, the current evidence only demonstrated that MT could improve cancer-related side effects on the basis of reducing cancer pain, but whether MT had long-term effects needed further study.

## 5. Conclusion

The current evidence demonstrated that MT was an effective intervention, which may have immediate effect on cancer

pain and may improve physical function and global well-being. Although MT achieved positive results on fatigue, nausea, anxiety, and depression, the current evidence cannot support the effectiveness. In the view of follow-up effects, MT had good effects for the reduction of pain and the recovery of physical function. However, because of limitations, the seemingly promising results should be interpreted with caution.

It was necessary to establish relevant standards for the intervention of MT on cancer pain, such as frequency, duration, and course of treatment, to ensure the normalization of treatment. In addition, for some important outcome indicators, it was better to use a unified measurement method and added special scales if necessary.

## Data Availability

The data used to support the findings of this study are available from public databases, and more details also can be obtained from the corresponding author on request. He can be reached at fm-tn0510@shutcm.edu.cn.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Chongjie Yao and Yanbin Cheng contributed equally to this work.

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*Research Article*

# Efficacy and Safety of Sahastara Remedy Extract Capsule in Primary Knee Osteoarthritis: A Randomized Double-Blinded Active-Controlled Trial

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Sahastara (SHT) remedy is a Thai traditional medicine described in the Thai National List of Essential Medicine (NLEM) for the relief of muscle pain. The purpose of this study was to investigate the efficacy and safety of SHT remedy extract capsule for treating primary OA. A phase 2, double-blind, randomized, and controlled trial study was used to determine the clinical efficacy and safety of SHT in comparison with diclofenac for the treatment of knee OA. The outcome of reduce pain was measured from VAS, 100 meter time walk, and the WOMAC score of day 14 and day 28 which should reduce significantly when compared with day 0 and should be equal with or better than diclofenac. Blood pressure and blood chemistry values at day 14 and day 28 did not change when compared with day 0. The results found that SHT remedy ethanolic extract capsule can reduce all OA knee scores at day 14 and day 28 significantly when compared with day 0 and also no significant difference with diclofenac ($P > 0.05$). The SHT also showed safety values on blood pressure and blood chemistry. The SHT was observed that it had no serious side effect. The results of this study are the first report of using the SHT ethanolic extract capsule in the treatment of primary osteoarthritis of the knee. It can be recommended as an anti-inflammatory herbal drug for reducing pain in knee osteoarthritis patients.

## 1. Introduction

Osteoarthritis (OA) is the most common joint disease which occurs more frequently in males before the age of 45 and more frequently in females after 55 years of age. Osteoarthritis commonly occurs in the hands, feet, spine, and large weight-bearing joints, such as hips and knees. OA with no known cause is referred to as primary osteoarthritis. When the cause is known, it is referred to as secondary osteoarthritis. Cartilage changes when the dynamics of biology, biochemistry, and bone structure around it changed. In the elderly, involvement of synovial fluid is most common. The pain is an important clinical complaint [1]. The oral and topical NSAIDs are most commonly used to relieve pain in OA knee patients. Diclofenac was used as an active control which showed a superior effect for osteoarthritis [2]. Not only NSIADs were used for OA treatment but alternative medicine was also commonly used, including acupuncture, Thai massage, sesame oil, yellow oil, and herbal medicine [3–5].

Sahastara (SHT) is a remedy in the National Essential Drugs List (2011) which comprises 21 species of herbs as shown in Table 1. Previous research studies supported anti-inflammatory activity of SHT remedy. $IC_{50}$ values of SHT ethanolic extract on nitric oxide and COX-2 inhibition were 2.81 $\mu$g/ml and 16.97 $\mu$g/ml, respectively [6]. SHT ethanolic extract exerted similar activity to NSAIDs. It was also found that SHT extract had no mutagenic effects on *Salmonella typhimurium* TA98 and TA100 isolated from patients [7]. The effectiveness and safety of the treatment with 3000 mg/day of powder SHT for primary osteoarthritic knee, as compared to 75 mg/day of diclofenac over 28 days, indicated that the SHT powder formulation was as effective as diclofenac. Furthermore, the SHT powder remedy was not toxic to the liver and kidney [8].

A previous study showed that SHT ethanolic extract exerted a higher inhibitory effect on nitric oxide (NO) release in activated murine macrophages cell line (RAW 264.7) than indomethacin ($IC_{50}$ = 2.81 and 20.32 $\mu$g/mL, respectively) [6]. SHT ethanolic extract and its components were also tested for anti-inflammation by their inhibitory effects on LPS-stimulated $PGE_2$ release in RAW 264.7 cells [9]. The SHT extract and indomethacin (positive control) had $IC_{50}$ values of 16.97 and 1.00 $\mu$g/mL, respectively. *P. nigrum* [10, 11] and *P. retrofractum* [12, 13] which are the main ingredients (33.42%) of SHT preparation also showed high anti-inflammatory activity on $PGE_2$ release with $IC_{50}$ of 17.70 and 23.08 $\mu$g/mL, respectively [6]. Piperine, a main component of both *Piper* species, exerted anti-inflammatory activity on human OA chondrocytes by inhibiting the IL-1$\beta$ which induces the production of $PGE_2$ and NO [14, 15]. Moreover, previous clinical studies on both SHT remedy (in powder form) and diclofenac had shown a significant reduction of VAS pain scores [8, 16]. The ethanolic extract of SHT in the capsule form was also studied on healthy volunteers with 300 and 600 mg/day for 28 days and showed safety on the kidney, liver, and blood [17]. The pharmacokinetic of piperine was also studied with oral administration dosing of 100–200 mg of SHT extract to healthy Thai volunteers and demonstrated that piperine was detectable in plasma for at least 48 hours with evidence of enterohepatic recirculation [18]. Thus, the objective of this research was to investigate the clinical efficacy of SHT ethanolic extract capsule compared with the conventional anti-inflammatory drug, diclofenac, in osteoarthritic knee patients and its safety over the four weeks of continuous treatment. This is the first study to report on clinical efficacy and safety of SHT ethanolic extract capsule in OA knee patients in order to determine whether further development of the SHT ethanolic extract capsule as medicine for OA is warranted. This study was also performed to determine whether the SHT ethanolic extract capsules can reduce the number of SHT powder capsules currently taken by the patients and hence increase the efficacy of the treatment.

## 2. Materials and Methods

*2.1. Research Design.* The research was a randomized, double-blind, and controlled trial (phase 2) designed to study the clinical efficacy and safety of the SHT remedy ethanolic extract capsule in comparison to diclofenac for the treatment of knee OA at Thammasat University Hospital, Pathum Thani Province, Thailand. It was approved by the Medical Ethics Committee of the Faculty of Medicine, Thammasat University, which was accepted by the Thai FDA (MTU-EC-TM-2-116_2/59). It was also registered at the Clinical Trials.gov (NCT04591795).

*2.2. Subjects.* Sixty-six outpatients from Department of Orthopedics, Thammasat University Hospital, between 40 and 70 years of age were recruited for this study. They were diagnosed with primary osteoarthritis of the knee according to the American College of Rheumatology's clinical and radiological criteria [19]. The patients who were included in this study had no plan for knee arthroplasty in three months, and their minimum pain symptom severity was ensured by the visual analogue scale (VAS) score of at least 20 mm from 100 mm. The exclusion criteria were patients rated with severe knee osteoarthritis (grade 4, based on the Kellgren and Lawrence radiographic system) [20], patients with serious medical conditions such as uncontrolled hypertension (BP > 140/90 mmHg), severe GI disease, congestive heart disease, liver and renal dysfunction, and obese patients with body mass index (BMI) more than 32 kg/m$^2$.

*2.3. Sample Size.* The estimate of the sample size was based on the previous phase 2 clinical trial of the SHT powder capsule in the treatment of primary osteoarthritis [8]. The lower level of average VAS pain scores from the previous clinical trial was 44.1 (S.D. = 23.5) for SHT powder capsules and 31.8 (S.D. = 22.8) for diclofenac, based on statistical calculations with Stata computer programs at the power of 80 and type I (alpha) error of 0.05. The calculated number of volunteers for each of the two treatments was 28, thus requiring 56 people for both treatments. Allowing for a dropout of 10%, i.e., six additional volunteers, a minimum of 62 recruits would be required to satisfy the clinical trial protocol. The following equation was used in the calculation:

$$n_1 = \frac{(z_1 - (\alpha/2) + z_1 - \beta)^2 \left[\sigma_1^2 + \left(\sigma_2^2/r\right)\right]}{\Delta^2},$$
$$r = \frac{n_2}{n_1}, \quad (1)$$
$$\Delta = \mu_1 - \mu_2.$$

In this experiment, a total of 75 volunteers were screened and 66 were chosen, with 33 each allocated to SHT and diclofenac treatment groups.

*2.4. Drug Preparation.* The SHT remedy was prepared according to Thai National List of Essential Medicine 2011 (NLEM). The proportion of medicinal plant ingredients and their sources is shown in Table 1. All plants were cleaned immediately of extraneous materials, dried at 50°C, weighed according to the SHT recipe, mixed together, and ground to

Table 1: Medicinal plants in Sahastara remedy formulation (for 1,000 g of powder drug).

| Thai name | Scientific name | Specimen voucher | Part used | Weight (g) | Source |
|---|---|---|---|---|---|
| Prik-Thai | *Piper nigrum* Linn. | SKP146161401 | Fruit | 240 | Chanthaburi, Thailand |
| Jet-Ta-Mul-Plerng-Dang | *Plumbago indica* Linn. | SKP148160901 | Root | 224 | Laos |
| Sa-Mhor-Thai | *Terminalia chebula* Retz. | SKP049200301 | Fruit | 104 | Sa Kaeo, Thailand |
| Dee-Plee | *Piper retrofractum* Vahl. | SKP146160301 | Fruit | 96 | Chanthaburi, Thailand |
| Tong-Tank | *Baliospermum montanum* Muell. A. | SKP121021301 | Root | 80 | Kanchanaburi, Thailand |
| Wan-Nam | *Acorus calamus* Linn. | SKP015010301 | Rhizome | 88 | Nonthaburi, Thailand |
| Has-Sa-Khun-Tade | *Kleinhovia hospita* Linn. | SKP183110801 | Root | 48 | Kanchanaburi, Thailand |
| Ka-Ra-Boon | *Cinnamomum camphora* Linn. | SKP096030301 | — | 14 | China |
| Dok-Chan | *Myristica fragrans* Houtt. | SKP121130601 | Aril of seed | 13 | China |
| Luk-Chan | *Myristica fragrans* Houtt. | SKP121130601 | Seed | 12 | China |
| Tien-Dang | *Lepidium sativum* Linn. | SKP057121901 | Seed | 11 | India |
| Tien-Ta-Tuk-Ka-Tan | *Anethum graveolens* Linn. | SKP199010701 | Fruit | 10 | India |
| Ma-Ha-Hing | *Ferula asafoetida* Linn. | SKP199060101 | Resin | 10 | India |
| Tien-Sut-Ta-but | *Pimpinella anisum* Linn. | SKP199160101 | Fruit | 9 | China |
| Tien-Khao | *Cuminum cyminum* Linn. | SKP199030301 | Fruit | 8 | India |
| Jing-Jor | *Merremia vitifolia* | SKP054132201 | Root | 8 | Kanchanaburi, Thailand |
| Tien-Dum | *Nigella sativa* Linn. | SKP160141901 | Seed | 7 | China |
| Kote-Kag-Kra | *Anacyclus pyrethrum* (L.) DC. | SKP051011601 | Root | 6 | China |
| Kote-Ka-Mao | *Atractylodes lancea* (Thunb) DC. | SKP051011201 | Rhizome | 5 | China |
| Kote-Kan-Prao | *Picrorhiza kurroa* Benth. | SKP177161101 | Root | 4 | India |
| Kote-Pung-Pla | *Terminalia chebula* Retz. (Gall) | SKP019200301 | Gall | 3 | India |

pass through sieve number 80. The ground SHT was macerated at room temperature with 95% ethanol (L/S = 2 : 1) for three days and filtered through a Whatman number 1 filter paper. The residue was further macerated with the same solvent two more times. The extracts were combined and concentrated with a rotary evaporator (Rotavapor R-205, Buchi, Switzerland). Quality control of all plant ingredients and SHT preparation was based on Thai herbal pharmacopoeia (appearance, chemical fingerprints, disintegration, microbial contamination, heavy metal contamination, and loss on drying). Piperine in SHT powder and extract was analyzed by high-performance chromatography (HPLC) to ensure that the piperine content in the SHT ethanolic extract was not less than 19 mg/g. The dry extract was pulverized and filled in 500 mg capsules with excipient (the concentration of SHT extract is 100 mg per capsule). Diclofenac sodium, 25 mg enteric-coated tablets (Voltaren®, Novartis), for oral administration, was filled into 500 mg capsules (1 tablet of diclofenac sodium per capsule). Lactose monohydrate as a placebo was also prepared in capsules (500 mg per capsule). Omeprazole (20 mg) (Miracid®, Berlin) was used as an open labeled medication.

*2.5. Procedures.* The patients who met the inclusion criteria were informed, signed a consent form, and divided randomly into two groups of treatment, using a computer-generated program, by an individual who did not have any contact with the investigators. The patients received a randomized number sequentially from a secret random list. Treatment assignment was also concealed from all investigators involved in the trial. Each patient received the same appearance of the treatment that contains treatment code, which was opened only in medical emergency condition. The masking was opened after data analysis. In the clinical trial, demographic data, clinical signs and symptoms, laboratory tests (complete blood count, fasting blood sugar, lipid profile, the liver function test, the renal function test, and urine analysis), visual analogue scale (VAS) for pain, 100 meter walk times, and the Western Ontario and McMaster Universities (WOMAC) index scores were collected on the first visit for baseline data and after receiving the treatment on days 14 and 28 [21, 22].

*2.6. Drug Administration.* The patients were divided randomly into two groups. Those in group 1 received the SHT extract 300 mg/day (one capsule of 100 mg SHT ethanolic extract three times daily before meals). This was the equivalent dose of SHT remedy that was indicated in NLEM at 3,000 mg/day of SHT remedy in the powder form. The patients in group 2 received diclofenac sodium 75 mg/day (1 capsule of 25 mg diclofenac three times daily after meals) [22]. In addition, the patients in both groups received 20 mg of omeprazole, 1 capsule before breakfast for the prevention of adverse gastrointestinal effects [8].

Clinical assessment: the treatments were completed in 28 days with the clinical and laboratory assessments at the $14^{th}$ and $28^{th}$ days. After treatments, a global assessment was performed on the patients at the last visit. The clinical efficacy was evaluated from the VAS pain scores, the 100 meter walk times, the WOMAC index scores (ranging from 0 to 96) at days 0, 14, and 28, and the global assessment on a 0–4 Likert scale (0, none; 4, excellent). The clinical efficacy and safety outcomes were evaluated by clinical examinations and laboratory analyses.

Toxicity of the drugs was considered in excluding patients following the USFDA guidance for the industry in the toxicity grading scale such as creatinine more than 1.7 mg/

dL, BUN more than 26 mg/dL AST, and ALT more than 2.5x upper limit of normal (ULN) or ALP more than 2.0x ULN.

*2.7. Statistical Analysis.* The changes in mean values from baseline to days 14 and 28 for each group were analyzed by the repeated measured analysis of variance (ANOVA) or Friedman's test. The mean values between the two groups were compared using Student's $t$ test or the Mann–Whitney $U$ test. The comparison of the global assessments of the two groups was analyzed by the chi-square test, with $P < 0.05$ indicating a significant difference. SPSS software (version 16.0, USA) was used to analyze the data.

## 3. Results

In this clinical trial, 75 volunteers were screened and 66 patients were chosen and divided into two groups of 33 people each, one group receiving SHT ethanolic extract capsules and the other receiving diclofenac capsules. Both groups showed no significant differences in their baseline characteristics and the radiographic grading (Table 2). Originally, 66 patients were chosen for the study, but only 63 (95.45%) patients completed the study (32 in the SHT group and 31 in the diclofenac group). Three patients dropped out at the follow-up visits with unrelated intervention and nonserious reasons (two patients missed appointments, and one patient suffered from a traumatic wrist injury that required surgery) (Figure 1).

*3.1. Efficacy of SHT Extract Capsule in Primary Osteoarthritis Patients.* The clinical trial results showed that the SHT ethanolic extract capsule had the ability to relieve pain, reduce inflammation, improve daily life activities, decrease the WOMAC scores, and 100 meter walk times (Table 3). Comparison of all criteria (WOMAC scores on all physical index and 100 meters walk) between SHT ethanolic extract capsule and diclofenac showed no significant difference ($P > 0.05$), but the VAS score of SHT at day 28 was higher than diclofenac and was significantly different ($P = 0.01$).

*3.2. Evaluation of the Overall Treatment (Global Assessment).* To evaluate the overall effectiveness of the OA treatment with SHT extract in comparison with diclofenac (global assessment), the symptoms were monitored at day 28, using Likert scale scores, and the results showed no significant difference between the two groups (Table 4).

*3.3. Safety Evaluation.* The safety data of SHT and diclofenac groups shown as the results of blood pressure, blood chemistry of liver function, renal function, and the other blood chemistry such as complete blood count, fasting blood sugar, and lipid profile are shown in Table 5.

The systolic and diastolic blood pressure measurements were not significantly different from the baseline nor between the two groups. All patients were examined for blood urine nitrogen (BUN) and creatinine in renal function tests and for aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in liver function tests at days 14 and 28. The renal function was similar in both groups when compared with their baseline values. The liver function tests of the SHT group showed no effect in the AST, ALT, and ALP values. In contrast, the AST and ALT values increased significantly after the treatment with diclofenac. The results of other blood chemistry such as complete blood count, fasting blood sugar, and lipid profile showed no significant difference between the two drug groups.

*3.4. Adverse Effect.* The adverse effects found in both groups were abdominal discomfort, 31.25% among the subjects in the SHT group and 22.58% in the diclofenac group. However, the common side effect of the SHT extract-treated group was belching, while that in the diclofenac-treated group was gastric pain (Table 6).

## 4. Discussion

The clinical efficacy of SHT might be described from the previous studies of five anti-inflammatory markers in Sahastara remedy such as piperine, ellagic acid, gallic acid, $\beta$-asarone, and plumbagin. Piperine as the main component of the SHT remedy was found to reduce the percentages of iNOS, elastin, and smooth muscle cells actin and was shown to decrease blood pressure from the third week of treatment [23]. The ethanolic extract of pepper showed anti-inflammatory activity in vitro, and piperine as the isolated compound was tested on interleukin 1 $\beta$- (IL-1$\beta$-) stimulated fibroblast-like synoviocytes derived from the OA knee patients [15]. Ellagic acid as an active compound could reduce inflammation on rat paw edema at 4 hours [24]. Gallic acid (10 $\mu$M) isolated from *Terminalia chebula* Retz. blocked TNF-$\alpha$ and IL-6 secretion induced by PMA plus and A23187 in HMC-1 cells (68.4% inhibition in TNF-$\alpha$ and 49.8% inhibition in IL-6) [25]. $\beta$-Asarone from *Acorus calamus* at the dose of 50 $\mu$M inhibited the production of proinflammatory cytokines, especially IL-1$\beta$ and TNF-$\alpha$ ($P < 0.05$). [26]. Plumbagin from *Plumbago indica* inhibited inflammatory cytokine (IL-2, IL-4, IL-6, and IFN-$\gamma$) production in activated lymphocytes which was stimulated with con. A (5 $\mu$g/ml) following which plumbagin was added at the indicated times, and the cells were cultured for 24 h at 37°C ($P < 0.01$) [27]. Asafoetida as a component in SHT has ever been reported that it showed good efficacy on reducing inflammation from dental plaque and gingivitis compared with chlorhexidine gluconate [28].

Diclofenac is an NSAID used as a control group. It is slightly better than a placebo over all of the specific treatments and increased with greater baseline pain severity ($P < 0.001$) [2].

Five parameters were measured in this study: the level of knee pain by VAS (mm) after walk on 100 meters, the 100 meters walking time, and the 3 WOMAC index scores (pain index, stiffness index, and physical function index). The levels of knee pain in the SHT extract group ($n = 32$) were reduced significantly

Table 2: Baseline characteristics of volunteers.

| Characteristics | Sahastara ($n = 32$) | Diclofenac ($n = 31$) | $p$ value* |
|---|---|---|---|
| Female, no. (%) | 29 (87.87) | 30 (90.90) | $0.68^{C}$ |
| Age, mean (SD) | 58.48 (6.98) | 59.00 (6.1) | $0.75^{t}$ |
| Weight (Kg), mean (SD) | 64.15 (7.6) | 60.53 (9.9) | $0.10^{t}$ |
| Height (cm), mean (SD) | 1.52 (0.08) | 1.54 (0.06) | $0.23^{m}$ |
| BMI, mean (SD) | 27.55 (3.45) | 25.25 (4.13) | $0.19^{m}$ |
| Walk 100 meters | | | |
| Knee pain level VAS (mm), mean (SD) | 57.84 (2.19) | 54.61 (14.50) | $0.28^{t}$ |
| 100 meters walk duration. (m/s), mean (SD) | 138.86 (40.38) | 130.41 (5.96) | $0.44^{m}$ |
| WOMAC index, mean (SD) | | | |
| Pain index | 8.33 (2.23) | 8.15 (4.21) | $0.23^{m}$ |
| Stiffness index | 1.72 (1.79) | 1.71 (1.95) | $0.83^{m}$ |
| Physical index | 32.66 (8.77) | 30.93 (10.83) | $0.44^{m}$ |
| Total score | 42.72 (10.53) | 40.81 (15.27) | $0.27^{m}$ |
| Kellgren and Lawrence X-ray grade | | | |
| Grade 1 | 7 (21.88) | 4 (12.90) | |
| Grade 2 | 7 (21.88) | 15 (48.39) | $0.10^{C}$ |
| Grade 3 | 18 (56.25) | 12 (38.70) | |
| Total | 32 (100) | 31 (100) | |

*Statistical analysis. $^{t}$Independent sample $t$ test; $^{c}$chi-square test; $^{m}$ = Mann–Whitney $U$ test.

($p < 0.01$) in both follow-ups ($14^{th}$ and $28^{th}$ days). However, the levels of knee pain in the diclofenac-treated group ($n = 31$) was significant after 28 days of treatment ($p < 0.01$). Comparison between SHT extract and diclofenac groups on the VAS score which evaluated the pain score after 100 meters walk showed that they were not significantly different at day 0 and day 14 but differed significantly at day 28 ($p < 0.01$). The results showed that SHT extract surpassed diclofenac in reducing knee pain.

The 100 meters walk durations were reduced in both follow-ups ($14^{th}$ and $28^{th}$ days). However, in the SHT extract group ($n = 32$), the reduction was significant after 28 days of treatment ($p < 0.05$). Comparison between SHT and diclofenac groups showed that they were not significantly different at days 0, 14, and 28 ($p > 0.05$).

The WOMAC index scores (pain index, stiffness index, and physical function index) in both groups were reduced significantly ($p < 0.01$) in both follow-ups ($14^{th}$ and $28^{th}$ days). Comparison between SHT and diclofenac groups showed that they were not significantly different at days 0, 14, and 28. These results were consistent with those in the study on knee pain using SHT powder capsules which showed significant reduction in the mean VAS pain scores sooner than diclofenac [18]. Another study on office syndrome using SHT powder capsules in comparison with diclofenac also showed equal effectiveness in reducing pain [16]. However, this study found that SHT extract showed good reduction in stiffness index, significantly different with day 0 and no significant difference with diclofenac. SHT extract showed different results when compared with SHT powder; in that, SHT extract showed better efficacy on stiffness reduction than SHT powder [5]. In addition, the total stiffness score of SHT extract decreased on days 14 and 28, equal to that of diclofenac. However, the previous result of SHT powder showed a significant difference of the total score only on day 28. These results indicated that SHT extract was more effective on OA knee patients than SHT powder. Both extract and powder of SHT showed no significant difference when compared with diclofenac [5].

For the safety evaluation, blood pressure was shown to increase in the diclofenac group by 50% [29]. This is similar to the results of this study which showed increased blood pressure and relief of pain. However, the SHT group did not show any significant change in blood pressure as indicated by the study with SHT powder drug in rat which showed no effect on blood pressure and the vasculoprotective effect in hypertensive and NO-impaired rats [30], which is similar to another SHT powder drug study in OA knee patients [7].

With regard to the adverse side effects of SHT powder and diclofenac, a previous study [8] reported that both groups developed abdominal discomfort. However, the side effect from the SHT extract group was minor (Table 6), i.e., only belching which is normally the effect of spicy taste of plant components, such as *Piper* spp., while that of diclofenac was a more serious gastric pain.

SHT ethanolic extract has no effect on blood pressure and renal and liver functions, which indicates that it is safe. Similar results have been reported previously on SHT powder. Diclofenac in this study showed an increase in the liver function value but remained within the normal range. This is similar to two previous reports [7, 18].

Important criteria for drugs to be included in the National Essential Drugs List are efficacy and human safety. SHT powder capsule is included in the National Essential Drugs List because it is shown to be a safe and effective drug for pain relief. Taking the drug in the form of powder at the dose of $6 \times 500$ mg capsules/day might affect patient compliance with the medication. This study used the SHT ethanolic extract capsules at a much smaller dose of $3 \times 100$ mg capsules/day and thus significantly reduced the number of capsules taken by the patients. This should increase the efficacy of the treatment since it would

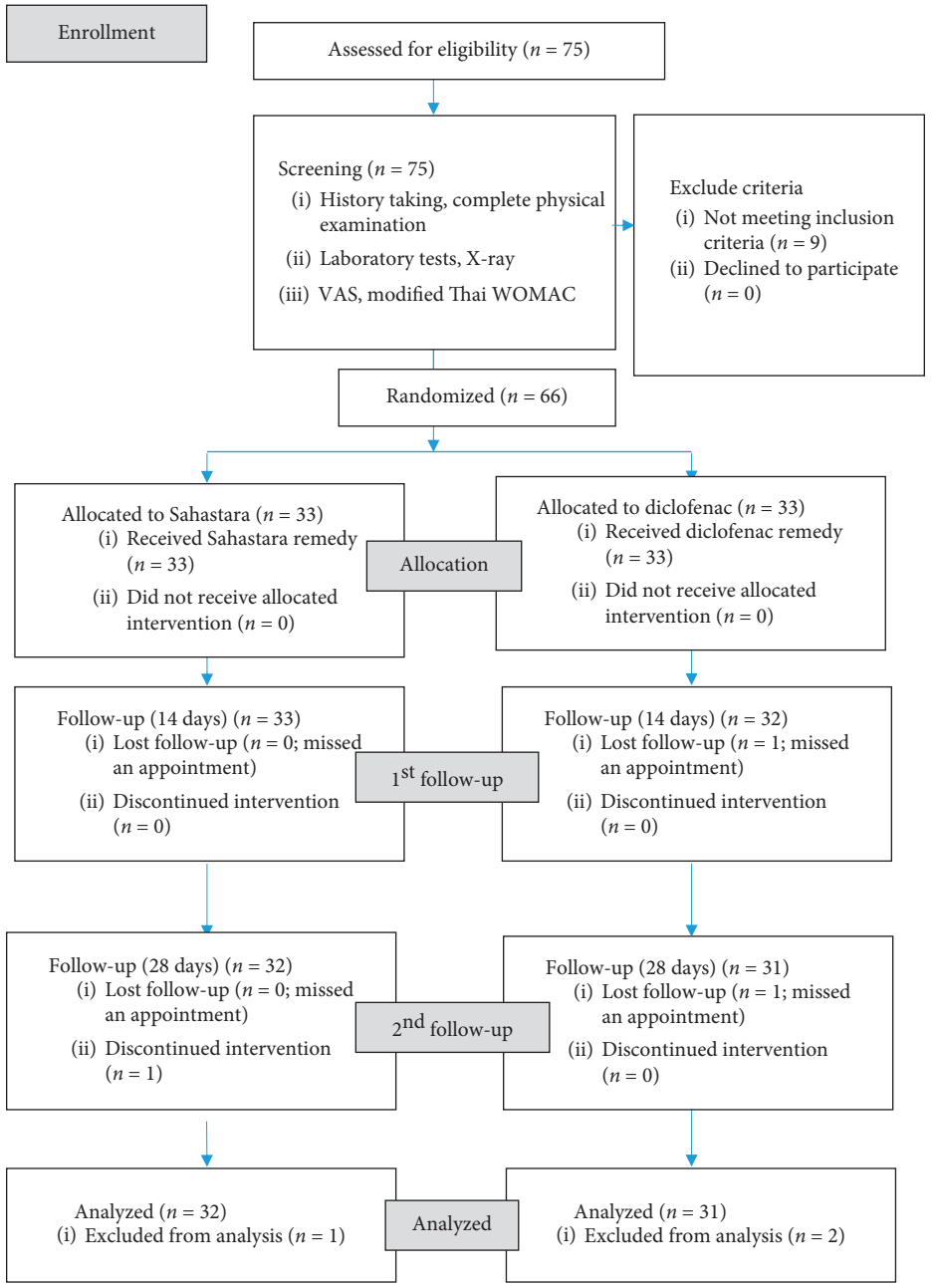


Figure 1: Flow chart of the study design.

Table 3: Experimental results of Sahastara ethanolic extract and diclofenac.

| Data | Follow-up | Treatment* | | $p$ value** |
|---|---|---|---|---|
| | | Sahastara ($n = 32$) | Diclofenac ($n = 31$) | |
| Walk 100 meters | | | | |
| Knee pain levels: VAS (mm) | Day 0 | 57.84 (2.19) | 54.61 (14.50) | $0.28^{m}$ |
| | Day 14 | $46.12\ (2.57)^{\dagger\dagger}$ | 41.09 (11.01) | $0.82^{m}$ |
| | Day 28 | $36.46\ (10.90)^{\dagger\dagger}$ | $29.77\ (8.86)^{\dagger\dagger}$ | $0.01^{m}$ |
| 100 meters walk (seconds) | Day 0 | 138.86 (40.38) | 130.41 (5.96) | $0.44^{m}$ |
| | Day 14 | 129.37 (29.28) | $120.70\ (4.14)^{\dagger}$ | $0.11^{m}$ |
| | Day 28 | $122.65\ (26.46)^{\dagger}$ | $111.51\ (3.83)^{\dagger}$ | $0.13^{m}$ |
| WOMAC index scores | | | | |
| Pain index | Day 0 | 8.25 (2.21) | 7.64 (3.02) | $0.23^{m}$ |
| | Day 14 | $5.93\ (2.52)^{\dagger\dagger}$ | $5.61\ (2.4)^{\dagger}$ | $0.64^{m}$ |
| | Day 28 | $3.81\ (2.05)^{\dagger\dagger}$ | $3.51\ (1.94)^{\dagger\dagger}$ | $0.63^{m}$ |

Table 3: Continued.

| Data | Follow-up | Treatment* | | $p$ value** |
|---|---|---|---|---|
| | | Sahastara ($n = 32$) | Diclofenac ($n = 31$) | |
| Stiffness index | Day 0 | 1.75 (1.81) | 1.69 (1.81) | 0.83[m] |
| | Day 14 | 0.7 (1.34)[†] | 0.57 (1.11)[††] | 0.44[m] |
| | Day 28 | 0.09 (0.39)[††] | 0.09 (0.34)[††] | 0.64[m] |
| Physical function index | Day 0 | 32.53 (8.8) | 31.66 (9.8) | 044[m] |
| | Day 14 | 24.87 (9.02)[††] | 24.87 (9.02)[††] | 0.19[m] |
| | Day 28 | 17.65 (7.78)[††] | 16.92 (7.6)[††] | 0.51[m] |
| Total score | Day 0 | 42.53 (1.88) | 40.06 (14.22) | 0.27[m] |
| | Day 14 | 32.96 (1.97)[††] | 28.61 (11.41)[††] | 0.21[m] |
| | Day 28 | 21.93 (1.75)[††] | 19.77 (8.75)[††] | 0.37[m] |

*Data represent mean (SD). **Statistical analysis. [m]Mann–Whitney $U$ test. For all within group statistical analysis: repeated measure ANOVA. [†]Significant difference from day 0 within the group ($P < 0.05$); [††]significant difference from day 0 within the group ($P < 0.01$).

Table 4: Overall assessment of treatments evaluated at day 28.

| Evaluate overall treatment (global assessment) | Sahastara, no. (%) | Diclofenac, no. (%) | $p$ value between groups |
|---|---|---|---|
| 0 (none) | 0 (0) | 0 (0) | 0.75[c] |
| 1 (mild better) | 1 (3.12) | 1 (3.23) | |
| 2 (moderate better) | 11 (34.37) | 8 (25.80) | |
| 3 (very much better) | 20 (62.50) | 22 (70.96) | |
| 4 (excellent) | 0 (0) | 0 (0) | |
| Total | 32 | 31 | |

[C]Chi-square.

Table 5: Effect of SHT and diclofenac on blood pressure, renal functions, liver functions and blood chemistry.

| Data | Follow-up | Treatment* | | $p$ value** |
|---|---|---|---|---|
| | | Sahastara ($n = 32$) | Diclofenac ($n = 31$) | |
| Blood pressure | | | | |
| Systolic (normal< 140 mmHg) | Day 0 | 128.39 (8.43) | 129.55 (7.57) | 0.57[m] |
| | Day 14 | 127.36 (7.63) | 130.94 (8.32)[†] | 0.66[m] |
| | Day 28 | 127.31 (7.32) | 131.71 (8.09)[††] | 0.01[m] |
| Diastolic (normal 90 mmHg) | Day 0 | 80.45 (7.25) | 81.21 (6.88) | 0.67[m] |
| | Day 14 | 80.45 (5.06) | 81.81 (6.96) | 0.38[m] |
| | Day 28 | 79.19 (5.01) | 80.52 (7.40)[†] | 0.02[m] |
| Renal functions | | | | |
| Blood urea nitrogen, BUN (mg/dL) (normal range = 7.0–18.0) | Day 0 | 14.89 (4.3) | 14.41 (5.87) | 0.27[m] |
| | Day 14 | 16.68 (5.76) | 14.97 (4.11) | 0.40[m] |
| | Day 28 | 15.19 (3.90) | 15.38 (5.00) | 0.91[m] |
| Creatinine (normal range = 0.8–1.3) | Day 0 | 0.74 (0.12) | 0.72 (0.22) | 0.14[m] |
| | Day 14 | 0.76 (0.13) | 0.75 (0.12) | 0.41[m] |
| | Day 28 | 0.74 (0.15) | 0.94 (1.11) | 0.45[m] |
| Liver function tests | | | | |
| AST (U/L) (normal range = 15–37U/L) | Day 0 | 24.71 (6.56) | 24.03 (6.53) | 0.55[m] |
| | Day 14 | 26.75 (10.88) | 28.25 (7.7) | 0.11[m] |
| | Day 28 | 27.59 (9.39) | 29.74 (11.33)[†] | 0.48[m] |
| ALT (U/L) (normal range = 30–65U/L) | Day 0 | 32.18 (9.55) | 28.55 (8.4) | 0.86[m] |
| | Day 14 | 35.56 (15.39) | 34.06 (11.59)[†] | 0.93[m] |
| | Day 28 | 33.87 (15.81 | 38.61 (20.15)[†] | 0.25[m] |
| ALP (U/L) (normal range = 30–120U/L) | Day 0 | 74.27 (20.18) | 78.96 (22.18) | 0.59[m] |
| | Day 14 | 75.66 (18.22) | 80.54 (18.24) | 0.21[m] |
| | Day 28 | 76.09 (19.19) | 82.00 (19.61) | 0.19[m] |
| The result of another laboratory | | | | |
| FBS (74–106 mg/dL) | Day 0 | 104.606 (27.06) | 116.48 (61.15) | 0.59[m] |
| | Day 14 | 105.96 (29.34) | 114.18 (62.12) | 0.39[m] |
| | Day 28 | 104.65 (29.41) | 113.58 (54.26) | 0.73[m] |

Table 5: Continued.

| Data | Follow-up | Treatment* | | $p$ value** |
|---|---|---|---|---|
| | | Sahastara ($n = 32$) | Diclofenac ($n = 31$) | |
| | Day 0 | 55.66 (15.18) | 58.00 (14.31) | 0.48[m] |
| HDL-cholesterol (40–60 mg/dL) | Day 14 | 55.90 (15.51) | 57.31 (15.55) | 0.71[m] |
| | Day 28 | 57.28 (13.58) | 53.70 (16.09) | 0.52[m] |
| | Day 0 | 220.63 (40.34) | 222.03 (44.27) | 0.85[m] |
| Total cholesterol (40–60 mg/dL) | Day 14 | 214.35 (35.12) | 220.65 (38.35) | 0.37[m] |
| | Day 28 | 212.00 (37.78) | 224.51 (41.71) | 0.18[m] |
| | Day 0 | 133.39 (34.47) | 132.63 (40.85) | 0.82[m] |
| LDL-cholesterol (0–150 mg/dL) | Day 14 | 130.27 (32.02) | 132.00 (32.00) | 0.74[m] |
| | Day 28 | 130.25 (30.02) | 132.54 (36.00) | 0.76[m] |
| | Day 0 | 152.66 (98.81) | 144.64 (79.68) | 0.98[m] |
| Triglyceride (0–150 mg/dL) | Day 14 | 159.90 (114.84) | 145.13 (85.90) | 0.84[m] |
| | Day 28 | 135.09 (84.13) | 158.68 (16.47) | 0.53[m] |
| | Day 0 | 4.60 (3.98) | 3.93 (0.25) | 0.44[m] |
| Live function total protein (6.4–8.2 mg/dL) | Day 14 | 4.30 (3.80) | 3.75 (0.34) | 0.44[m] |
| | Day 28 | 4.20 (3.80) | 3.76 (0.28) | 0.24[m] |
| | Day 0 | 3.98 (0.25) | 3.93 (0.25) | 0.51[m] |
| Albumin (3.4–5 mg/dL) | Day 14 | 3.80 (0.27) | 3.75 (0.34) | 0.66[m] |
| | Day 28 | 3.80 (0.20) | 3.76 (45 .28) | 0.54[m] |
| | Day 0 | 3.70 (0.30) | 3.85 (0.47) | 0.51[m] |
| Globulin (1.5–3.5 mg/dL) | Day 14 | 3.65 (0.35) | 3.76 (0.51) | 0.66[m] |
| | Day 28 | 3.56 (0.28) | 3.73 (0.48) | 0.54[m] |
| | Day 0 | 0.63 (0.37) | 0.53 (0.28) | 0.11[m] |
| Total bilirubin (0.2–1.0 mg/dL) | Day 14 | 0.53 (0.34) | 0.43 (0.16) | 0.25[m] |
| | Day 28 | 0.52 (0.23) | 0.47 (0.21) | 0.13[m] |
| | Day 0 | 0.13 (0.07) | 0.14 (0.12) | 0.87[m] |
| Direct bilirubin (0.0–0.2 mg/dL) | Day 14 | 0.11 (0.05) | 0.10 (0.03) | 0.30[m] |
| | Day 28 | 0.10 (0.04) | 0.10 (0.04) | 0.75[m] |
| | Day 0 | 74.27 (20.18) | 78.96 (22.18) | 0.59[m] |
| Total alkaline phosphatase (50–136 U/L) | Day 14 | 75.66 (18.22) | 80.54 (18.24) | 0.21[m] |
| | Day 28 | 76.09 (19.19) | 82.00 (19.61) | 0.19[m] |
| | Day 0 | 6.37 (1.42) | 6.25 (1.67) | 0.68[m] |
| CBC WBC (4.9–11.0 K/cumm) | Day 14 | 5.83 (1.88) | 5.77 (1.75) | 0.92[m] |
| | Day 28 | 6.09 (1.43) | 5.98 (1.57) | 0.81[m] |
| | Day 0 | 52.45 (12.26) | 52.45 (12.26) | 0.40[m] |
| Neutrophil (45–75%) | Day 14 | 51.15 (9.11) | 51.15 (9.11) | 0.35[m] |
| | Day 28 | 52.00 (6.99) | 52.00 (6.99) | 0.39[m] |
| | Day 0 | 37.93 (8.27) | 37.93 (8.27) | 0.54[m] |
| Lymphocyte (20–45%) | Day 14 | 39.54 (8.341) | 39.54 (8.34) | 0.62[m] |
| | Day 28 | 37.66 (6.84) | 37.66 (6.84) | 0.74[m] |
| | Day 0 | 3.52 (01.58) | 3.55 (01.64) | 1.00[m] |
| Monocyte (2–10%) | Day 14 | 3.69 (01.9) | 3.59 (01.67) | 0.99[m] |
| | Day 28 | 3.70 (01.52) | 3.48 (02.09) | 0.18[m] |
| | Day 0 | 4.14 (3.47) | 4.16 (4.04) | 0.74[m] |
| Eosinophil (4–6%) | Day 14 | 4.26 (2.41) | 4.97 (3.87) | 0.70[m] |
| | Day 28 | 4.83 (2.48) | 6.22 (4.43) | 0.18[m] |
| | Day 0 | 0.53 (0.25) | 0.57 (0.75) | 0.33[m] |
| Basophil (0-1%) | Day 14 | 0.71 (01.17) | 0.49 (0.44) | 0.30[m] |
| | Day 28 | 0.47 (0.24) | 0.42 (0.22) | 0.62[m] |
| | Day 0 | 4.40 (0.36) | 4.36 (0.59) | 0.25[m] |
| RBC ($4.5–6.0 \times 10^6$/cumm) | Day 14 | 4.27 (0.65) | 4.34 (0.63) | 0.73[m] |
| | Day 28 | 4.32 (0.40) | 4.48 (1.10) | 0.99[m] |
| | Day 0 | 12.4 (0.98) | 14.82 (17.60) | 0.03[m] |
| Hb (14–18 gm/dL) | Day 14 | 12.14 (0.92) | 11.66 (1.12) | 0.14[m] |
| | Day 28 | 12.01 (1.00) | 11.71 (1.13) | 0.33[m] |
| | Day 0 | 37.20 (2.42) | 35.58 (3.08) | 0.36[m] |
| Hct (41–51%) | Day 14 | 36.92 (2.41) | 35.81 (3.52) | 0.35[m] |
| | Day 28 | 36.63 (3.08) | 35.60 (3.50) | 0.20[m] |
| | Day 0 | 275.60 (58.63) | 284.06 (78.23) | 0.99[m] |
| Platelets (150–400 K/cumm) | Day 14 | 286.90 (63.08) | 283.87 (67.26) | 0.61[m] |
| | Day 28 | 288.00 (64.26) | 290.21 (68.90) | 0.99[m] |

*Data represent mean (SD); **statistical analysis; [m]Mann–Whitney $U$ test. For all within group statistical analysis: repeated measure ANOVA. [†]Significant difference from day 0 within the group ($P < 0.05$); [††]significant difference from day 0 within the group ($P < 0.01$).

Table 6: Adverse reaction comparison between Sahastara and diclofenac.

| Adverse events | Sahastara, no. (%) | Diclofenac, no. (%) | $p$ value[C] |
|---|---|---|---|
| Gastric pain | 0 (0) | 7 (22.58) | 0.11 |
| Belching | 10 (31.25) | 0 (0) | 0.001** |
| Constipation | 2 (6.25) | 1 (3.22) | 0.81 |
| Dry lips and throat | 4 (12.50) | 2 (6.45) | 0.65 |
| Sweating | 1 (3.12) | 1 (3.22) | 0.98 |
| Dizziness | 1 (3.12) | 2 (6.45) | 0.81 |

[C]Chi-square.

be easier for the patients to take. Moreover, taking SHT ethanolic extract which has been standardized [31] enables accurate amount of drug administration with known amount of active ingredient. All these should support the inclusion of the SHT ethanolic extract capsules in the National Essential Drugs List.

However, this research had some limitations with respect to sex of the patients, since we could not do block design to balance the numbers of female and male patients as suggested in the previous study. This was because almost all orthopedic patients in our hospital were female (more than 90%). Nevertheless, this is the first study on clinical trial phase II of SHT ethanolic extract capsule. Results from this study will form the basis for the continuation of the clinical study into phase III and will encourage further development of this drug.

## 5. Conclusion

This study is the first to evaluate the clinical efficacy and safety of SHT ethanolic extract capsules for the treatment of osteoarthritic knee patients in comparison with the conventional NSAID drug, diclofenac. The SHT ethanolic extract capsule can relieve the inflammation symptoms in OA knee patients almost as effectively as diclofenac, with less minor side effects. Therefore, it can be considered a safe alternative anti-inflammatory drug for the treatment of OA.

## Data Availability

The data supporting the findings of the study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

NK did the whole clinical study. PP and AI are NK's advisors. PP helped in recruiting orthopedic patients and designing the clinical study. PK helped to evaluate the clinical results. AI helped with the preparation and quality control of the drugs for the research and helped write the manuscript. BO proofread, made suggestions on revision, and edited the manuscript. AI provided the research grant and helped in all aspects of the experiments including evaluation of the results on drug preparations, writing of the manuscript, and acting as the corresponding author.

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*Research Article*

# An Investigation of the Molecular Mechanisms Underlying the Analgesic Effect of Jakyak-Gamcho Decoction: A Network Pharmacology Study

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Herbal drugs have drawn substantial interest as effective analgesic agents; however, their therapeutic mechanisms remain to be fully understood. To address this question, we performed a network pharmacology study to explore the system-level mechanisms that underlie the analgesic activity of Jakyak-Gamcho decoction (JGd; Shaoyao-Gancao-Tang in Chinese and Shakuyaku-Kanzo-To in Japanese), an herbal prescription consisting of *Paeonia lactiflora* Pallas and *Glycyrrhiza uralensis* Fischer. Based on comprehensive information regarding the pharmacological and chemical properties of the herbal constituents of JGd, we identified 57 active chemical compounds and their 70 pain-associated targets. The JGd targets were determined to be involved in the regulation of diverse biological activities as follows: calcium- and cytokine-mediated signalings, calcium ion concentration and homeostasis, cellular behaviors of muscle and neuronal cells, inflammatory response, and response to chemical, cytokine, drug, and oxidative stress. The targets were further enriched in various pain-associated signalings, including the PI3K-Akt, estrogen, ErbB, neurotrophin, neuroactive ligand-receptor interaction, HIF-1, serotonergic synapse, JAK-STAT, and cAMP pathways. Thus, these data provide a systematic basis to understand the molecular mechanisms underlying the analgesic activity of herbal drugs.

## 1. Introduction

Pain is a major healthcare and socioeconomic issue worldwide that severely affects the overall health, quality of life, daily activities, and productivity of patients, and it places a substantial financial burden on healthcare systems and society [1–6]. Based on the pathophysiological mechanisms, pain is classified into (i) nociceptive and (ii) nonnociceptive neuropathic pain [7–21]. Nociceptive pain is caused by the activation and stimulation of nociceptors and pain pathways driven by inflammation, chemicals, or physical events, and it is subdivided into somatic and visceral [7–21]. Neuropathic pain develops due to damage, injury, dysfunction, or disease of the somatosensory nervous system, and it is further classified into central and peripheral [7–21]. At present, opioid analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), and non-anti-inflammatory antipyretic analgesic agents serve as primary therapies for pain alleviation [6, 15, 22–28]. However, current treatment options for pain management are still associated with limited efficacy and unwanted adverse effects [6, 15, 22–28]. Meanwhile, herbal drugs and multicomponent-multitarget-multipathway polypharmacological therapeutics have received considerable attention for pain treatment because of their important analgesic effects with fewer side effects and toxicity [29–36].

Jakyak-Gamcho decoction (JGd; Shaoyao-Gancao-Tang in Chinese and Shakuyaku-Kanzo-To in Japanese) is an herbal drug that consists of *Paeonia lactiflora* Pallas and

*Glycyrrhiza uralensis* Fischer, which has been prescribed for the treatment of various types of pain, gynecological diseases, arthritic diseases (e.g., osteoarthritis and arthralgia), and muscular diseases (myalgia, muscle tension, spasm, and cramps) [30, 37–48]. Previous studies have demonstrated the various therapeutic properties of JGd, including its analgesic, anti-inflammatory, antispasmodic, and anti-allergic effects [30, 37–40, 43, 44, 46–55]. Among the diverse effects of this herbal drug, the most common therapeutic use of JGd is to alleviate pain arising from cancer, diabetes, neuropathy, and muscle and arthritis diseases [30, 37–40, 44, 46–48], which makes it one of the most frequently prescribed oral analgesic agents in East Asia [56]. The analgesic mechanisms of JGd include the modulation of spinal $\alpha$2-adrenoceptors, transient receptor potential vanilloid 1 (TRPV1) channels, calcium and Sirt1 signalings, muscle contraction and relaxation, and chemokine and cytokine expression [39, 44, 46, 51, 57–60]. However, the pharmacological properties of JGd at the systemic level need to be explored.

Because of the complex pharmacological nature of multicompound-multitarget-multipathway agents, there is often a fundamental limitation in investigating their comprehensive mechanisms of action based only on conventional biological experimental methodologies [61–68]. To overcome such challenges, network pharmacology, an integrative research field that systematically combines computational systems biology, network science, medicine, pharmacology, mathematics, and physics, has emerged as one of the most effective approaches for the mechanistic exploration of polypharmacological drugs, such as herbal medicines [61–68]. The goal of this integrative science is to unveil the mechanisms of disease pathogenesis and drug activity that are coordinated through the interactions among diverse biological components such as genes, proteins, cells, tissues, and organs [61–68]. Previous network pharmacology studies successfully investigated the polypharmacological properties of herbal drugs by identifying their active compounds and key therapeutic targets and further elucidating the distinct system-level pharmacological effects and mechanisms (e.g., therapeutic modulation of biological processes such as proliferation, apoptosis, cell cycle regulation, angiogenesis, oxidation and reduction, insulin metabolism, and inflammation) for the treatment of various diseases, including cancer, diabetes, arthritis, and ischemic stroke, which are exerted by the synergistic interplay between multiple compounds and targets contained in herbal drugs [61–79]. In the present network pharmacology study, we aimed to uncover the molecular mechanisms that underlie the analgesic properties of JGd with a system's perspective.

## 2. Materials and Methods

*2.1. Screening of Active Chemical Compounds in Jakyak-Gamcho Decoction.* Information on chemical compounds comprising the herbal constituents of JGd was investigated using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database [80]. Then, based on their absorption, distribution, metabolism, and excretion (ADME) properties (i.e., oral bioavailability (OB), Caco-2 permeability, and drug-likeness (DL)), chemical compounds that satisfy the following criteria were screened and determined to be bioactive as previously suggested [63, 80, 81] using the TCMSP [80]: OB $\geq$ 30%, Caco-2 permeability $\geq$ -0.4, and DL $\geq$ 0.18. In brief, OB is the proportion of orally administered drug compounds that enter the general circulation, and it is one of the most crucial considerations in the design and development of a drug [80, 82]. Of note, compounds with an OB larger than 30% are commonly regarded as effectively absorbed in the human body [80, 82]. Caco-2 permeability is an important index for the investigation of intestinal permeability and drug efflux that is based on an evaluation of the rate of absorption and diffusion of a compound across Caco-2 human intestinal cells [80, 83–85]. In general, a chemical compound is considered not permeable in the intestinal epithelium if its Caco-2 permeability is lower than −0.4 [86, 87]. DL is a widely used measurement that qualitatively assesses whether a certain compound is physicochemically and structurally suitable for use as a drug [80, 88]. Note that the average DL of all drugs is 0.18, and therefore, it is commonly used as the threshold to determine the pharmacological potential of a compound [80, 88].

*2.2. Target Identification.* Human targets of the active chemical compounds of JGd were investigated using various databases and models, including the PharmMapper [89], search tool for interactions of chemicals (STITCH) 5 [90], Swiss Target Prediction [91], similarity ensemble approach (SEA) [92], systematic drug targeting tool (SysDT) [93], and weighted ensemble similarity (WES) [94]. The pain-associated human genes and proteins were investigated from the DisGeNET [95], Therapeutic Target Database [96], GeneCards [97], Comparative Toxicogenomics Database [98], Human Genome Epidemiology Navigator [99], Online Mendelian Inheritance in Man [100], Pharmacogenomics Knowledgebase [101], and DrugBank [102], using the medical subject headings term "Pain" (ID: D010146) for *Homo sapiens* species.

*2.3. Network Construction.* The herbal medicine-active chemical compound (H-C), active chemical compound-target (C-T), and target-pathway (T-P) networks were generated by connecting the herbal medicines with their active chemical compounds, the compounds with their targets, and the targets with the signaling pathways in which they are enriched. The protein-protein interaction (PPI) network was generated using the STRING database (interaction confidence score $\geq$ 0.9) [103]. Analysis and visualization of networks were performed with Cytoscape software [104]. A network is composed of nodes (e.g., herbal medicines, chemical compounds, targets, or pathways) and edges (or links) describing the interactions among the nodes [105]. The degree is defined as the number of links of a node [105].

*2.4. Contribution Index Evaluation.* The network-based efficacy-based contribution index (CI) of active chemical

compounds of JGd was evaluated following previous procedures as follows [81]:

$$\mathrm{NE}(j) = \sum_{i=1}^{n} d_i,$$
$$\mathrm{CI}(j) = \frac{c_j \times \mathrm{NE}(j)}{\sum_{i=1}^{m} c_i \times \mathrm{NE}(i)} \times 100\%, \quad (1)$$

where $m$ is the number of chemical compounds, $n$ is the number of targets of chemical compound $j$, $d_i$ is the number of links of target $i$ of chemical compound $j$, and $c_i$ (or $c_j$) is the number of previous studies having "pain" and component $i$ (or $j$) in their title or abstract searched from the PubMed database (https://pubmed.ncbi.nlm.nih.gov/). The chemical compounds with the highest CIs were regarded as contributing more to the pharmacological activity of a certain herbal drug [81].

*2.5. Functional Enrichment Analysis.* Gene ontology (GO) enrichment analysis was performed with g:Profiler [106]. Pathway enrichment analysis was performed with Kyoto Encyclopedia of Genes and Genomes database [107]. Functional association analysis was conducted using GeneMANIA [108].

*2.6. Molecular Docking Analysis.* The structures of chemical compounds of JGd and their targets were obtained from the PubChem [109] and RCSB Protein Databank [110] databases, respectively. Then, the molecular docking scores between the chemical compounds and the targets were assessed using AutoDock Vina [111]. Of note, a certain chemical compound is regarded as having high binding affinity to a target if the corresponding docking score is less than or equal to −5.0 [112, 113].

## 3. Results

The network pharmacology study for the exploration of analgesic mechanisms of JGd was conducted as follows (Figure 1). Detailed information regarding the chemical constituents of JGd was obtained from the comprehensive biomolecular databases, and the bioactive compounds were investigated using their ADME characteristics (Figure 1). The human targets of the active chemical compounds were identified from various databases and models that assess chemical-protein interactions (Figure 1). Then, we integrated the extensive herbal drug-related data into networks and performed network pharmacology analysis (Figure 1).

*3.1. Active Chemical Compounds of Jakyak-Gamcho Decoction.* Detailed information regarding the chemical compounds present in JGd was obtained from TCMSP [80] (Supplementary Table S1), and the active compounds were defined as those with OB ≥ 30%, Caco-2 permeability ≥ −0.4, and DL ≥ 0.18, as described previously [63, 80, 81]. Some components were also determined to be active because of the

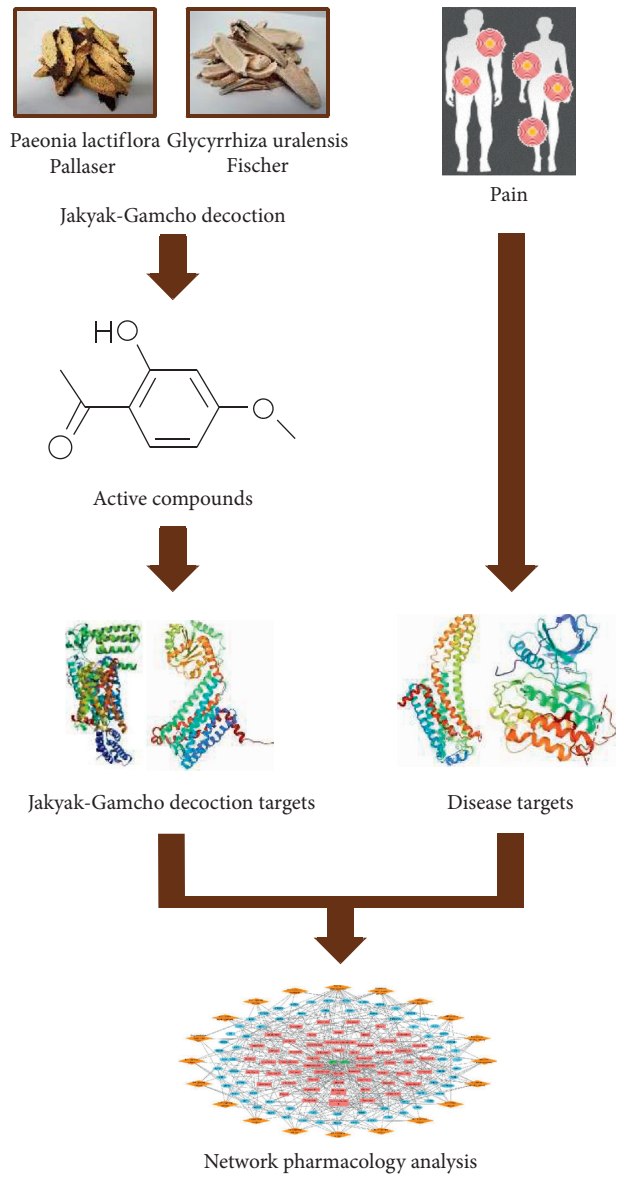


Figure 1: A schematic diagram illustrating the network pharmacology exploration of the analgesic mechanisms of Jakyak-Gamcho decoction.

substantial amount contained in JGd and their reported relevant pharmacological activity [42, 57, 114–128], although they did not meet the criteria. As a result, 111 active chemical compounds were obtained for JGd (Supplementary Table S2).

*3.2. Targets of Jakyak-Gamcho Decoction.* We identified the targets of the active chemical compounds of JGd using the following databases and models for the investigation of chemical-protein interactions: Swiss Target Prediction [91], STITCH 5 [90], PharmMapper [89], SEA [92], SysDT [93], and WES [94]. Therefore, 70 human pain-associated and 137 nonpain-associated targets were obtained for JGd (Supplementary Table S3).

*3.3. Network Pharmacology-Based Analysis of Jakyak-Gamcho Decoction.* To perform network pharmacology-based analysis of the pharmacological features of JGd, we constructed an herbal medicine-active chemical compound-target (H-C-T) network composed of 129 nodes (two herbal medicines, 57 active chemical compounds, and 70 pain-associated targets) and 217 links (Figure 2 and Supplementary Table S3) using comprehensive information regarding the herbal drug. We found that quercetin (number of targets = 36) and kaempferol (number of targets = 11) have relatively many targets (Figure 2 and Supplementary Table S3), implying that they might be important active compounds for the therapeutic activity of JGd. In addition, 27 human genes/proteins were found to be targeted by two or more active chemical compounds of JGd (Figure 2), suggesting a polypharmacological mechanism.

To investigate the biological interaction relationship between the JGd targets, we generated a PPI network (58 nodes and 174 links) comprising the targets (Figure 3). Next, we searched for hubs, specific nodes with a high degree in the network that are shown to have crucial biological functions and promising therapeutic potential [129, 130]. In the analysis, hubs were determined as nodes for which the degree was greater than or equal to twice the average node degree of the network [131, 132]. The results showed that PIK3R1 (degree = 25), HSP90AA1 (degree = 15), EGFR (degree = 14), AKT1 (degree = 13), LPAR1 (degree = 13), LPAR2 (degree = 13), and LPAR3 (degree = 13) were hubs (Figure 3), implying that they might be the key targets responsible for the analgesic activity of JGd. These hubs were shown to be involved in the regulation of pain-related processes and could function as potent targets to induce analgesic effects. The *PIK3R1* gene was suggested to have the potential to function as a pain-related regulator according to the genetic interaction analysis [133], and its expression level might be associated with osteoarthritis pathogenesis [134]. Upregulation of the *HSP90AA1* gene was observed in patients with fibromyalgia [135–137], and pharmacological inhibition of heat shock protein 90 (HSP90; encoded by *HSP90AA1*) was shown to alleviate monoarthritis-induced pain [138]. The activation of epidermal growth factor receptor (EGFR; encoded by *EGFR*) and AKT (encoded by *AKT1*) is associated with the development and enhancement of diverse types of pain, and their therapeutic modulation might be associated with analgesic properties [139–156]. Lysophosphatidic acid receptor 1 (encoded by *LPAR1*) activity is involved in pain behavior arising from bone cancer, inflammation, diabetes, and neuropathy, and its pharmacological or genetic ablation might reduce the pain response [157–165]. Lysophosphatidic acid receptor 3 (encoded by *LPAR3*) plays crucial roles in the development and maintenance of neuropathic pain, and its blockade exerts analgesic effects [163, 166, 167].

We further assessed the CIs of the active chemical compounds of JGd to assess their pharmacological contribution to the analgesic effect of the herbal drug as described earlier [81, 168]. As a result, quercetin was shown to have the highest CI (91.83%) (Supplementary Figure S1), which suggests that this chemical compound might be the primary contributor to the analgesic activity of JGd.

Together, these data indicate the system-level pharmacological properties of the analgesic activity of JGd.

*3.4. Functional Enrichment Investigation of Jakyak-Gamcho Decoction Networks.* To investigate the molecular mechanisms underlying the analgesic effect of JGd, we carried out GO enrichment analysis of the targets. As a result, the JGd targets were enriched in GO terms involved in the modulation of a variety of biological activities, such as calcium- and cytokine-mediated signalings, calcium ion concentration and homeostasis, cellular behaviors of muscle and neuronal cells, inflammatory response, and response to chemical, cytokine, drug, and oxidative stress (Supplementary Figure S2), which are in accordance with the previously reported molecular mechanisms of the herbal drug [40, 41, 44, 46, 49, 55, 58–60, 169–172]. In addition, GeneMANIA analysis indicated that the JGd targets might functionally interact via diverse mechanisms (Supplementary Figure S3), implying the similarity in their pharmacological roles.

Because various signaling pathways were reported to be associated with the initiation, transmission, perception, and maintenance of pain [12, 14, 20, 144, 155, 173–186], we carried out pathway enrichment analysis. We found that the JGd targets were enriched in the following signalings: "PI3K-Akt signaling pathway," "Neuroactive ligand-receptor interaction," "Estrogen signaling pathway," "cAMP signaling pathway," "Chemokine signaling pathway," "JAK-STAT signaling pathway," "Neurotrophin signaling pathway," "AMPK signaling pathway," "Dopaminergic synapse," "ErbB signaling pathway," "HIF-1 signaling pathway," "Insulin signaling pathway," "mTOR signaling pathway," "Serotonergic synapse," "Adipocytokine signaling pathway," "Drug metabolism - cytochrome P450," "IL-17 signaling pathway," "TNF signaling pathway," "Arachidonic acid metabolism," and "VEGF signaling pathway" (Figure 4 and Supplementary Figure S2). These signalings are well-known pain-regulating pathways and function as therapeutic targets of analgesic and pain-relieving drugs. The activities of adenosine monophosphate-activated kinase (AMPK), ErbB, mammalian target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K)-Akt, tumor necrosis factor (TNF), or vascular endothelial growth factor (VEGF) signaling pathways are involved with the development and maintenance processes of various types of pathological pain, and their functional modulation might relieve neuropathic, nociceptive, and bone cancer pain [144, 149, 150, 155, 156, 187–226]. Furthermore, the activity of PI3K-Akt and the adipocytokine pathway further correlates with the severity of neuropathic and inflammatory pain, and their targeting agents exert analgesic effects [227–229]. The estrogen pathway serves as a modulator of the processing and sensitivity of visceral and mechanical pain responses [230–235]. Previous studies have shown the involvement of cyclic adenosine monophosphate (cAMP), chemokine, Janus kinase-(JAK-) signal transducer and activator of transcription (STAT), neurotrophin, and hypoxia-inducible factor (HIF) pathways in the initiation and persistence of inflammatory, cancer, and neuropathic pain, as well as their role as pharmacological mediators of analgesic approaches [141, 224, 236–265]. The

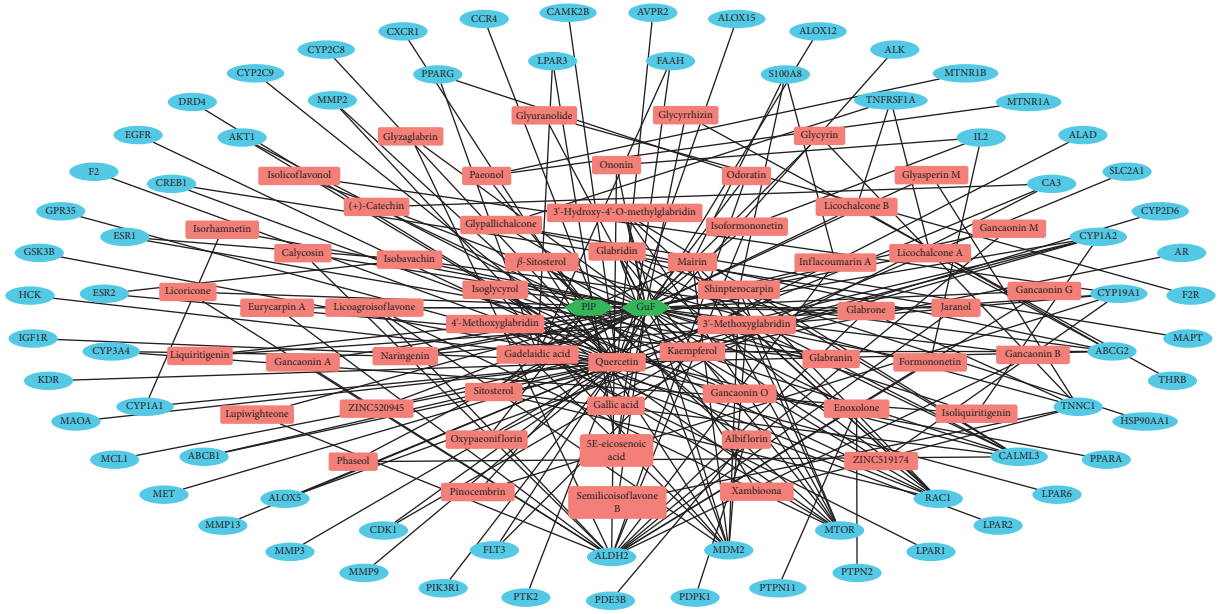


Figure 2: The herbal medicine-active chemical compound-target network of Jakyak-Gamcho decoction. Green nodes, herbal medicines; red nodes, active chemical compounds; blue nodes, pain-related targets.

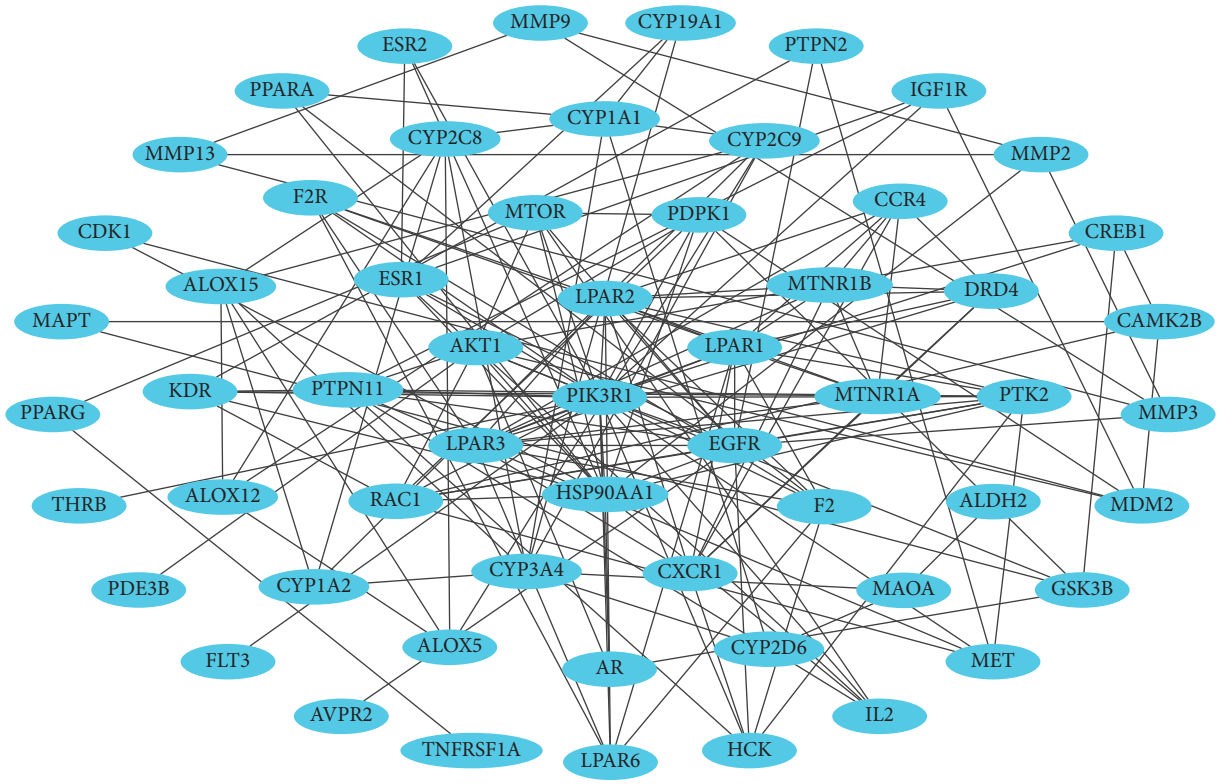


Figure 3: The protein-protein interaction network for the pain-related targets of Jakyak-Gamcho decoction. Blue nodes, pain-related targets.

impaired regulation of insulin signaling might promote the development of and pain sensation with diabetic neuropathy, which can be alleviated by its functional restoration [266–269]. The interleukin- (IL-) 17 pathway plays a crucial role in cellular mechanisms of pain pathogenesis and maintenance in various diseases including multiple sclerosis, prostatitis, intervertebral disk degeneration, femoral head osteonecrosis, and neuropathy; its inhibition might block the generation and persistence of pain [270–282]. Arachidonic acid metabolism is associated with the generation and secretion of diverse biomolecular substances

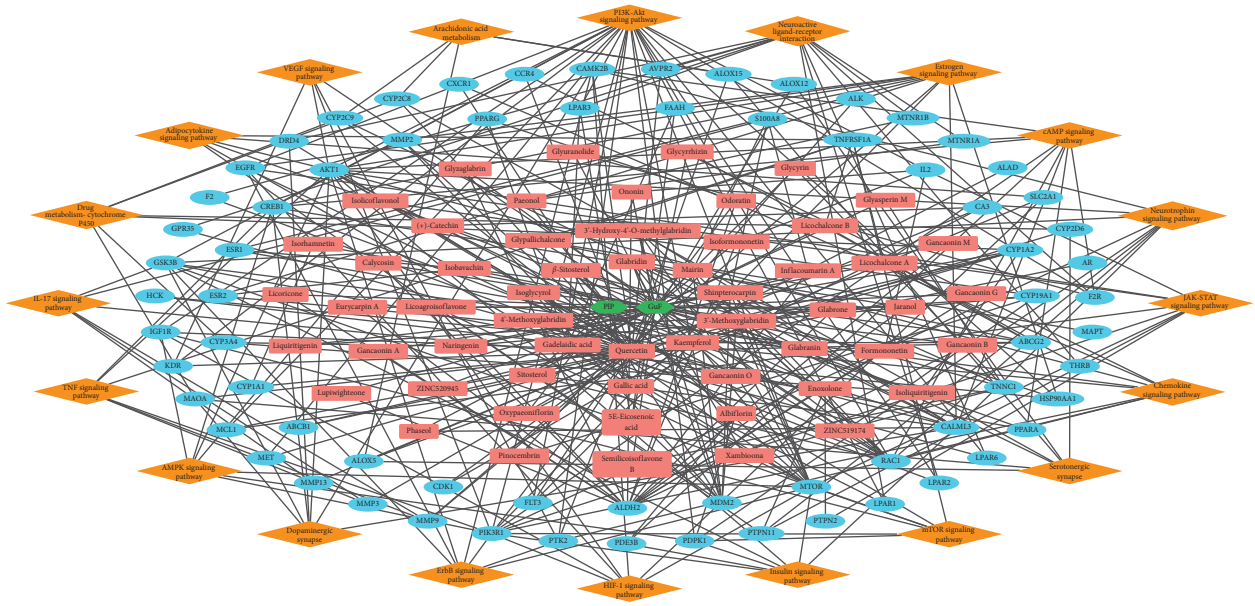


FIGURE 4: The herbal medicine-active chemical compound-target-pathway network of Jakyak-Gamcho decoction. Green nodes, herbal medicines; red nodes, active chemical compounds; blue nodes, pain-related targets; orange nodes, signaling pathways.

responsible for the induction of inflammation and pain, and it is mainly involved in the mechanisms of action of NSAIDs [283–288]. Moreover, the serotonergic and dopaminergic synapse pathways are key neurotransmitters responsible for modulating the intensity and duration of pain, and their therapeutic interventions have been shown to attenuate pain behaviors [289–292].

Collectively, these results demonstrate the molecular- and pathway-level mechanisms underlying the analgesic activity of JGd.

*3.5. Molecular Docking Evaluation.* To investigate the binding potential of the chemical compounds of JGd components for the targets, we evaluated their molecular docking activity. As a result, 95.09% of the binding interactions between the active chemical components of JGd and the hub targets was found to have docking scores equal to or lower than −5.0 (Figure 5 and Supplementary Table S4), indicating their therapeutic binding potential. Of note, the protein structures for LPAR2 and LPAR3 were unavailable in the RCSB Protein Databank [110]; therefore, they were excluded from the analysis.

## 4. Discussion

Herbal medicines are increasingly being acknowledged as effective analgesic and pain-relieving agents owing to their promising therapeutic activity with fewer side effects [29–36]. JGd is a well-known herbal drug that alleviates pain induced by multiple diseases such as peripheral neuropathy, myalgia, arthralgia, and diabetes [30, 37–40, 44, 46–48], and it is one of the most frequently prescribed oral analgesics in East Asia [56]. Previous studies have attempted network pharmacology analyses to investigate the mechanisms underlying JGd for the treatment of osteoarthritis and Parkinson's disease [293, 294]; however, its network-perspective analgesic properties have not been fully elucidated. Therefore, this network pharmacology study attempted to investigate system-level mechanisms that underlie the analgesic activity of JGd. The ADME evaluation and network pharmacology investigation identified 57 active chemical compounds in JGd and their 70 pain-associated human molecular targets. Further enrichment analysis indicated that JGd targets were enriched with GO terms related to the modulation of biological activities, involving calcium- and cytokine-mediated signalings, calcium ion concentration and homeostasis, cellular behaviors of muscle and neuronal cells, inflammatory response, and response to chemical, cytokine, drug, and oxidative stress, consistent with the previously reported molecular mechanisms of the herbal drug [40, 41, 44, 46, 49, 55, 58–60, 169–172]. We further showed that JGd might target various pain signalings to exert its analgesic and pain-relieving effects, which involve the PI3K-Akt, estrogen, ErbB, neurotrophin, neuroactive ligand-receptor interaction, HIF-1, serotonergic synapse, JAK-STAT, and cAMP pathways.

The analgesic activity of the chemical components of JGd has been previously reported. (+)-Catechin and pinocembrin produce analgesic, antineuropathy, and antinociceptive effects [295–297]. Albiflorin might play a pharmacological role as an analgesic, antineuropathy, and antinociceptive compound that can reduce pain intensity via the functional modulation of calcium channels, mitogen-activated protein kinase (MAPK) pathways, and various cytokines and chemokines [127, 298]. Moreover, formononetin, glabridin, glycyrrhizin, and paeonol exhibit anti-inflammatory, antinociceptive, and analgesic activities by inhibiting the generation of inflammatory cytokines and signaling molecules, thereby attenuating the pain responses [117, 120, 299–302]. Gallic acid could also have potential anti-

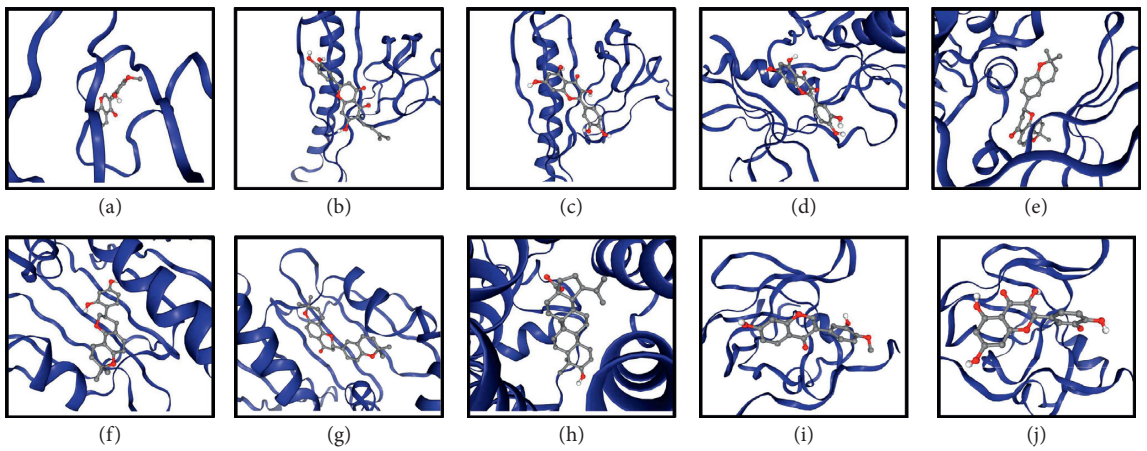


Figure 5: Molecular docking analysis of interactions between the active chemical compounds of Jakyak-Gamcho decoction and hub targets. (a) Calycosin-AKT1 (score = −7.5). (b) Gancaonin O-AKT1 (score = −5.7). (c) Quercetin-AKT1 (score = −6.4). (d) Quercetin-EGFR (score = −8.0). (e) Xambioona-EGFR (score = −10.5). (f) Glabridin-HSP90AA1 (score = −8.3). (g) Xambioona-HSP90AA1 (score = −9.1). (h) Mairin-LPAR1 (score = −8.4). (i) Calycosin-PIK3R1 (score = −7.9). (j) Quercetin-PIK3R1 (score = −6.3).

inflammatory, antioxidant, and neuroprotective effects that could improve neuropathic pain, neuronal damage, and injury [119, 303, 304]. Glycyrrhizin and naringenin reduce inflammatory and neuropathic pain-like behaviors by modulating the secretion of inflammation-associated cytokines and mediators, as well as the activities of cyclic guanosine monophosphate (cGMP) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$\kappa$B) signalings [120, 301, 305–314]. Isoliquiritigenin was reported to possess analgesic, antispasmodic, and relaxant properties [315, 316]. Isorhamnetin ameliorates the pain intensity of diabetic neuropathy via its neuroprotective, antioxidative, and anti-inflammatory effects [317]. Kaempferol shows anti-inflammatory, antioxidant, and analgesic effects, which relieve the pain symptoms of gastritis, pancreatitis, and diabetic neuropathy [318–320]. In addition, liquiritigenin might suppress neuropathic pain by improving thermal, cold, and mechanical hyperalgesia [116]. Mairin (betulinic acid) has been shown to exert anti-inflammatory, antinociceptive, antipyretic, and analgesic effects, thereby alleviating visceral pain and chemotherapy-, infection-, and diabetes-associated neuropathies [321–326]. Quercetin reduces pain arising from inflammation, cancer, chronic prostatitis/chronic pelvic pain syndrome, arthritis, and muscle injury by inhibiting the induction of oxidative stress and activating inflammatory and adrenergic pathways, neurotransmitters, and cytokines [327–334]. In addition, quercetin further modulates the activity of a variety of pathways, including Toll-like receptor, mTOR, protein kinase C$\varepsilon$ (PKC$\varepsilon$)-TRPV1, p70 ribosomal S6 kinase (p70S6K), and $P2X_4$ receptor signalings, as well as oxidative stress- and inflammation-associated mediators to exert its analgesic effects against diverse types of neuropathic pain [214, 335–347]. $\beta$-Sitosterol shows analgesic, antinociceptive, and anti-inflammatory activities [348–353]. These studies regarding the chemical components of JGd provide the pharmacological basis for the analgesic activities of this herbal drug.

Based on the network pharmacological analyses, the following studies would contribute to the improvement of herbal drug therapies: (i) an assessment of the therapeutic efficacy of JGd analgesic activity in specific diseases that are associated with distinct types of pain, such as cancer, osteoarthritis, myalgia, arthralgia, and diabetes; (ii) a comprehensive exploration of the system-level mechanisms of analgesic properties of the herbal drug from diverse pharmacological perspectives, involving antinociceptive, anti-inflammatory, muscle relaxant, and antipyretic effects; and (iii) an investigation of the safety and effectiveness of combined treatment with JGd and widely used analgesic agents, including celecoxib, tramadol, and acetaminophen [24, 56, 354].

To conclude, we investigated the systems' perspective pharmacological properties of JGd, a widely prescribed analgesic herbal drug [56]. Based on the network pharmacological approach, we investigated 57 active chemical compounds and their 70 pain-related targets responsible for the analgesic activity of JGd. The targets of JGd were associated with the modulation of biological functions such as calcium- and cytokine-mediated signalings, calcium ion concentration and homeostasis, cellular behaviors of muscle and neuronal cells, inflammatory response, and response to chemical, cytokine, drug, and oxidative stress, which suggests the molecular mechanisms of JGd treatment. In addition, the enrichment analysis indicated that the targets are involved in various pathways that are associated with the pathophysiology of pain, including the PI3K-Akt, estrogen, ErbB, neurotrophin, neuroactive ligand-receptor interaction, HIF-1, serotonergic synapse, JAK-STAT, and cAMP pathways. The overall data offer a novel systematic view of the polypharmacological characteristics of herbal drugs and a mechanistic basis for their clinical implications for pain treatment.

## Data Availability

The data used to support the findings of this study are included within the article and supplementary materials file.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Supplementary Materials

Supplementary Figure S1: contribution index analysis for the analgesic activity of active chemical compounds of Jakyak-Gamcho decoction. Supplementary Figure S2: functional enrichment analyses for the pain-associated targets of Jakyak-Gamcho decoction. Supplementary Figure S3: functional interaction analysis of the pain-associated targets of Jakyak-Gamcho decoction. Supplementary Table S1: list of the chemical compounds contained in Jakyak-Gamcho decoction. Supplementary Table S2: list of the active chemical compounds contained in Jakyak-Gamcho decoction. Supplementary Table S3: list of the targets of active chemical compounds of Jakyak-Gamcho decoction. Supplementary Table S4: docking scores of the active chemical compounds of Jakyak-Gamcho decoction with the hub targets. (*Supplementary Materials*)

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*Research Article*

# Effects of Acupuncture on Explosive Force Production by the Healthy Female Shoulder Joint

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*Background*. Acupuncture is often used to treat chronic conditions, such as pain. In recent years, given the importance of the explosive forces generated by shoulder muscles for the completion of motor tasks, studies in which nerves were stimulated through acupuncture to increase the explosive forces were conducted. This study explored the effect of acupuncture on explosive force production by the muscles of the female shoulder joint. *Methods*. Eighteen healthy women underwent shoulder adduction (Add), abduction (Abd), flexion (Flex), and extension (Ext) tests with an isokinetic measurement system. Acupuncture was used to stimulate the Zhongfu (LU1), Tianfu (LI3), Xiabai (LU4), Binao (LI14), Naohui (SJ13), Jianliao (SJ14), and Xiaoluo (SJ12) points, and electromyography (EMG) signals were recorded before and after acupuncture. *Results*. After acupuncture, there was a significant difference in the average maximum work, the average maximum power, the average maximum speed, the total work in Add/Abd and Flex/Ext, the EMG signals, and the stiffness of the muscles in Abd and Ext ($P < 0.05$). There were no significant differences in the average maximum torque in Abd or Flex. *Conclusion*. Based on the results, there may be a significant correlation between the manipulation of different acupoints by acupuncture and the average maximum torque and stiffness. Acupuncture may stimulate nerves to activate muscles and induce a postactivation potentiation effect that improves explosive force production. Therefore, acupuncture as an auxiliary tool may increase the explosive forces generated by acupoint-related muscles by stimulating nerves.

## 1. Introduction

Acupuncture was introduced more than 2,000 years ago [1], and it remains an important part of traditional Chinese medicine (TCM). In 2013, acupuncture was used in 183 countries, according to a survey by the World Federation of Acupuncture-Moxibustion Societies (World Health Organization (WHO) report) [2]. Recently, acupuncture has been used to enhance recovery from sports competitions [3]; for example, it has been shown to enhance balance [4] and reduce spasticity [5]. As these examples show, the use of acupuncture is increasingly widespread.

In sports medicine, acupuncture has been preliminarily used to control pain and relieve muscle aches caused by exercise [6], lateral epicondylitis (tennis elbow) [7], knee osteoarthritis [8], low back and neck pain [9], and rotator cuff tendinitis [10]. Previous studies have shown that acupuncture also has positive effects on strength, aerobic training, flexibility, and athletic performance [11]. Overall, the application of acupuncture in sports medicine can improve athletic performance in domains such as muscle strength. Neurochemical, histological, and neurophysiological studies have attempted to elucidate the mechanisms of action of acupuncture [12]. Additionally, many studies in animals and humans have demonstrated that acupuncture can cause multiple biological responses [13]. For example, needling may cause receptors to send neural impulses to the spinal cord or may act on ascending pathways to the brain,

causing the release of neurotransmitters that subsequently modulate functions in the brain as well as in the periphery [14]. Therefore, there is a reasonable physiological basis for acupuncture to improve motor function.

Explosive force, the ability to overcome a certain resistance in a short time and with great acceleration, is considered an important indicator of athletic performance [15]. Given the important role of explosive force and movement in athletic performance, many researchers have extensively studied methods related to increased muscle strength and explosive force training methods, which may be useful for improving athletic performance. For example, studies have shown that whole-body vibration (WBV) training can improve strength, power, and jump height [16], and changes in muscle stiffness are believed to be the mechanism through which vibration training improves functional performance [17]. Additionally, there exists a phenomenon of postactivation potentiation (PAP); that is, previous muscle contraction increases subsequent strength and muscle power output beyond the baseline level [18]. Past research on PAP has mainly focused on exploring the effect of the dynamic stimulation of PAP on the performance of the lower extremities, and stimulation can help increase the subsequent explosive force produced by the lower extremities [19]. One of the mechanisms underlying PAP is an increase in the recruitment of higher-order motor units [20]. The same effect may occur when acupuncture stimulates the surface of the skin to induce accelerated extremity reflexes and increased muscle strength [21]. Therefore, acupuncture may induce PAP by stimulating nerves to improve explosive force production.

In a recent study of the effect of acupuncture on the endurance of the female shoulder joint muscles, it was found that acupuncture can increase the excitability of the shoulder joint muscles, delaying muscle fatigue, and increasing muscle endurance [22]. However, the effectiveness in increasing the explosive force of the female shoulder joint has not been confirmed until now. Therefore, this study aimed to explore the effect of acupuncture on explosive force production by the muscles of the female shoulder joint. In this study, we hypothesized that acupuncture can improve the explosive forces generated by the shoulder joint muscles after isokinetic exercise through the corresponding neurophysiological responses.

## 2. Methods

*2.1. Study Design.* The study was registered prospectively at the Chinese Clinical Trial Registry (Registration number: ChiCTR1900025407). Eighteen healthy female subjects (age: $21.2 \pm 7.2$; weight: $57.6 \pm 6.3$ kg; height: $164 \pm 4$ cm) were recruited at JLSU (September 1, 2019, to September 30, 2019). All participants signed informed consent before they participated in the study.

*2.2. Subjects.* Inclusion in this study was restricted to participants meeting the following criteria: age >18 years, the absence of pain in the upper limbs, the absence of a history of muscle disease, and the absence of acupuncture or any medical treatments within the last 6 months. The patients were encouraged not to perform physical exercise for at least 48 hours before the test [6, 23]. The exclusion criteria were upper limb pain, a history of trauma, neuromuscular impairment, uncontrolled epilepsy, epithelial allergy, or any adverse reactions to needles [24]. Participants with a history of significant trauma or systemic inflammatory conditions, such as rheumatoid arthritis, polymyalgia rheumatica, and fracture, were also excluded [25]. The final analysis conducted in this study included eighteen subjects who met the above criteria.

*2.3. Instruments.* An isokinetic training system (Con-Trex MJ; CMV AG, Dübendorf, Switzerland) was used to collect kinetic data on adduction/abduction (Add/Abd) and flexion/extension (Flex/Ext) of the shoulder joint. A portable surface electromyography (EMG) machine with six channels (BTS FreeEMG 300, BTS SpA, Milan, Italy) and disposable circular electrodes with a diameter of 10 mm was used to collect EMG signals (1000 Hz) before and after acupuncture. Disposable stainless-steel needles ($0.25\,\text{mm} \times 40\,\text{mm}$, Suzhou Medical Appliance Factory, Suzhou, People's Republic of China) were used for acupuncture.

*2.4. Acupuncture.* Acupuncture needling was performed by an experienced acupuncturist. Perpendicular needling was carried out bilaterally using sterile disposable needles. The following classical acupuncture points were used in the following order: Zhongfu (LU1), Tianfu (LI3), Xiabai (LU4), Binao (LI14), Naohui (SJ13), Jianliao (SJ14), and Xiaoluo (SJ12) (see Figure 1). The needle was left in place for 20 minutes and then promptly removed. Each needle was rotated at 2 minutes, 5 minutes, and 10 minutes after insertion. The depth of needle insertion depended on the anatomical location of each point and the physical characteristics of each subject (e.g., skin thickness and subcutaneous fat layer thickness) and varied from 5 to 30 mm. De Qi sensation was provoked by manual stimulation (rotation) at the beginning of each session. In TCM, De Qi is a unique sensation of numbness, soreness, heaviness, or tingling that develops at the site of acupuncture, often spreading toward nearby cutaneous areas [21].

*2.5. Reasons for Acupoint Selection.* Studies have shown that the main muscles responsible for shoulder joint Add, Abd, Flex, and Ext are the deltoid anterior (DA), deltoid posterior (DP), and pectoralis (PS), which are all distributed around the shoulder joint [26]. In this study, the Zhongfu (LU1), Tianfu (LU3), Xiabai (LU4), and Binao (LI4) acupoints were located in the anterior Flex and Abd muscle groups of the shoulder joint, while the Jianliao (SJ14), Naohui (SJ13), and Xiaoluo (SJ12) acupoints were located on the Abd and Ext muscle groups of the shoulder joint. Overall, acupuncture points are selected to stimulate nerves and produce PAP according to the principles of TCM.

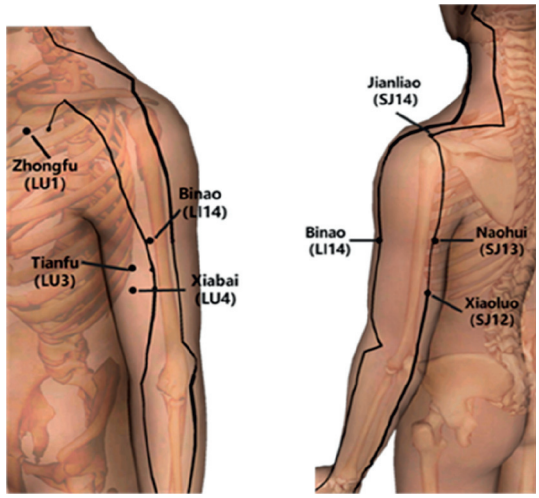


Figure 1: Acupuncture points.

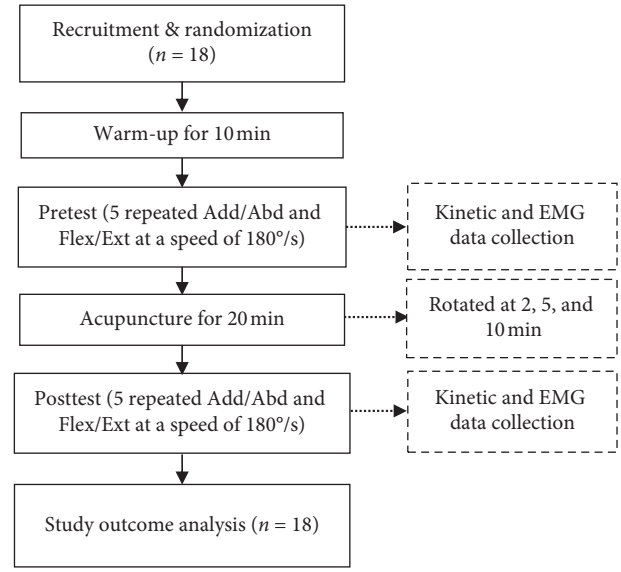


Figure 2: Study flowchart. Note: shoulder joint adduction and abduction (Add/Abd), flexion, and extension (Flex/Ext).

*2.6. Protocol.* Each subject completed isokinetic strength tests of the shoulder joint muscle group before and after acupuncture, including a pretest and a posttest. We introduced each participant to the entire experimental process to familiarize them with the experimental settings, equipment, and procedures. We simulated the explosive use of muscles of the shoulder joint at a high speed of 180°/s and used isokinetic equipment for standardization. Participants were randomly selected and instructed to warm up for 10 minutes and then rest for 2 minutes. During the break, the skin was cleaned with 75% alcohol. The electrode plates were placed at the approximate center of the belly of each muscle, including the deltoid anterior (DA), deltoid posterior (DP), pectoralis (PS), infraspinatus (ID), and triceps (TC). The electrodes remained attached between trials, so the electrode positions were the same for all trials in each subject. When the electrodes were attached, the participants were asked to relax to avoid the influence of premature muscle activation on the experimental results. The subject took a lateral position: the angle between the seat back and the seat was adjusted to 85°, the rotation angle was adjusted to 15°, the shoulder abduction angle was 90°, the forearm was in a neutral position, the rotation axis of the isokinetic equipment was aligned with the center of the shoulder joint, and the range of motion (ROM) boundary was set. Before testing, the subject first performed three Flex and Ext exercises of the shoulder at an angular speed of 60°/s to become familiar with the movement. Then, the subject performed a group of 15 shoulder Add/Abd and Flex/Ext movements using the isokinetic test system at a speed of 180°/s with verbal encouragement to collect EMG signals as the pretest. After 20 minutes of acupuncture, as with the pretest, EMG signals were collected during another isokinetic test with verbal encouragement. The experimental process is shown in Figure 2.

*2.7. Data Analysis.* The data were further analyzed using MATLAB (version R2016a; MathWorks, Inc., Natick, MA) in terms of the average maximum torque, the average maximum work, the average maximum power, the average maximum speed, the total work and stiffness of the shoulder joint Add/Abd, and Flex/Ext muscle groups and including the EMG signals. Past studies have shown that torque, work, power, and speed can be used as measures of explosive force [27]. Stiffness is another measure of explosive force [28]. Therefore, in this study, we choose these parameters to assess the changes in the explosive forces generated by the shoulder joint muscles. The middle section of the selected EMG signal, lasting 5 s, was recorded as the isometric maximum voluntary contraction (MVC) of each muscle. All EMG signals were processed using specific routines carried out in MATLAB [29]. The EMG signal data are presented as MVC %. The stiffness of the shoulder joint ($K_{\text{joint}}$) tested was determined by the ratio of the change in the average torque ($\Delta T_{\text{joint}}$) to the change in the angle of the shoulder joint ($\Delta\theta_{\text{joint}}$) and was calculated using the following formula: $K_{\text{joint}} = (\Delta T_{\text{joint}}/\Delta\theta_{\text{joint}})$.

*2.8. Statistical Analysis.* All data were analyzed using SPSS 23.0 software (Chicago, IL, USA). The data are reported as the mean ± standard deviation (SD). The pretest and posttest kinetic data and EMG signals of the shoulder joint muscles were compared using paired $t$-tests. The significance level for all statistical analyses was set at $P < 0.05$.

## 3. Results

All participants successfully completed the study, and the EMG results are presented in Figure 3. There was a significant difference after stimulation in the Add/Abd and Flex/Ext of the DA, Deltoid DP, PS, ID, and TC of shoulder joint ($P < 0.05$) (see Figure 3).

Kinetic data of shoulder joint Add/Abd are shown in Table 1. The average maximum torque of Add significantly increased following acupuncture (+Δ45%, $P < 0.001$). The average work of Add/Abd significantly increased following

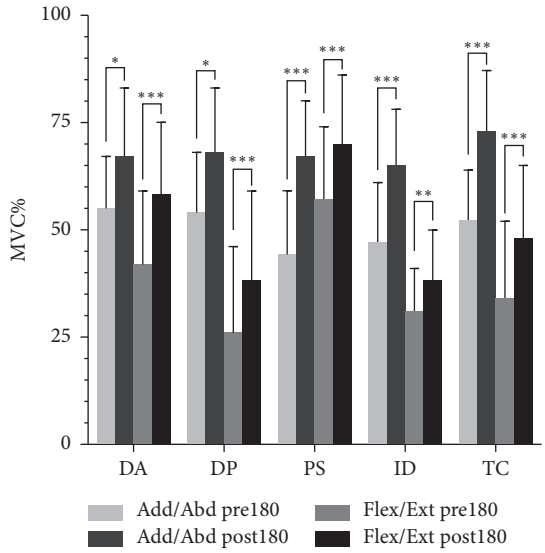


Figure 3: Differences in Add/Abd and Flex/Ext before and after acupuncture. Notes: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Shoulder joint adduction and abduction (Add/Abd), flexion, and extension (Flex/Ext). Deltoid anterior (DA); deltoid posterior (DP); pectoralis (PS); infraspinatus (ID); triceps (TC).

Table 1: Mean (SD) shoulder joint Add/Abd before and after acupuncture.

| Add/Abd | Pre 180 Mean ± SD | Post 180 Mean ± SD | $P$ |
|---|---|---|---|
| Average max torque, Add (Nm/kg) | 0.42 ± 0.12 | 0.61 ± 0.13 | <0.001 |
| Average max torque, Abd (Nm/kg) | 0.34 ± 0.13 | 0.33 ± 0.25 | 0.76 |
| Average work, Add (J/kg) | 0.50 ± 0.19 | 0.70 ± 0.15 | <0.001 |
| Average work, Abd (J/kg) | 0.44 ± 0.17 | 0.55 ± 0.15 | 0.001 |
| Average power, Add (W/kg) | 0.34 ± 0.15 | 0.59 ± 0.13 | <0.001 |
| Average power, Abd (W/kg) | 0.28 ± 0.10 | 0.41 ± 0.11 | 0.001 |
| Average max speed, Add (deg/s ∗ kg) | 1.98 ± 0.52 | 2.68 ± 0.35 | <0.001 |
| Average max speed, Abd (deg/s ∗ kg) | 1.73 ± 0.38 | 2.15 ± 0.27 | 0.002 |
| Total work, Add (J) | 426.76 ± 151.15 | 602.52 ± 105.61 | <0.001 |
| Total work, Abd (J) | 376.23 ± 127.02 | 468.94 ± 122.31 | 0.002 |
| Total work, Add + Abd (J) | 802.99 ± 274.49 | 1071.46 ± 209.04 | <0.001 |
| Stiffness, Add | 0.003 ± 0.007 | 0.936 ± 1.267 | 0.02 |
| Stiffness, Abd | 0.002 ± 0.001 | 0.049 ± 0.090 | 0.09 |

Differences were considered significant when $P < 0.05$. Add/Abd indicates shoulder joint adduction/abduction.

acupuncture (+Δ40%, +Δ25%, $P < 0.05$). The average power of Add/Abd significantly increased following acupuncture (+Δ74%, +Δ46%, $P < 0.05$). The average maximum speed of Add/Abd significantly increased following acupuncture (+Δ35%, +Δ24%, $P < 0.001$). The total work of Add/Abd significantly increased following acupuncture (+Δ41%, +Δ25%, $P < 0.05$). The total work (Add + Abd) increased following acupuncture (+Δ33%, $P < 0.001$). The stiffness of the joint during Add also significantly increased following acupuncture ($P = 0.02$). However, there was no significant difference in the average maximum torque of Abd ($P = 0.076$) or the stiffness of the joint during Abd ($P = 0.09$).

Kinetic data of shoulder joint Flex/Ext are shown in Table 2. The average maximum torque of Ext significantly increased following acupuncture (+Δ30%, $P < 0.001$). The average work of Flex/Ext significantly increased following acupuncture (+Δ26%, +Δ33%, $P < 0.001$). The average power of Flex/Ext significantly increased following acupuncture (+Δ47%, +Δ52%, $P < 0.001$). The average maximum speed of Flex/Ext significantly increased following acupuncture (+Δ23%, +Δ25%, $P < 0.001$). The total work of Flex/Ext significantly increased following acupuncture (+Δ27%, +Δ35%, $P < 0.001$). The total work (Flex + Ext) increased following acupuncture (+Δ31%, $P < 0.001$). The stiffness of the joint during Ext also significantly increased following acupuncture ($P = 0.002$). There was no significant difference in the average maximum torque of Ext ($P = 0.081$) or the stiffness of the joint during Flex ($P = 0.112$).

## 4. Discussion

*4.1. Analysis of Kinetic Data before and after Acupuncture.* In this study, the average maximum torque of the shoulder joint Add and Ext muscle groups increased after

Table 2: Mean (SD) shoulder joint Flex/Ext before and after acupuncture.

| Flex/Ext | Pre 180<br>Mean ± SD | Post 180<br>Mean ± SD | $P$ |
|---|---|---|---|
| Average max torque, Flex (Nm/kg) | 0.3 ± 0.11 | 0.36 ± 0.19 | 0.081 |
| Average max torque, Ext (Nm/kg) | 0.47 ± 0.12 | 0.61 ± 0.07 | <0.001 |
| Average work, Flex (J/kg) | 0.43 ± 0.09 | 0.54 ± 0.10 | <0.001 |
| Average work, Ext (J/kg) | 0.55 ± 0.16 | 0.73 ± 0.12 | <0.001 |
| Average power, Flex (W/kg) | 0.30 ± 0.09 | 0.44 ± 0.10 | <0.001 |
| Average power, Ext (W/kg) | 0.46 ± 0.18 | 0.70 ± 0.12 | <0.001 |
| Average max speed, Flex (deg/s ∗ kg) | 1.77 ± 0.32 | 2.18 ± 0.31 | 0.002 |
| Average max speed, Ext (deg/s ∗ kg) | 2.25 ± 0.56 | 2.81 ± 0.32 | <0.001 |
| Total work, Flex (J) | 364.91 ± 79.07 | 462.34 ± 93.19 | <0.001 |
| Total work, Ext (J) | 467.99 ± 127.00 | 632.92 ± 118.68 | <0.001 |
| Total work, Flex + Ext (J) | 832.90 ± 194.67 | 1095.26 ± 203.42 | <0.001 |
| Stiffness, Flex | 0.002 ± 0.002 | 0.154 ± 0.304 | 0.112 |
| Stiffness, Ext | 0.004 ± 0.005 | 0.112 ± 0.096 | 0.002 |

Differences were considered significant when $P < 0.05$. Flex/Ext indicates shoulder joint flexion/extension.

acupuncture. This may indicate that the stimulation of muscle contraction by acupuncture reflects an increase in the ability of the nervous system to drive the muscle to produce maximum torque. Acupuncture stimulates nerves to recruit more motor units and/or stimulate currently active motor units at higher frequencies, and segmental reflex rings may also be involved [30]. Therefore, in this study, acupuncture stimulation of the nerves may have accelerated limb reflexes and recruited additional motor units to increase muscle contraction and increase the average maximum torque. However, the average maximum torque of the shoulder joint Flex and Abd muscle groups was not effectively improved after acupuncture. This may be related to the specificity of acupuncture points. A previous study has shown that acupuncture stimulation of muscles can induce changes in motor cortex excitability and that the level of motor cortex excitability is related to the selected muscle and the needle insertion point [21]. Additionally, stimulating different acupuncture points will trigger different activation modes in the brain [31]. Therefore, in this study, the average maximum torque of the shoulder joint Flex and Abd muscle groups could not be effectively improved, possibly because of acupoint specificity.

The average maximum power, average maximum work, average maximum speed, and total work of the shoulder joint Add/Abd and Flex/Ext muscle groups increased after acupuncture. This is likely because acupuncture can effectively increase muscle function, such as power, work output, and contraction speed. Previous studies have suggested that the stimulation of nerves by acupuncture can change the excitability of cortical motor neurons [32] as well as lead to the recruitment of more motor units and/or the generation of higher-frequency activity of already active motor units [30]. The effect was similar to that of PAP. A study has shown that PAP can increase work and power output [18]. One mechanism for this phenomenon is the phosphorylation of myosin regulatory light chains. Additionally, myosin light chain kinase is responsible for the formation of the actin-myosin complex to provide more adenosine triphosphate (ATP) [20], which increases the muscle's ability to do work. Another study used PAP principles to explain why short-term exercise improves performance. The reason is that the stimulation of motor nerves can enhance the tolerance of muscles to aerobic exercise, delay muscle fatigue, and improve exercise performance [33]. Another possible mechanism is that the PAP phenomenon causes an increase in the recruitment of motor units, increasing the efficiency and degree of neurotransmitter transfer and recruiting higher-level motor units (type II muscle fibers), thereby increasing the ability of muscles to produce force and increasing subsequent explosive performance [34]. When acupuncture is applied to specific points on the body surface for stimulation, it can activate multiple pathways in the nervous system, accelerate the feedback speed of neurons, and stimulate muscles to respond quickly [35]. Therefore, in this study, acupuncture of the acupoints of the shoulder joint Add/Abd and Flex/Ext muscle groups improved the ability of the muscles to perform work and increased the explosive force of the shoulder joint, which may be related to the benefits of PAP.

*4.2. Analysis of Surface EMG Signals before and after Acupuncture.* EMG signals were significantly enhanced after acupuncture. Research suggests that manual acupuncture (MA) or electroacupuncture (EA) at particular acupoints activates afferent fibers that send signals to the spinal cord [1]. The regulation of motor neuron activity is realized through the input of the spinal cord and the upper spinal cord. Short-term and long-term acupuncture stimulation of somatosensory afferent nerves can induce neuromuscular excitability changes at the level of the spinal cord, especially the upper spinal cord [36]. Additionally, acupuncture stimulates peripheral $\alpha$ motor neurons by stimulating peripheral nerves to generate nerve impulses, increasing the transmission rate of spinal nerve impulses and the firing frequency of motor units [20, 34]. In another study, the excitability of spinal motor neurons increased significantly after repeated acupuncture with H reflex measurement in the musculus soleus [37]. Therefore, in this study, acupuncture may have increased nerve impulses and activated acupuncture-related muscles by stimulating nerves, eventually enhancing the EMG signal at the acupuncture site.

*4.3. Effect of Joint Motion on Muscle Stiffness.* In this study, the stiffness of the shoulder Add and Ext muscle groups significantly increased after acupuncture. Past studies have shown that, among the factors that affect joint stiffness, the most important is the degree of muscle activation [38]. Additionally, soft tissues, such as tendons, also increase muscle stiffness as they contract or stretch [39]. Therefore, in this study, acupuncture stimulated nerves to activate related muscles and increased the average maximum torque and stiffness of the shoulder joint Add and Ext muscle groups.

*4.4. Limitations.* The limitations of this study are that the effects of fake acupuncture were not assessed, and muscle blood flow was not measured to determine the mechanism of acupuncture.

## 5. Conclusion

In this study, isokinetic testing and acupuncture were used to investigate the effects of acupuncture on the explosive muscle force of the female shoulder joint during Add/Abd and Flex/Ext. The results show that acupuncture may induce PAP and increase the average work, power, torque, and speed of the muscles related to the acupoints, thereby increasing the explosive forces generated by the shoulder joint muscle groups. The results support the hypothesis that acupuncture can improve the explosive forces generated by the shoulder joint muscles after isokinetic exercise through corresponding neurophysiological responses. Therefore, acupuncture may be used as an alternative medicine to improve physical performance in sports medicine. However, the average maximum torque of the shoulder Flex and Abd groups did not show a statistically significant difference after acupuncture. This may be related to the selected acupoints, so the specificity of acupuncture and potentially related factors need to be further explored in future studies.

## Data Availability

The datasets used and analyzed in the current study are included in this article.

## Ethical Approval

The protocol used with the subjects was reviewed and approved by the Joint Institutional Review Board of Jilin Sport University (JLSU; Changchun, China; JLSU-IRB no. 2018004).

## Disclosure

All authors have read and approved the final manuscript.

## Conflicts of Interest

The authors declare there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Authors' Contributions

ILW and YMC designed the experiments. JW interpreted the results, prepared figures, and wrote the manuscript. HR and KKZ prepared and performed the laboratory experiments. ILW, YMC, and HCS contributed reagents and materials and analyzed the data. All authors have read and approved the final version of the manuscript.

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