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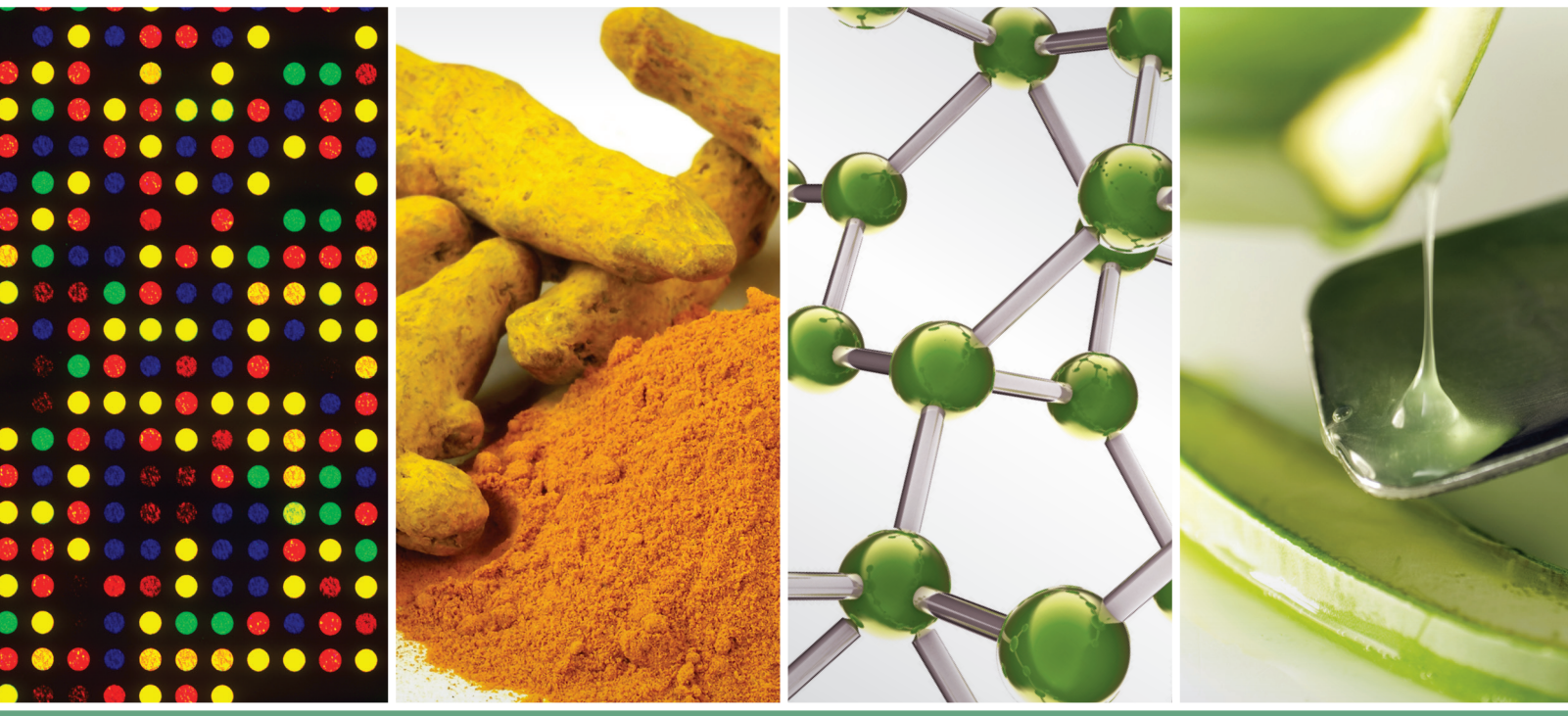
Guest Editors: Musa T. Yakubu, Taofik O. Sunmonu, Francis B. Lewu, Anofi O. T. Ashafa, Femi J. Olorunniji, and Mohamed Eddouks



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Editorial

Medicinal Plants Used in the Management of Diabetes Mellitus 2015

Musa T. Yakubu,[1] Taofik O. Sunmonu,[2] Francis B. Lewu,[3] Anofi O. T. Ashafa,[4] Femi J. Olorunniji,[5] and Mohamed Eddouks[6]

[1] *Phytomedicine, Toxicology and Reproductive Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin 240003, Nigeria*
[2] *Phytomedicine, Environmental Toxicology and Plant Biochemistry Research Laboratory, Department of Biological Sciences, Al-Hikmah University, Ilorin 240001, Nigeria*
[3] *Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Wellington Campus, Wellington, Western Cape 7655, South Africa*
[4] *Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba 9866, South Africa*
[5] *Institute of Molecular, Cell, and System Biology, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*
[6] *Faculty of Sciences and Techniques Errachidia, Moulay Ismail University, 52000 Errachidia, Morocco*

Correspondence should be addressed to Musa T. Yakubu; tomuyak@yahoo.com

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Diabetes mellitus is one of the common endocrine disorders prevalent in almost all of the countries. This chronic pathology is characterized by hyperglycemia caused by defective insulin action, insulin secretion, or the combination of both. Prolonged persistence of elevated blood glucose level consequently caused a series of complications such as nephropathy, retinopathy, and cardiomyopathy. Currently available synthetic drugs for treating this disease are found to be associated with many adverse effects. The use of plants in medicine is an age-long practice in various parts of the globe for both preventive and curative purposes. Several warnings have been issued over lack of quality control, scientific evidence for the efficacy, and potential adverse effects of herbal remedies including hepatotoxicity, nephrotoxicity, cardiotoxicity, and reproductive toxicity among others. Despite all of these, reliance on herbs as medicine for the management of diabetes mellitus is still much practiced by a large proportion of the world population because they are readily available and affordable with perceived reduced toxicity. Therefore, with the upsurge of interests in medicinal plants, there is a need for thorough scientific investigations of these plants for both efficacy and potential toxicity. In this issue, we present some recent advances in the use of medicinal plants for treating diabetes mellitus. B. Pang et al. ("Innovative Thoughts of Treating Diabetes from the Perspective of Traditional Chinese Medicine")presented a review article on the contribution of traditional Chinese medicine to the development of alternative and complementary medicine for the treatment and prevention of diabetes mellitus. In another paper ("Effect of Rhizoma Coptidis (Huang Lian) on Treating Diabetes Mellitus"), B. Pang et al. discussed the efficacy and safety of Rhizoma Coptidis in the treatment of diabetes mellitus. In another study ("Evaluation of the Effects of *Cornus mas* L. Fruit Extract on Glycemic Control and Insulin Level in Type 2 Diabetic Adult Patients: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial"), R. Soltani et al. reported the results of a clinical trial on the effect of *Cornus mas* L. fruit extract on hyperglycemia in type 2 diabetic patients. In addition, W. Liu et al. ("The Effects of Chinese Medicine on Activation of Wnt/β-Catenin Signal Pathway under High Glucose Condition") present a valuable review on some compounds implicated in the regulation of Wnt/β-catenin signal pathway as a mechanism of action involved in the antihyperglycemic activity from

Chinese medicine. Furthermore, A. O. T. Ashafa and M. I. Kazeem ("Toxicopathological Evaluation of Hydroethanol Extract of *Dianthus basuticus* in Wistar Rats") reported on the effects of *Dianthus basuticus* (a Basotho plant with acclaimed antidiabetic activity) on some biochemical parameters and histology of Wistar rats. Finally, X.-J. Li et al. ("TCM Formula Xiaoyaosan Decoction Improves Depressive-Like Behaviors in Rats with Type 2 Diabetes") evaluated the effect of traditional medicine formula, Xiaoyaosan, on the cognitive function of diabetic rats. After the first volume of this special issue that was published in 2014, we hope that this issue will present additional valuable information for scientists and clinicians.

Acknowledgments

We would like to appreciate all the authors for their contributions to this issue; 16 manuscripts were submitted and only 6 were finally accepted for publication. We would like to appreciate all the reviewers for their expertise and time.

Musa T. Yakubu
Taofik O. Sunmonu
Francis B. Lewu
Anofi O. T. Ashafa
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Mohamed Eddouks

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Research Article

TCM Formula Xiaoyaosan Decoction Improves Depressive-Like Behaviors in Rats with Type 2 Diabetes

Na Li, Qun Liu, Xiao-Juan Li, Xiao-Hui Bai, Yue-Yun Liu, Hong-Bo Zhao, Zhong-Ye Jin, Yu-Xia Jing, Zhi-Yi Yan, and Jia-Xu Chen

School of Basic Medical Science, Beijing University of Chinese Medicine, Beijing 100029, China

Correspondence should be addressed to Jia-Xu Chen; chenjiaxu@hotmail.com

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The mechanism of depression with type 2 diabetes remains elusive, requiring further study. *Objective*. To evaluate the effect of TCM formula Xiaoyaosan on depressive-like behaviors in rats with type 2 diabetes. *Methods*. Rats were divided into 5 groups and drugs were administered during the model period of 21 days. The model of depressive-like behaviors in rats with type 2 diabetes was induced by a high fat diet, low doses of STZ injection, and chronic restraint stress for 21 days. The body weight, fasting blood glucose, ITT, OGTT, 5-HT, DA, depression behaviors, and morphological changes of formation were measured and observed. *Results*. After modeling, marked changes were found in model rats; behavioral analyses of rats indicated that this modeling method negatively impacts locomotor function. In the H&E staining, changes were found predominately in the CA1 and DG subregions of the hippocampus. After 21 days of treatment by fluoxetine and Xiaoyaosan, rats' body weights, behaviors and fasting blood glucose, and hippocampal formation were modified. *Conclusions*. A new model of depressive-like behaviors in rats with type 2 diabetes was successfully created. Xiaoyaosan and fluoxetine in this study independently contribute to exacerbate the disease progression.

1. Introduction

Depression in people with diabetes is globally prevalent. The condition affects quality of life [1], glucose control [2], nonadherence to treatment [3], cost of living [4], mortality, and life expectancy. According to the published studies, up to 45% of psychiatric disorders are not detected among the people suffering from diabetes. It has been identified that race, advanced age, and lack of health insurance were closely related to increased undertreatment for psychiatric disorders [5]. Chronically depressed individuals are more likely to have adverse effects associated with diabetes. One out of ten diabetes patients have been reported to suffer from apparent depressive symptoms, and as high as 25–30% of patients have inconspicuous depressive symptoms [6, 7]. It has been shown that people with depression and diabetes have higher mortality rates than individuals with diabetes exclusively, and the occurrence of self-harm and committing suicide is more frequent in patients with diabetes than in healthy population [8]. This indicates that the high prevalence of depression in diabetes patients needs further studies of the mechanism and medical treatment.

Xiaoyaosan decoction originated in *Taiping Huimin Heji Jufang*, created in the Song Dynasty of China (960–1127 AD). The decoction composition is composed of 8 crude herbs, Radix Angelicae Sinensis, Radix Paeoniae Alba, Poria, Radix Bupleuri, Radix Glycyrrhizae, Rhizoma Atractylodis Macrocephalae, Herba Menthae, and Rhizoma Zingiberis Recens. Xiaoyaosan decoction contains various chemical compounds, such as paeoniflorin, liquiritin, curcumin, and saikosaponins [9]. The decoction has been extensively used to treat mental diseases such as depression and the syndromes such as liver stagnation and spleen deficiency in traditional Chinese medicine clinical practice. Also, Xiaoyaosan decoction is used for the prevention and treatment of multiple-system diseases such as psychiatric disorders, neurological diseases, digestive system diseases, gynecologic diseases, and endocrine diseases [10–14]. Additionally, modified Xiaoyaosan has a significant effect to regulate diabetes [15].

While clinical studies provide evidence for the assessment of physiological context, an animal model is needed to provide the appropriate mechanistic characterization for a systemic pathology.

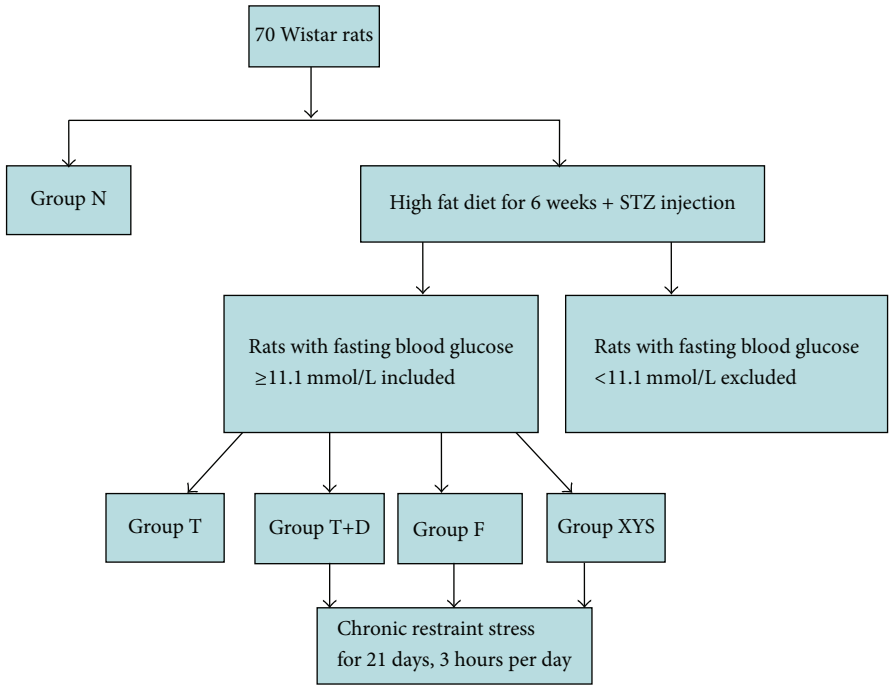


Figure 1: Study design.

The objective of this research was to observe the effect of Xiaoyaosan decoction on depressive-like behaviors in rats with type 2 diabetes. Despite decades of research, little knowledge is recognized about the mechanisms supporting the depression combined with diabetes, which is quite complex. We expect our study in this paper will be able to provide empirical evidences with animal experiment.

2. Materials and Methods

2.1. Animal. 170 ± 20 g male Wistar rats (SCXK(Jing)2012-0001) were housed under standard laboratory conditions 24 ± 1°C, 45 ± 15% relative humidity, and 12 h/12 h dark/light cycle with food and water freely available (SYXK(Jing)2011-0024).

2.2. Study Design. Wistar rats were divided into two groups, 8 rats for normal group stressor (group N, no stressor plus deionized water), fed with standard diet ([SCXK(Jing)2009-0012]); 62 rats for the high fat diet group (compound feed: 68.8% of basic feed, 10% of sugar, 10% of lard oil, 10% of yolk powder, 1% of cholesterol, 0.2% of cholate [SCXK(Jing)2009-0008]). After six weeks of dietary treatment, rats in the high fat diet group were intraperitoneally injected with a single dose of streptozotocin (35 mg/kg; Sigma, USA). Fasting blood glucose was measured after three days and seven days. Forty-six rats with fasting blood glucose higher than 11.1 mmol/L were selected from the diabetes group. The other rats with fasting blood glucose lower than 11.1 mmol/L were excluded. The rats in the diabetes group were divided into four groups, including the type 2 diabetes group (group T, no stressor and deionized water), the depressive-like behaviors in rats with type 2 diabetes group (group T+D, stressor plus deionized water), the depressive-like behaviors in rats with type 2 diabetes model with fluoxetine group (group F, stressor plus fluoxetine), and the depressive-like behaviors in rats with type 2 diabetes model with Xiaoyaosan group (group XYS, stressor plus Xiaoyaosan), with 8 rats in each group. The model in group T+D was established on the basis of characteristics of emotional diseases and methods in the literature [16]. Some rats were randomly selected for chronic immobilization stress [17]. The rats were bound to a type T binding platform, which consists of the base (10 cm × 20 cm × 2.8 cm) and the upper part of the binding platform (22 cm × 6.6 cm). The front end had small frames for fixing the head and small grooves for limbs; the upper binding platform had two adjustable soft belts for, respectively, fixing the abdomen and chest. The rats were bound for 3 hours per day, randomly selected from 8 am to 7 pm to prevent the adaptation to a fixed binding time [18]. Study design see (Figure 1).

Experimental procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals. The animal protocol was approved by the Committee on the Ethics of Animal Experiments of Beijing University of Chinese Medicine.

2.3. Preparation of Extracts of Xiaoyaosan Decoction. Xiaoyaosan decoction consists of 300 g of *Poria cocos* (Schw.) Wolf (*Poria*), 300 g of *Paeonia lactiflora* Pall. (Radix Paeoniae Alba), 150 g of *Glycyrrhiza uralensis* Fisch. (Radix Glycyrrhizae), 300 g of *Bupleurum chinense* DC. (Radix Bupleuri), 300 g of *Angelica sinensis* (Oliv.) Diels (Radix Angelicae Sinensis), 300 g of *Atractylodes macrocephala* Koidz. (Rhizoma Atractylodis Macrocephalae), 100 g of *Mentha haplocalyx* Briq. (Herba Menthae), and 100 g of *Zingiber officinale*

Rosc. (Rhizoma Zingiberis Recens). These eight herbs were purchased from Beijing Tongrentang Co., Ltd. The 8 herbs were processed into dry extract in the Chinese medicine preparation room of the China-Japan Friendship Hospital (Beijing), following the Regulation on Processing of Traditional Chinese Medical Herbal Pieces of Beijing. All raw materials were extracted by boiling water three times, and then the decoction was dehydrated in vacuo (70°C) and ground into powder for use. The extraction rate of the dry extract was 18.8%, dosage of Xiaoyaosan = 6.17 × crud herbs ÷ 60 kg (normal human body weight) × extraction rate (actual dry powder/actual crude herbs). Xiaoyaosan dissolved in deionized water was gavaged at a dose of 3.854 g/Kg·d [19], one time per day, 1 mL/100 g bodyweight. 20 mg/capsule of fluoxetine dissolved in deionized water was gavaged based on body weight. Group N, group T, and group T+D were gavaged with deionized water.

2.4. Instruments and Method of High Performance Liquid Chromatography Coupled with LTQ Orbitrap Mass Spectrometry. 1 g Xiaoyaosan powder was put into 25 mL of 70% methanol-water solution, then the mix solution was ultrasonic extracted for 30 minutes at room temperature, filtered at 0.22 μm filter, stored at 4°C.

Accela High performance liquid chromatography and LTQ Orbitrap XL were purchased from Thermo Fisher Scientific Company (America); methanol (HPLC Grade) and formic acid (HPLC Grade) were purchased from Thermo Fisher Scientific Company (America); reference standards were purchased from the Chengmust Company, Sichuan Province, China.

Xiaoyaosan was performed on high performance liquid chromatography (HPLC) Accela 600 pump, LTQ Orbitrap XL (Thermo Fisher Scientific Company, America) using a SB-Aq column (4.6 × 250 mm, 5 micron, Agilent Technologies, USA), Capillary Voltage 2500 V–3000 V, Tubeleu 110 V, Scan range 100–1500, Sheath Gas 30 psi, and Aux Gas Flow 10 psi.

Method. The mobile phases comprised eluent A (0.1% formic acid) and eluent B (methanol). The gradient flow was as follows: 0~5 minutes, 30% B; 5~40 minutes, 30–90% B; 40~45 minutes, 90% to 100% B; 45~50 minutes, 100% B. The analysis was performed at a flow rate of 1.0 mL/min. The injection volume was 10 μL.

2.5. Body Weight, Fasting Blood Glucose, Oral Glucose Tolerance (OGTT), and Insulin Tolerance Test (ITT). Body weights were monitored and measured every 7 days.

Fasting blood glucose was measured via tail vein after overnight fasting using an ultrasensitive hand-held glucometer (Johnson and Johnson, USA).

For OGTT, rats were fasted overnight and gavaged with glucose (2.5 g/kg body weight). Glucose levels were measured both before and 30, 90, 120, and 180 minutes after glucose administration.

For ITT, rats were intraperitoneally injected with insulin (0.6 units/kg body weight), and blood glucose levels were measured both before and 30, 90, 120, and 180 minutes after insulin administration.

2.6. Open Field Test. The open field test was monitored by camera on day 21 of chronic stress. The activity of the rats was measured in a 100 cm × 100 cm × 40 cm cube with wood walls and wood floor, without ceiling (handmade), which is covered by black paint. The chambers are individually divided into 25 squares by yellow paint. The activity monitor camera was on the top of the middle square (Panasonic, Japan), and both horizontal and vertical movements were analyzed by Observer 5.0 software (Noldus, Netherlands) and EthoVision 3.0 software (Noldus, Netherlands). Each rat was placed in the central square and observed for 5 minutes, testing in the apparatus once. Scores were calculated by the amount of time, including the rat's movement speed, out zone definition (s), spent rearing (defined as standing upright on its hind legs), the number of crossings in the grid lines (it crossed with at least three paws), and licking frequencies.

2.7. Preparation of Serum. Venous blood samples were obtained after anesthetization from all the groups and collected in tubes and then centrifuged at 3000 r/min for 10 minutes at 4°C. Serum was collected after 4 hours and then quick-frozen in liquid nitrogen and stored in a −80°C freezer for measuring 5-hydroxytryptamine and dopamine.

2.8. 5-Hydroxytryptamine and Dopamine. 5-Hydroxytryptamine (5-HT) (Lot: 20141130, 60087R) and dopamine (DA) (Lot: 20141130, 60088R) levels were measured by ELISA (Thermo Multiskan MK3, Finland) and the concentration was calculated according to the standard curves.

2.9. Hematoxylin and Eosin Staining (H&E Staining). All rats were processed by deep anaesthesia with 10% chloral hydrate (0.4 mL/kg) and were transcardially perfused through the ascending aorta with 200–300 mL of cold saline, followed by 300 mL of 4% cold paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 25 minutes. The brain was taken out and postfixed in the same fixative at 4°C for 6–10 hours before immersing in a 20% sucrose solution in 0.1 M PB at 4°C. The brains were frozen in liquid nitrogen and sectioned (30 μm) using a freezing microtome (Leica-CM 1900, Germany) at −20°C. Brain sections were then thawed and mounted onto microscope slides that were previously coated with 10% polylysine. The slides were stored in a freezer (−70°C) prior to use [20].

H&E staining was performed as follows: after dewaxing with xylene, sections were stained with hematoxylin (Sigma, America) and eosin solution (Sigma, America).

2.10. Image Analysis. Image analysis was completed by the use of an image analyzer (MIAS99) with a color video camera (JVC TK-C1381) and an Olympus BX50 microscope.

2.11. Statistical Analysis. All numerical data were expressed as mean ± standard deviation ($\overline{x} \pm s$). A mixed design analysis of variance (ANOVA) by SPSS 17.0 for Windows was used to analyze significant differences, with $P < 0.05$ considered significant.

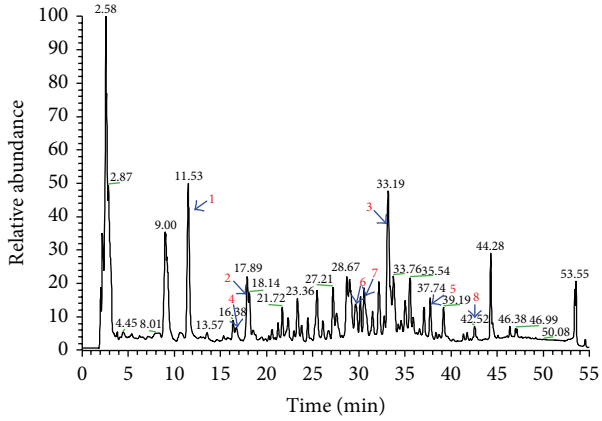


Figure 2: HPLC-LTQ-Orbitrap-MS chromatogram of eight ingredients from Xiaoyaosan samples. In Figures 2 and 3, HPLC-LTQ-Orbitrap-MS chromatogram of eight matched references in Xiaoyaosan sample from Tongrentangs 1, 2, 3, 4, 5, 6, 7, and 8 represents paeoniflorin, liquiritin, glycyrrhizic acid, ferulic acid, saikosaponins A and C, curcumin, and *Poria cocos* alcohol.

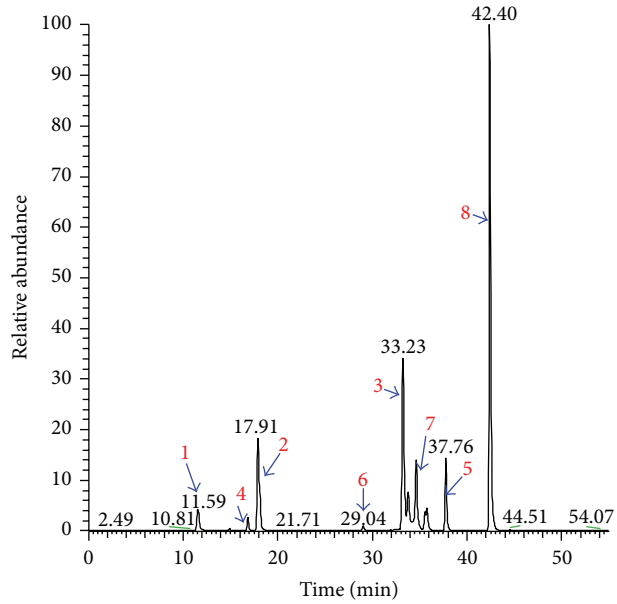


Figure 3: HPLC-LTQ-Orbitrap-MS chromatogram of eight reference standards. In Figures 2 and 3, HPLC-LTQ-Orbitrap-MS chromatogram of eight matched references in Xiaoyaosan sample from Tongrentang 1, 2, 3, 4, 5, 6, 7, and 8 represents paeoniflorin, liquiritin, glycyrrhizic acid, ferulic acid, saikosaponins A and C, curcumin, and *Poria cocos* alcohol.

3. Results

3.1. Compositional Analysis of Xiaoyaosan by HPLC-LTQ-Orbitrap-MS. Eight compounds, including paeoniflorin, liquiritin, glycyrrhizic acid, ferulic acid, saikosaponins A and C, curcumin, and *Poria cocos* alcohol in Xiaoyaosan samples were determined by HPLC-LTQ-Orbitrap-MS. The eight compounds are active ingredients in Radix Paeoniae Alba, liquorice, *Angelica sinensis*, Radix Bupleuri, fresh ginger, and *Poria cocos*, respectively. The alignment of the compounds with extracts of Xiaoyaosan indicated that eight compounds could match the corresponding peaks of Xiaoyaosan by the same HPLC-LTQ-Orbitrap-MS eluted system (Figures 2 and 3). The results suggested that the eight compounds might be quality control references of Xiaoyaosan.

3.2. Body Weight at Days 0, 7, 14, and 21. Compared with group N, rats in the other groups showed a significant decrease in the body weight at days 0, 7, 14, and 21 ($P < 0.05$, $P < 0.01$). Rats in group XYS showed a significant increase in body weight at days 14 and 21 compared with group T+D ($P < 0.05$), but there was no significant difference between group F and group T+D ($P > 0.05$) (Figure 6).

3.3. Fasting Blood Glucose. Compared with group N, rats in the other groups showed a significant increase in the fasting blood glucose at days 0, 7, 14, and 21 ($P < 0.01$). Rats in group XYS and group F showed a significant decrease in the fasting blood glucose at day 21 compared with group T+D ($P < 0.05$) (Figure 7).

3.4. Insulin Tolerance Test (ITT). Compared with group N, rats in other groups showed a significant increase in the fasting blood glucose at 0, 30, 60, 120, and 180 minutes ($P < 0.01$), and it did not return to normal at 180 minutes. Compared with group T+D, there were significant differences in group XYS and group F at 30, 60, 120, and 180 minutes ($P < 0.05$, $P < 0.01$) (Figure 8).

3.5. Oral Glucose Tolerance Test (OGTT). Compared with group N, rats in other groups showed a significant increase in the fasting blood glucose at 0, 30, 60, 120, and 180 minutes ($P < 0.01$), and it did not return to normal at 180 minutes. Compared with group T+D, there were significant differences in group XYS and group F at 30, 120, and 180 minutes ($P < 0.05$, $P < 0.01$), but the blood glucose did not recover to normal after 180 minutes (Figure 9).

3.6. 5-Hydroxytryptamine (5-HT) and Dopamine (DA) in Serum. Compared with group N, rats in the other groups showed no significant change in 5-HT and DA ($P > 0.05$). Rats in group T showed a significant increase in 5-HT compared with group T+D ($P < 0.05$). Rats in group XYS showed a significant increase in DA compared with group T+D ($P < 0.05$) (Figures 10(a) and 10(b)).

3.7. Scores on Open Field Activity. Open field tests were conducted at day 21 of the chronic restraint stress (Figures 11(a), 11(b), 11(c), 11(d), 11(e), and 4). In these behavioral tests, there were significant differences ($P < 0.01$, $P < 0.05$) among the rats in group N and group T+D, group T+D and group XYS, and group T+D and group F, with no differences between group N and group XYS, indicating that the model of depressive-like behaviors in rats with type 2 diabetes was successfully established, and the behavior of rats in group XYS and group F resumed to normal levels after 21 days

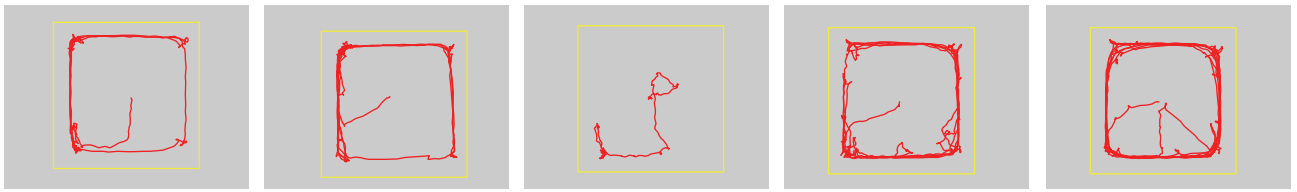


Figure 4: Representative behavior track plot reports of rats in different groups as assessed by open field test using video tracking software, indicating that this modeling method negatively impacts locomotor function. In group XYS and group F, rats showed improvement in locomotor function.

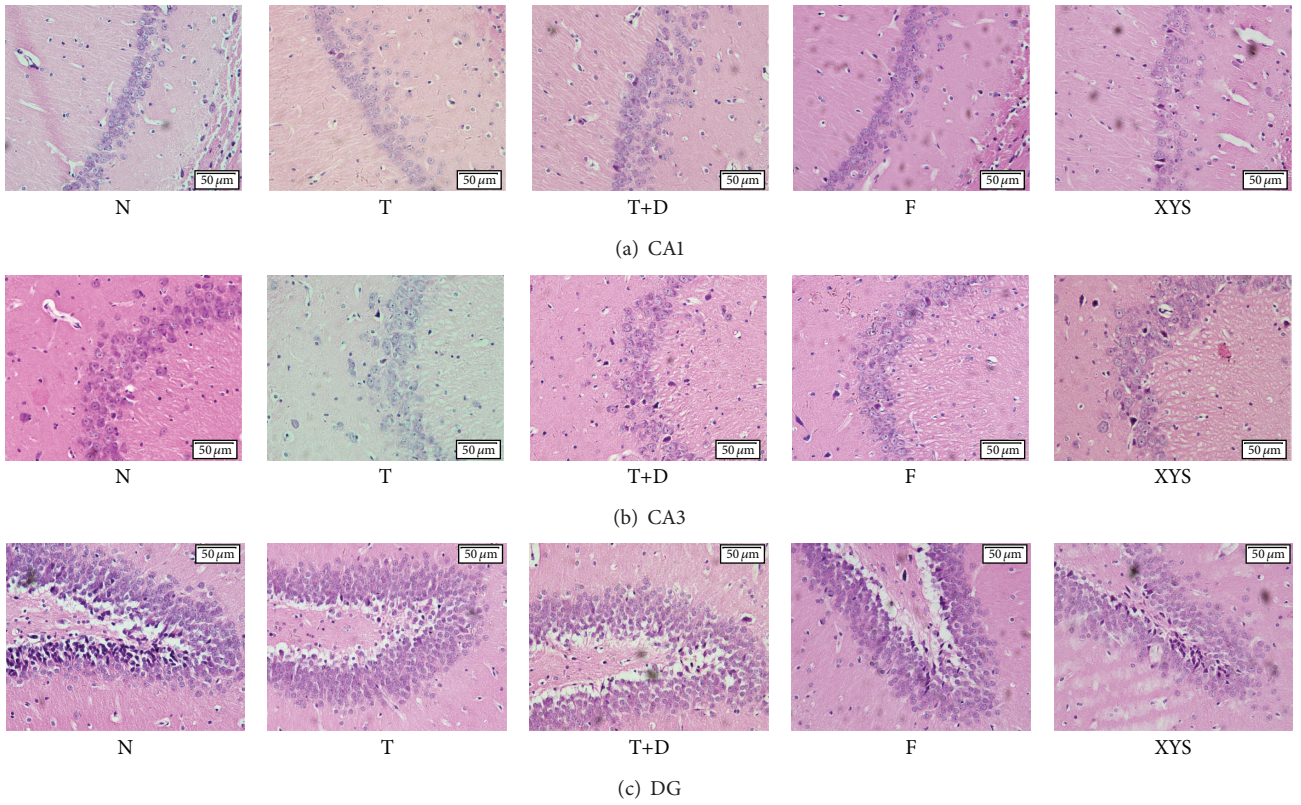


Figure 5: Histology of rat hippocampal structure in CA1, CA3, and DG subregions. Changes were found predominately in the CA1 and dentate gyms (DG) region of the hippocampus, rather than the CA3 subregion as seen in the pictures. Compared with group N and group T, obvious damage in hippocampal neurons appeared condensed and pyknotic in group T+D after high fat diet, STZ injection, and chronic restraint stress. The chromatins were in order, neurons increased, and less condensed and damaged neurons were found in group XYS and group F, compared with group T+D.

treatment of Xiaoyaosan or fluoxetine in spite of the exposure to chronic restraint stress.

3.8. H&E Staining. Paraffin embedded brain sections were dewaxed and stained with hematoxylin and counterstained with eosin. The hippocampal structure was observed by electron microscope. Changes were found predominately in the CA1 and dentate gyms (DG) region of the hippocampus, rather than the CA3 subregion as seen in the pictures. The effect of chronic restraint stress on the histology of rat hippocampal structure (CA1, CA3, and DG) showed that the formation of the rat model in the rat's hippocampus induced neuronal loss or death and impacted locomotor function. Compared with group N and group T, obvious damage in hippocampal neurons in other groups appeared condensed and pyknotic. It indicated that marked neuronal degeneration, the death of CA1 pyramidal neurons in the hippocampus, is consistent with stress-related psychiatric illnesses [21]. Compared with group T+D, the chromatins were in order, neurons increased, and less condensed and damaged neurons were found in group XYS and group F. Compared with group T+D, there were signs of recovery in group F and group XYS after treatment, indicating that Xiaoyaosan and fluoxetine had an effect on model rats (Figure 5(a) CA1, Figure 5(b) CA3, and Figure 5(c) DG).

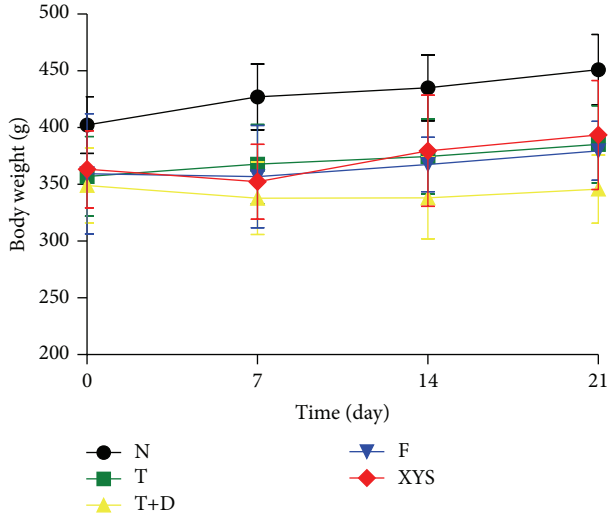


Figure 6: Body weight (g) of the rats in each group. Data are expressed as $\overline{x} \pm s$, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus group N; $^{*}P < 0.05$, $^{**}P < 0.01$ versus group T+D.

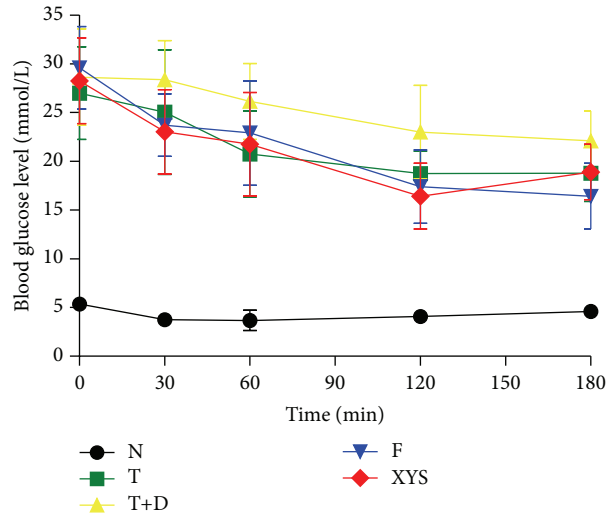


Figure 8: ITT (mmol/L) of rats in each group. Data are expressed as $\overline{x} \pm s$, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus group N; $^{*}P < 0.05$, $^{**}P < 0.01$ versus group T+D.

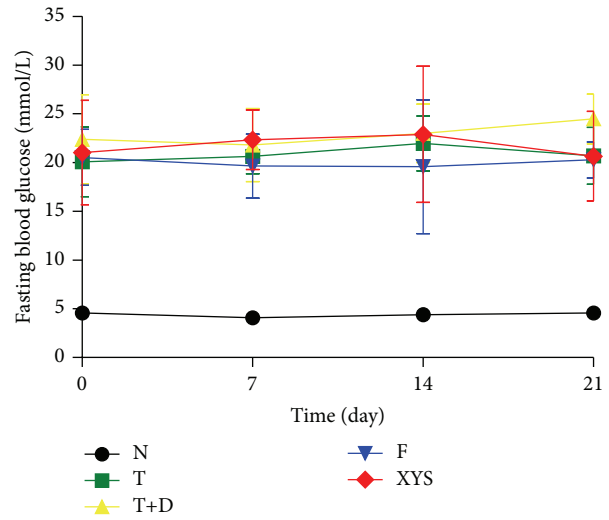


Figure 7: Fasting blood glucose (mmol/L) of rats in each group. Data are expressed as $\overline{x} \pm s$, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus group N; $^{*}P < 0.05$, $^{**}P < 0.01$ versus group T+D.

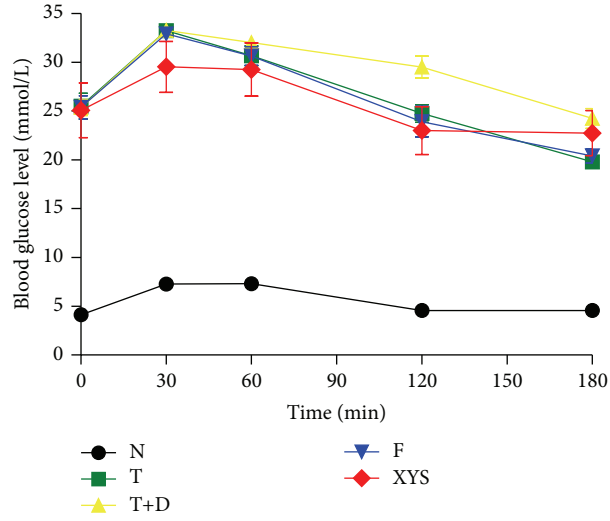
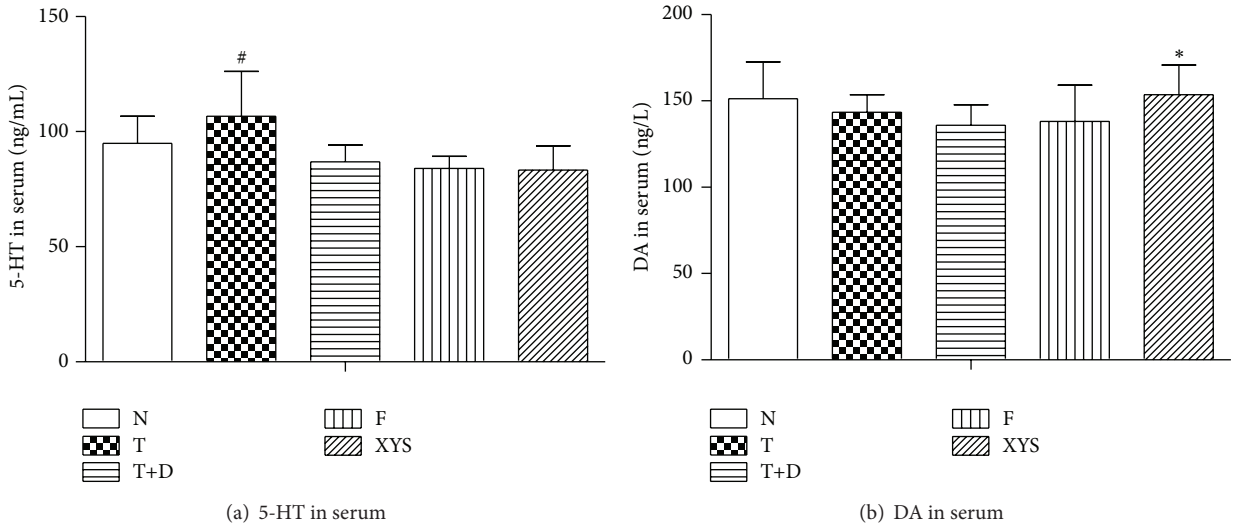


Figure 9: OGTT (mmol/L) of rats in each group. Data are expressed as $\overline{x} \pm s$, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus group N; $^{*}P < 0.05$, $^{**}P < 0.01$ versus group T+D.

4. Discussion

As a traditional Chinese formula consisting of multiple compounds, Xiaoyaosan targets both depression and diabetes. The constituent chemical compounds, such as paeoniflorin, liquiritin, curcumin, and saikosaponins A and C, are active ingredients as antidepressants [22–26], while curcumin and paeoniflorins have antidiabetic effects [27–30]. The HPLC-LTQ-Orbitrap-MS chromatogram results showed that the eight compounds, including paeoniflorin, liquiritin, glycyrrhizic acid, ferulic acid, saikosaponins A and C, curcumin, and *Poria cocos* alcohol derived from Radix Paeoniae Alba, liquorice, *Angelica sinensis*, Radix Bupleuri, fresh ginger, and *Poria cocos*, respectively, could match corresponding peaks of reference standards by the same HPLC-LTQ-Orbitrap-MS eluted system. The results suggested that the 8 compounds might be quality control references of Xiaoyaosan. As the components are complicated, the analytical work and the evaluation of the disassembled prescription in detail should be further studied. The work is still ongoing.

In this study, after a high fat diet intake and STZ injection, the model rats' sugar tolerance was impaired. A significant body weight decrease has been observed after 21 days of chronic restraint stress. The neurotransmitter 5-hydroxytryptamine and dopamine, which play a role in central fatigue [31], mood regulation, movement, learning,



(a) 5-HT in serum

(b) DA in serum

Figure 10: 5-Hydroxytryptamine (5-HT) and dopamine (DA): (a) 5-HT (ng/mL) of rats in each group. (b) DA (ng/L) of rats in each group. Data are expressed as $\overline{x} \pm s$, $^{\#}P < 0.05$ versus group N; $^{*}P < 0.05$ versus group T+D.

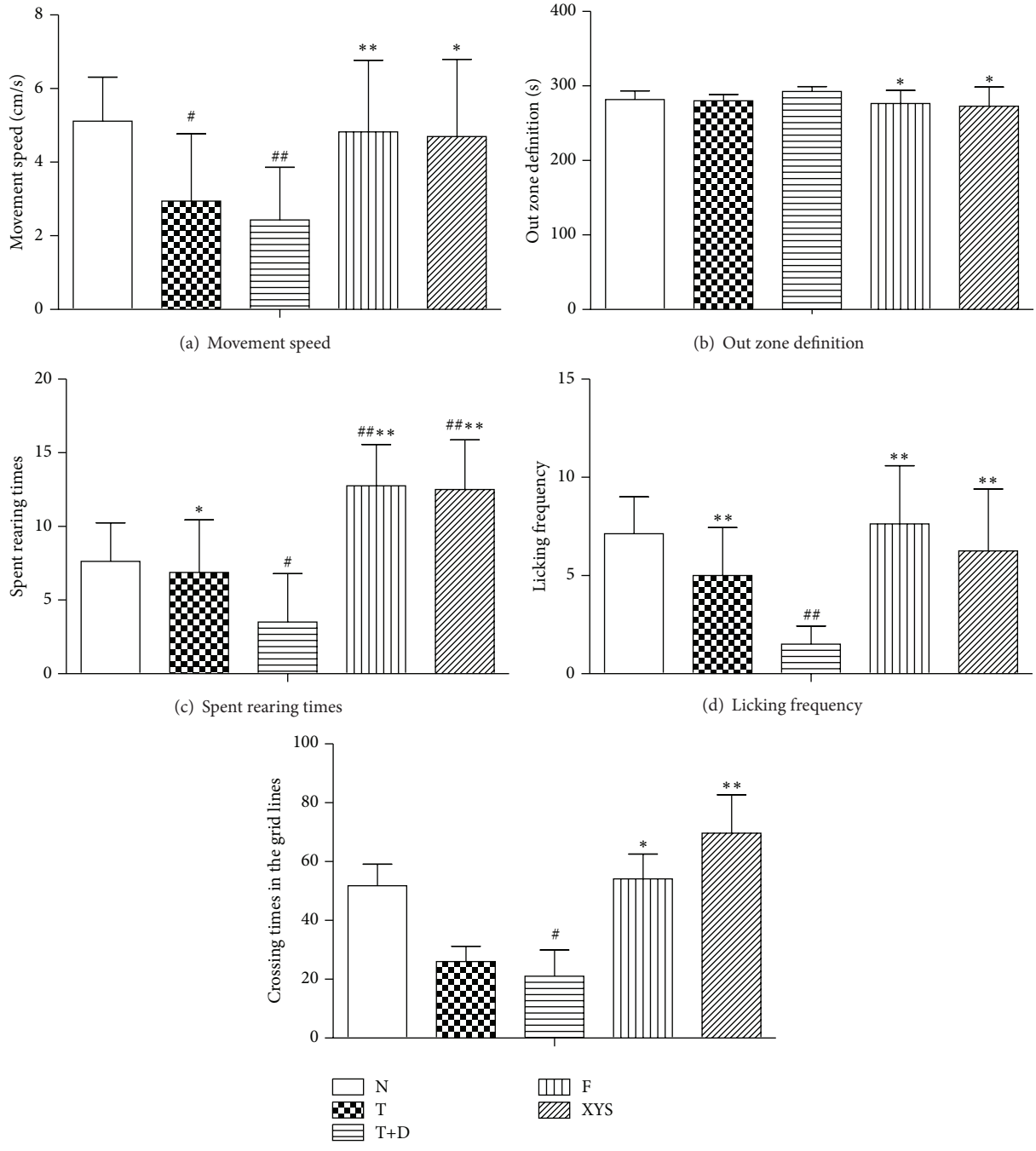
and memory including energy metabolism and diet intake [32], were changed. Moreover, behavioral studies that use open field test to measure locomotor activity and depression [33] showed a progressive decline in the exploration of environment and a negative impact on spontaneous locomotor activity, which also contribute to depression. The hippocampus is especially vulnerable to stress-induced damage [34] and plays a role in executive function and working memory, involving in extinction of learning [35]. In the H&E staining, changes were found predominately in the CA1 and DG subregions of the hippocampus, instead of the CA3 subregion as shown in the pictures. It is unclear why changes are revealed in CA1 and DG subregions of the hippocampus rather than in the CA3 subregion. It might be that CA1 pyramidal neurons and DG granule neurons have a greater propensity to facilitate induced condensed, pyknotic, and damaged neurons than do CA3 pyramidal neurons. These results are consistent with those in the previous works reporting that patients with depressive disorders are usually accompanied by changes in the hippocampus [36]. Therefore, in this study, a new model of depressive-like behaviors in rats with type 2 diabetes was successfully established to study depression with type 2 diabetes disease. This is more humane and milder than the chronic unpredictable stress, which may provide the information applicable for further human clinical research.

In the clinical trial, fluoxetine was reported to improve insulin-mediated glucose disposal in obese patients, without changes in body weight [37], or improves glycaemic control in elderly type 2 diabetic patients [38]. It was also reported that the utility of stress management training could establish long-term glycemic control in type 2 diabetes [20]. In our previous study, Xiaoyaosan had almost the same effect on depression in rodent animals. Results from our HPLC-LTQ-Orbitrap-MS confirmed that Xiaoyaosan consists of antidepressive and antidiabetic components such as paeoniflorin, liquiritin, glycyrrhizic acid, ferulic acid, saikosaponins A and C, curcumin, and *Poria cocos* alcohol. We therefore created the hypothesis indicating that Xiaoyaosan is able to downregulate the blood glucose in depressive-like behaviors in rats with type 2 diabetes.

After 21 days of treatment, the body weight and blood glucose in Xiaoyaosan and fluoxetine treatment groups changed, and dopamine increased. Furthermore, rats also showed the improvement in locomotor function, which is corresponding to the previous studies regarding the Xiaoyaosan decoction modifying impairment of cellular plasticity and improving mood disorders by exerting neuroprotective and neurotrophic properties [20]. With neuroprotective properties, Xiaoyaosan decoction may ameliorate the impairment of neuron injury in the morphological observation. At the end of chronic restraint stress, these behavioral indicators were back to baseline when the model rats were treated with Xiaoyaosan or fluoxetine. Based on behavior research and morphological observation, it proved that Xiaoyaosan can produce significant antidepressant-like effect. Despite these findings, the blood glucose did not return to normal levels after the treatment, but the blood glucose showed a decline after treatment that is consistent with the hypothesis. The mechanism underlying the pathophysiology of mood disorders and type 2 diabetes is still unclear, so more investigations at the molecular level will be provided in future studies.

5. Conclusion

The mechanism of depression with type 2 diabetes remains elusive. The depressive-like behaviors in rats with type 2 diabetes provide an animal model in this study, which is a tool for investigation of the mechanism. The key point of this study is to demonstrate that Xiaoyaosan has a potential antidepressive and antidiabetic effect on depressive-like behaviors in rats with type 2 diabetes. The results



(a) Movement speed

(b) Out zone definition

(c) Spent rearing times

(d) Licking frequency

(e) Crossing times in the grid lines

Figure 11: Scores on open field activity. (a) Movement speed (cm/s) in 5 minutes of open field test. (b) Out zone definition (s) in 5 minutes of open field test. (c) Spent rearing times in the grid lines times in 5 minutes of open field test. (d) Licking frequency in the grid lines times in 5 minutes of open field test. (e) Crossing in the grid lines times in 5 minutes of open field test. Data are expressed as $\overline{x} \pm s$, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$ versus group N; $^{*}P < 0.05$, $^{**}P < 0.01$ versus group T+D.

confirmed and extended previous findings, implying that Xiaoyaosan in this study independently contributes to exacerbating disease state or disease progression. Furthermore, these results are consistent with the results in previous reports that Xiaoyaosan plays an important role in the treatment of neurodegenerative diseases, helps to maintain neuronal survival, and enhances rehabilitation and regeneration of neurons after injury. In summary, this study provides new evidence for the clinical application of the Xiaoyaosan decoction. However, further studies at molecular level are still needed to elucidate the mechanism for Xiaoyaosan in the treatment of depressive-like behaviors with type 2 diabetes,

which will provide information for further evaluation in clinical trial.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Na Li and Qun Liu equally contributed to this paper. Jia-Xu Chen obtained funding, supervised this study, and helped revise the paper. Na Li conducted the experiments, conceived and designed the study, and wrote the paper. Qun Liu conducted the experiments and conceived the study. Xiao-Juan Li and Xiao-Hui Bai helped conduct the animal experiments and provided technical support. Yue-Yun Liu, Hong-Bo Zhao, Zhong-Ye Jin, Yu-Xia Jing, and Zhi-Yi Yan helped conduct the animal experiments.

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Research Article

Evaluation of the Effects of *Cornus mas* L. Fruit Extract on Glycemic Control and Insulin Level in Type 2 Diabetic Adult Patients: A Randomized Double-Blind Placebo-Controlled Clinical Trial

Rasool Soltani,[1] Abdollah Gorji,[1] Sedigheh Asgary,[2] Nizal Sarrafzadegan,[2] and Mansour Siavash[3]

[1]*Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran*
[2]*Isfahan Cardiovascular Research Center, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran*
[3]*Isfahan Endocrine & Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

Correspondence should be addressed to Sedigheh Asgary; sasgary@yahoo.com

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Background. The plant *Cornus mas* L. (cornelian cherry) is traditionally used as an antidiabetic supplement; however, there is no related clinical trial. In this study, we evaluated the effects of the fruit extract of this plant on biomarkers of glycemic control in adult patients with type 2 diabetes. *Methods*. Sixty patients with type 2 diabetes were randomly assigned to two groups to receive either the extract or placebo capsules (2 capsules twice daily) for 6 weeks. Each drug capsule contained 150 mg of anthocyanins. Fasting plasma levels of glucose, insulin, $HgbA_{1C}$, and triglyceride as well as 2-hour postprandial glucose level (2Hpp) were measured before and after the intervention and finally the mean values were compared between groups. *Results*. After 6 weeks of intervention, significant increase in insulin level (1.13 ± 1.90 versus -0.643 ± 1.82, $P < 0.05$) as well as decrease in $HgbA_{1C}$ (-0.24 ± 0.429 versus 0.023 ± 0.225, $P < 0.05$) and TG (-23.66 ± 55.40 versus 2.83 ± 15.71, $P < 0.05$) levels was observed in drug group compared to placebo. *Conclusion*. Daily consumption of the fruit extract of *Cornus mas* L. improves glycemic control by increasing insulin level and reduces TG serum level in type 2 diabetic adult patients.

1. Introduction

Diabetes mellitus (DM) is one of the most common endocrine disorders characterized by hyperglycemia. Type 2 DM, characterized by insulin resistance and a relative lack of insulin secretion, accounts for as much as 90% of all cases of DM and its prevalence is increasing [1]. DM is the leading cause of blindness in adults aged 20 to 74 years and end-stage renal disease (ESRD) and a main cause of cardiovascular events [1]. Optimal management of the patient with DM will reduce or prevent complications and improve quality of life [2]. Also, aggressive management of cardiovascular risk factors, including dyslipidemia, is needed to reduce the likelihood of development of macrovascular disease [2].

Medical nutrition therapy is recommended for all patients with DM and, along with activity, is a cornerstone of treatment [3]. *Cornus mas* L. (cornelian cherry) is a plant found in parts of central and southern Europe as well as western Asia including northern forests of Iran [4]. The fruits (berries) of this plant are rich in anthocyanins including delphinidin-3-glucoside, cyanidin-3-rhamnoglucoside, cyanidin-3-glucoside, cyanidin-3-galactoside, and pelargonidin-3-galactoside [4, 5]. It has been shown that anthocyanins increase insulin secretion from pancreatic β-cells

and improve insulin resistance [6–9]. Furthermore, a recent animal study has shown the effect of *Cornus mas* L. fruit on reduction of blood glucose level in diabetic rats [10]. Although this plant is traditionally used as an antidiabetic supplement, there is no clinical study about its effect. Therefore, this trial aimed to evaluate the effects of *Cornus mas* L. fruit extract on several markers of glycemic control in type 2 diabetic adult patients.

2. Materials and Methods

2.1. Plant Material and Extraction. Fresh ripe berries of *C. mas* were collected from the forests of Ghazvin, Iran, in July 2012. After washing and separation of the cores, the fruits were crushed by electric mixer (Moulinex, France) and filtrated by filter paper. The obtained material was then extracted by maceration with ethanol 70% (Stalk, Iran) repeated for 3 times. The extract was then filtrated and concentrated using rotary evaporator (Heidolph, Germany).

2.2. Extract Standardization. The obtained extract was standardized based on the total anthocyanin content using the pH differential method [11]. For this, two 1-g samples of dried extract were dissolved in 10 mL of buffer solution with pH = 1 composed of 125 mL of KCl 0.2 M (Merck, Germany) and 375 mL of HCl 0.2 M (Merck, Germany) and 10 mL of buffer solution with pH = 4.5 composed of 400 mL of sodium acetate 1 M (Merck, Germany), 240 mL of HCl 1 M, and 360 mL of water, respectively. Both solutions were diluted 10 times with the same buffer and their absorbance was read at 510 nm using spectrophotometer (PerkinElmer, USA). Total anthocyanin content was determined by the following equation:

$$\text{Anthocyanin concentration } (\text{mg/L}) = \frac{\left(\text{Abs}_{\text{pH 1}} - \text{Abs}_{\text{pH 4.5}}\right) \times 484.82 \times 1000 \times \text{DF}}{24825}, \quad (1)$$

where 484.82 is the molecular mass of cyanidin-3-glucoside chloride, 24825 is molar absorptivity of cyanidin-3-glucoside at 510 nm in pH = 1, and DF is the dilution factor.

2.3. Preparation of Drug and Placebo Capsules. The concentrated extract was mixed with tribasic calcium phosphate powder (Merck, Germany), then granulated, and dried. Each drug capsule was filled with 500 mg of the mixed granules equivalent to 150 mg of total anthocyanin. The placebo capsules with shape, color, and size similar to drug ones were filled only with dried granulated tribasic calcium phosphate.

2.4. Patient Selection. The inclusion criteria for participation of patients in the study were (1) being diagnosed with type 2 diabetes mellitus for at least 2 years according to the American Diabetes Association (ADA) diagnostic criteria [12], (2) age of 18 to 80 years, (3) serum glycosylated hemoglobin (HbA_{1C}) > 7% and <10%, (4) not being substance abuser (including alcohol), (5) not using any insulin preparation and/or any antidiabetic drug increasing endogenous insulin secretion (sulfonylureas, glinides, glucagon-like peptide-1 (GLP-1) analogs, and dipeptidyl peptidase IV (DPP-IV) inhibitors), (6) no change of the dose of antidiabetic drug within the last month, (7) free of either liver, kidney, or cardiovascular disease, (8) free of diabetic foot ulcer, and (9) not being pregnant or lactating (for women).

The exclusion criteria included (1) irregular use of the capsules, (2) change of the dose of antidiabetic drug during the study, and (3) the need to use any insulin preparation and/or any antidiabetic drug increasing endogenous insulin secretion (as mentioned above).

2.5. Study Design and Interventions. This was a randomized, double-blind, placebo-controlled clinical trial conducted in Isfahan Cardiovascular Research Center affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, from December 2012 to September 2013. Informed consent was obtained from all participants and the study protocol was approved by the Ethical Committee of Isfahan University of Medical Sciences. Patients who met the inclusion criteria were randomly and equally assigned to either the study drug (*Cornus mas*) or placebo groups. Before intervention, the demographic characteristics (including BMI) were recorded for all patients and, by receiving 5 mL of blood sample from each participant in fasting state and 2 hours after meal, fasting serum levels of glucose (FPG), insulin, and triglyceride (TG), glycosylated hemoglobin (HbA_{1C}), and serum level of 2-hour postprandial glucose (2Hpp) were determined. Also, to detect any possible side effect of the drug on the liver and kidney, the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine were obtained. The patients of drug and placebo groups were instructed to use 4 medicinal and placebo capsules, respectively, per day (2 capsules every 12 hours) with food for 6 weeks. All patients were advised to maintain their usual diet and physical activity and report any adverse effect during the study. The patients' compliance was evaluated by counting their capsules at the end of use and their results were applied for data analysis if they used more than 80% of their capsules. At the end of 6 weeks, all the above-mentioned variables were again determined and compared to baseline values. For randomization and blindness, each capsule container was given a code according to the type of its content (drug or placebo). When giving a container to each patient, its code was recorded on his/her own consent form. At the end of the intervention and after determination of the patient's own results, the recorded code was identified in terms of the type of intervention. All participants, the physician, and the laboratory personnel were blind to the intervention type.

2.6. Statistical Analysis. SPSS 20.0 software (SPSS Inc., Chicago, USA) was used for statistical analysis of obtained data. Kolmogorov-Smirnov test was performed to assess distribution pattern of continuous data. Because of normal distribution of all continuous data, Student's t-test was used for comparisons. Paired-samples t-test was performed for comparison of values at the beginning and end of intervention within each group. Independent-samples t-test was used for comparing the mean changes of each parameter form baseline between drug and placebo groups. Chi-square

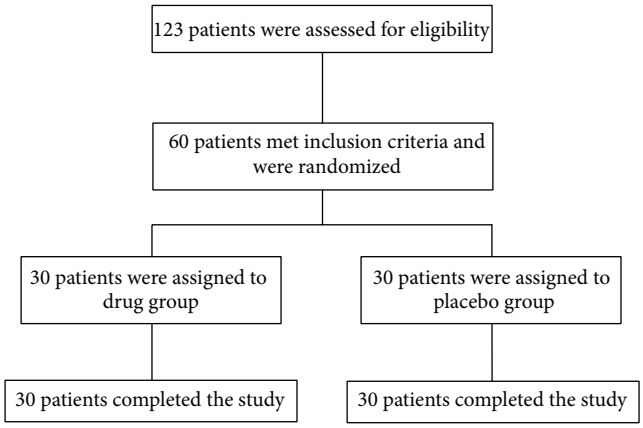

Figure 1: Flowchart of patients' enrollment in the study.

Table 1: Baseline demographic and clinical characteristics of the study subjects. The values are presented as mean ± SD.

Parameter (unit)	*Cornus mas* ($n = 30$)	Placebo ($n = 30$)	P value
Age (years)	49.16 ± 5.62	49.93 ± 6.12	0.616
Gender (%male)	63.33	66.70	0.723
BMI (kg/m^2)	29.40 ± 1.73	29.21 ± 2.01	0.621
FPG (mg/dL)	157.93 ± 41.38	174.67 ± 40.80	0.120
Insulin (μU/mL)	5.67 ± 2.85	5.91 ± 2.72	0.727
HbA_{1C} (%)	7.72 ± 0.75	7.78 ± 0.65	0.745
2Hpp (mg/dL)	228.40 ± 66.42	231.20 ± 60.27	0.865
TG (mg/dL)	198.37 ± 66.64	237.43 ± 148.95	0.615

BMI: body mass index; FPG: fasting plasma glucose; 2Hpp: 2-hour postprandial glucose; TG: triglyceride.

test was done for comparison of gender distribution in two groups. $P < 0.05$ was considered as significant.

3. Results

During the study, a total of 123 type 2 diabetic patients were assessed for participation in the study, of whom 60 patients (age range of 41 to 65 years) met the inclusion criteria that were randomly and equally divided into two intervention groups (30 in each group). All patients fully completed the trial (Figure 1).

Table 1 shows baseline demographic and clinical characteristics of the study patients. As shown, all subjects were matched regarding baseline values.

Table 2 shows comparatively the effects of interventions on evaluated variables after 6 weeks in the study patients. As seen, *C. mas* significantly reduced the serum levels of HbA_{1C} and TG and increased the serum level of insulin compared to placebo. Although *C. mas* reduced BMI, FPG, and 2Hpp, these effects were not statistically significant compared to placebo.

Table 3 presents the effects of *C. mas* and placebo on laboratory markers of liver and kidney function after 6 weeks of intervention. As shown, no significant changes were detected in these values during the study. Also, no complication or adverse effect was reported by the patients of both groups.

Table 2: The effects of interventions on tested parameters after 6 weeks in the study subjects. The values are presented as mean ± SD.

Parameter (unit)	*Cornus mas* ($n = 30$)	Placebo ($n = 30$)	P value (between groups)
BMI (kg/m^2)			
End	29.06 ± 1.60	29.31 ± 2.07	
Change	−0.33 ± 0.45	0.10 ± 0.45	0.062
P value	0.723	0.320	
FPG (mg/dL)			
End	143.30 ± 40.19	178.73 ± 38.80	
Change	−14.63 ± 36.87	4.06 ± 55.39	0.130
P value	0.038	0.691	
Insulin (μU/mL)			
End	6.80 ± 3.20	5.27 ± 2.53	
Change	1.13 ± 1.90	−0.643 ± 1.82	0.001
P value	0.003	0.064	
HbA_{1C} (%)			
End	7.49 ± 0.71	7.81 ± 0.64	
Change	−0.240 ± 0.429	0.023 ± 0.225	0.005
P value	0.005	0.621	
2Hpp (mg/dL)			
End	222.17 ± 55.17	244.83 ± 63.27	
Change	−6.23 ± 46.19	13.63 ± 80.76	0.247
P value	0.466	0.363	
TG (mg/dL)			
End	174.70 ± 78.28	240.26 ± 147.97	
Change	−23.66 ± 55.40	2.83 ± 15.71	0.014
P value	0.026	0.332	

BMI: body mass index; FPG: fasting plasma glucose; 2Hpp: 2-hour postprandial glucose; TG: triglyceride.

Table 3: The effects of interventions on the liver and kidney function tests of the study subjects after 6 weeks. The values are presented as mean ± SD.

(a)

Parameter (unit)	*Cornus mas* ($n = 30$)		
	Baseline	Week 6	P value
ALT (U/L)	16.43 ± 9.61	16.70 ± 6.47	0.848
AST (U/L)	22.76 ± 6.75	23.36 ± 7.07	0.673
BUN (mg/dL)	16.01 ± 2.77	15.88 ± 3.65	0.879
Creatinine (mg/dL)	0.633 ± 0.225	0.593 ± 0.316	0.335

(b)

Parameter (unit)	Placebo ($n = 30$)		
	Baseline	Week 6	P value
ALT (U/L)	17.46 ± 8.16	17.86 ± 7.44	0.830
AST (U/L)	22.16 ± 9.83	23.76 ± 14.13	0.447
BUN (mg/dL)	15.41 ± 2.73	15.73 ± 3.49	0.677
Creatinine (mg/dL)	0.620 ± 0.274	0.713 ± 0.278	0.232

4. Discussion

Our study showed that the fruit extract of *C. mas* could improve glycemic control characterized by reduction of HbA_{1C} as measurements of HbA_{1C} are the gold standard for following long-term glycemic control [13]. Although the effects of this plant on FPG and 2Hpp were not statistically significant compared to placebo, the significant decrease in FPG compared to baseline ($P < 0.05$) and slight decrease in 2Hpp show the potential ability of this extract for reduction of serum glucose level. Therefore, the use of higher doses of the extract for longer periods of time might have more significant effect on markers of glycemic control in type 2 diabetic patients. The observed effects of *C. mas* fruits may be due to its anthocyanin content. It has been shown that the anthocyanins cyanidin-3-glucoside and delphinidin-3-glucoside, found in the fruits, stimulate insulin secretion from rodent pancreatic beta-cells (INS-1 832/13) in vitro [7]. This is consistent with our results showing increased level of insulin by the extract. Also, the study of Zhang et al. showed that the compound ursolic acid in fruits of *C. mas* is capable of phosphorylation of insulin receptors and stimulation of glucose uptake by tissues [14]. In an animal study performed by Jayaprakasam et al. the anthocyanins and ursolic acid, purified from *C. mas* fruits, prevented glucose intolerance and increased insulin levels in high-fat-fed mice [6]. Furthermore, release of acetylcholine to raise insulin secretion through stimulation of muscarinic type 3 (M3) receptors by oleanolic acid contained in *Cornus* species, as suggested by Hsu et al. [15], may be another mechanism of increased insulin level and consequent reduction of glucose level.

To the best of our knowledge, there is no clinical trial about the effects of cornelian cherry on glycemic control in diabetic patients. However, several animal studies have been conducted on *Cornus mas* [6, 10, 16] and *Cornus officinalis* [17, 18]. In the study of Shamsi et al., daily use of 2 g of *Cornus mas* fruits by alloxan-induced diabetic rats for 4 weeks resulted in significant reduction of blood glucose levels, increased insulin levels, and increased size of pancreatic islets compared to control nondiabetic rats [10]. In the study of Yamabe et al., treatment with *Cornus officinalis* fruit extract for 10 days suppressed hyperglycemia, proteinuria, and renal advanced glycation end-product (AGE) formation in streptozocin-induced diabetic rats [18].

Since a common lipid abnormality in type 2 diabetes is hypertriglyceridemia (>150 mg/dL) [19] as was present in our study subjects, we evaluated the effect of *Cornus mas* on triglyceride levels too in the study subjects. The extract showed significant TG-lowering effect in type 2 diabetic patients. This effect could be due to anthocyanin content of the extract as the hypolipidemic effects of anthocyanins and some anthocyanin-containing plants have been confirmed in several studies [20–23]. The TG-lowering effect of anthocyanins may be through suppression of the expression of lipogenic enzymes (fatty acid synthase, acyl-CoA synthase 1, and glycerol-3-phosphate acyltransferase) in the liver and adipose tissue [24] as well as increasing lipoprotein lipase activity in skeletal muscle and reducing it in visceral adipose tissue [25].

It is noteworthy that the large standard deviation (SD) of change values from baseline (as shown in Table 2) could be due to several factors including relatively low number of patients in each group and large variations in the change of each parameter in several patients, both presenting some limitations of the current study.

In conclusion, our study shows that daily consumption of the fruit extract of *Cornus mas* L. (equivalent to 600 mg of anthocyanins daily) improves glycemic control by increasing insulin level and reduces TG serum level in type 2 diabetic adult patients. Therefore, this extract could be considered as a beneficial nutritional supplement for adult patients with type 2 diabetes. However, more studies with larger sample size and longer duration are required to confirm these results.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Effect of *Rhizoma coptidis* (Huang Lian) on Treating Diabetes Mellitus

Bing Pang,[1] Xiao-Tong Yu,[1,2] Qiang Zhou,[3] Tian-Yu Zhao,[1] Han Wang,[1] Cheng-Juan Gu,[1] and Xiao-Lin Tong[1]

[1]*Department of Endocrinology, Guang'anmen Hospital of China, Academy of Chinese Medical Sciences, Beijing 100054, China*
[2]*College of Acupuncture, Beijing University of Chinese Medicine, Beijing 100029, China*
[3]*Department of Digestion, Beijing Hospital of Traditional Chinese Medicine, Capital University of Medicine Sciences, Beijing 100010, China*

Correspondence should be addressed to Xiao-Lin Tong; xiaolintong66@sina.com

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The rapidly increasing diabetes mellitus (DM) is becoming a major public health issue globally; considerable progress has been made in the field of western hypoglycemic drug and insulin, but some shortages still exist. As one of the most important parts in complementary and alternative therapies, traditional Chinese medicine (TCM) performs a good clinical practice and is showing a bright future in the treatment of DM. TCM therapy has certain advantages of less toxicity and/or side effects, and Chinese herbal medicine which usually contains various active ingredients could provide multiple therapeutic effects. Huang Lian (*Rhizoma coptidis, RC*) is a herb frequently used in many traditional formulas for properties of "clearing damp-heat, quenching fire, and counteracting poison" in Asia for centuries. In this review, we summarize the application of *RC* in the treatment of DM from two aspects of contents. Firstly, theoretical principles are explained, including the properties and related records about *RC* in ancient references and modern pharmacological researches and pharmacokinetics on *RC* and its active components. Secondly, the clinical application of *RC* is mainly reviewed, such as applicable stage and syndrome, the reasonable dose range, the preparation formulations, and the toxicity and/or side effects and solutions to its adverse actions. This review provides scientific evidence about the effective components, pharmacological researches, and toxicity of *RC*, as well as introducing traditional Chinese medical theory and clinical experience, in order to guide clinician to use *RC* more suitably and reasonably in the clinical practice.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder in the endocrine system due to either an absolute deficiency in insulin secretion or reduction in the biological effectiveness of insulin. The global prevalence of DM among adults aged 20–79 years was 8.3% in 2013 [1]. As one of the largest developing countries, China has the biggest population of DM—92.4 million, which accounts for 9.7% of adult population [2]. In addition, 148.2 million adults (15.5%) have prediabetes [2]. DM is the 7th leading cause of mortality in the world, which can substantially result in a huge burden in terms of renal failure, nontraumatic lower-limb amputations, and newly diagnosed retinopathy and represent a major public health issue [3]. Type 2 diabetes mellitus (T2DM) is the predominant form of DM and accounts for 90–95% of diabetic people globally, due to an increased number of elderly people and a greater prevalence of obesity and sedentary lifestyle [4, 5]. Management of T2DM is still a challenge; currently, the standard therapy for T2DM includes diet, exercise, use of oral hypoglycemic drugs, and/or subcutaneous insulin injections [6]. These treatment methods frequently have side effects, such as weight gain, bone loss, and increased risk of cardiovascular events; treatment is costly as well, since T2DM is a chronic disease and long-term treatment is necessary [4–6]. In traditional Chinese medicine (TCM), DM may fall under the categories of "Xiaokezheng". It is characterized by excessive fluid drinking, excessive food-consumption, excessive urination, and weight loss; all of these symptoms are commonly called "three excesses and one loss" [7]. The main

pathogenesis of "Xiaokezheng" lies in yin deficiency leading to endogenous dryness-heat in the body, and blood stasis and phlegm retention often present. If prolonged yin deficiency impairs yang, this will result in dual deficiency of qi and yin as well as dual deficiency of yin and yang. Therefore, the main TCM treatment methods for Xiaokezheng are invigorating qi, nourishing yin, clearing away the heat, and promoting fluid production [3, 8, 9]. Famous formulas including Bai Hu Jia Renshen Tang, Jin Gui Shen Qi Wan, and Yuye Tang are widely used [3, 8–10]. TCM has a long history of more than 2,000 years to treat T2DM in China [11]. Treatment by TCM has certain advantages of less toxicity and/or side effects, and herbal medicine which usually contains various active components could provide multiple therapeutic effects on multiple targets, including enhancement of insulin sensitivity, stimulation of insulin secretion, or reduction of carbohydrate absorption [7, 12, 13]. There are 86 herbal medicines often used in the traditional Chinese formulas for T2DM and its complications; of these, *RC* is widely used for treatment of T2DM and its complications [12]. It is the rhizome of *Coptis chinensis* Franch that belongs to the Ranunculaceae family, which is recorded in the Chinese Pharmacopeia with the Chinese name of Huang Lian. It could clear the damp-heat, quench the fire, and counteract the poison, which was called as a holy herb to treat Xiaokezheng by Liu He-Jian in the Jin and Yuan Period [14]. In the Taiping Shenghui Formulary (*Tài Píng Shèng Huì Fāng*) of Song Dynasty, *RC* ranked in the first three among the frequently used ten medicines in the 177 prescriptions for Xiaokezheng [15]. Recent studies have indicated that *RC* possesses multispectrum therapeutic activities, including antihyperglycemia, antihyperlipidemia, antihypertension, anti-inflammatory, and antioxidant effects [12, 16–18]. In this review, we summarize the application of *RC* from two aspects of contents, including theoretical principles and the clinical practice of *RC* in both English and Chinese search engines, in order to guide clinician to use *RC* more suitably and reasonably in the clinical practice.

2. Theoretical Principles of Using *RC*

2.1. Properties of RC and Records in Ancient References. The use of *RC* in the treatment of DM is, first, to clear the damp-heat, quench the fire, and counteract the poison and, second, to lower the blood glucose. The combination of medicine properties and pharmacology ensures the good therapeutic effects [19, 20]. *RC* is a herb of bitter flavor and cold property, entering channels of heart, spleen, stomach, gallbladder, and large intestine. It could clear away the excess heat, removing dampness and eliminating toxins according to traditional Chinese pharmacology [21]. The Supplementary Records of Famous Physicians (*Míng Yī Bié Lù*) in Wei-Jin Period firstly recorded the "treat Xiaokezheng" function of *RC*. The Newly Revised Herbal Foundation (*Xīn Xīu Běn Cǎo*) recorded that "*RC* in Sichuan had a large size and the most bitter flavor, which was the best for treatment of Xiaokezheng." During Ming and Qing Dynasties, the Compendium of Materia Medica (*Běn Cǎo Gāng Mù*) written by Li Shi-Zhen, recorded that "*RC* was mainly used to deplete thirst and treat copious urine." Main medicinal processing methods included (1) making honey pills; (2) steaming with wine, soaking with wine, or decocting with water; and (3) processing with wax gourd [15].

2.2. Modern Pharmacological Researches on Berberine and Other Alkaloids. *RC* was mainly composed of a diversity of alkaloids, including berberine (6.88% to 13.64%), palmatine (1.28% to 2.12%), jateorrhizine (0.77% to 1.32%), coptisine (0.42% to 0.85%), epiberberine (0.42% to 0.92%), worenine, and magnoflorine, all of which are considered to be its active components [22]. Berberine (BBR), an isoquinoline alkaloid, is the major active component of *RC*. Modern pharmacological researches have showed multiple mechanisms of BBR to lower blood glucose, such as improvement of insulin sensitivity, increase of insulin secretion [23], promotion of intestinal glucagon-like protein-1 (GLP-1) secretion [24], inhibition of hepatic gluconeogenesis [25], induction of glycolysis in peripheral tissues [26], promotion of antioxidant activities [16, 17], regulation of lipid disorders [27], and modulation of the gut microbiota [28, 29]. Lee et al. [30] showed that BBR improved insulin sensitivity and increased insulin secretion possibly through activating adenosine monophosphate-activated protein kinase pathway (AMPK) activity. Zhou et al. [31] showed that BBR modulated glycolipid metabolism possibly through increasing peroxisome proliferator-activated receptors (PPARs) PPARα/δ expression and reducing PPARγ expression in liver. Kong et al. [32, 33] found that BBR could increase the expression of the low-density lipoprotein receptor (LDLR) through an extracellular signal–regulated kinase (ERK)—dependent way. Moreover, BBR may exert antidiabetes effects via regulating gut microbiota [28]. Xie et al. [29] showed that BBR significantly reduced the number of harmful microbiota and increased the number of beneficial ones in the feces of high-fat diet-fed (HFD) mice, which may have a relationship with the glucose-lowering and lipid-lowering effects.

In addition to BBR, coptisine (COP), palmatine (PAL), and jatrorrhizine (JAT) were generally considered as the main bioactive components in *RC* [34]. Coptisine (COP), an isoquinoline alkaloid isolated from *RC*, possesses evident pharmacological activities against diabetic complications-related symptoms, including hypoglycemic [35], antiradical, antioxidant [36], and antibiotic effects [37]. Jiang et al. showed stronger hypoglycemic capability of COP in vitro compared with JAT and epiberberine but relatively weaker than BBR [38]. Jung et al. indicated that COP had inhibitory activities against aldose reductase, which may be important way to treat DM and diabetic complications [35]. Yokozawa et al. reported that COP was the most effective for protecting oxidative stress [17]. In another study, COP significantly decreased the levels of blood lipid in high-fat and high-cholesterol diet mice (HFHC). Results demonstrated that a high dosage of COP (70.05 mg/kg) could inhibit cholesterol synthesis via suppressing the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) expression, as well as promoting the use and excretion of cholesterol via upregulating LDLR and cholesterol 7α-hydroxylase (CYP7A1) expression [39].

Some researchers investigated that COP exerted the protective effects on myocardial ischemia and reperfusion (I/R) damage in rat, which may relate to its strong antioxidant activity. COP also decreased the proinflammatory cytokines, such as interleukin- (IL-)1β, IL-6, and tumor necrosis factor-α (TNF-α), by inhibiting the rhodopsin (Rho)/Rho-kinase (ROCK) pathway [40, 41]. PAL is also a main isoquinoline alkaloid isolated from *RC*, which has been used in the treatment of hypertension, inflammation, jaundice, dysentery, cardiovascular diseases, and liver-related diseases [42, 43]. PAL also had therapeutic effects on related symptoms of diabetic complications due to its hypoglycemic, hypolipidemic, and cardiovascular protective effects. Yuan et al. [18] reported that PAL possesses hypoglycemic and hypocholesterolemic activities. A PAL-derivative, namely, 11-hydroxypalmatine, was evaluated for the hypoglycemic activity. The PAL-derivative was administered to alloxan-induced diabetic mice at doses of 25, 50, and 100 mg/kg orally. The blood glucose significantly decreased by 52% after treatment with the PAL-derivative compared with the positive control glibenclamide (54%) and the diabetic control (27%) [44]. PAL was considered as a potential agent for lowering the blood lipids. The mechanisms may relate to upregulation of LDLR and CYP7A1 mRNA and protein expression [45]. JAT is one of the major bioactive components isolated from *RC*. Some studies have demonstrated the hypoglycemic activity of JAT in alloxan-induced mice [46]. Wang et al. showed the effect of JAT on glucose uptake. JAT at different concentrations (265.65, 53.75, 10.75, 2.15, and 0.45 μmol/L) was administered to 3T3-L1 adipocytes for different periods (12, 24, 48, and 72 h), indicating that the optimal active concentration of JAT was 0.45 μmol/L and the preferable reaction time was 48 hours. Moreover, JAT can promote the fatty acid oxidation in 3T3-L1 adipocyte, which may be attributable to the upregulation of PPARα and PPAR β levels [47]. Wu et al. showed that JAT had a strong hypolipidemic effect in a dose-dependent way mainly through upregulating the mRNA and protein expression of LDLR and CYP7A1 [48]. Epiberberine is also a natural bioactive protoberberine alkaloid, which showed the mild hypoglycemic effects and low cytotoxicity of *RC* extract in HepG2 cells [34]. Yuan et al. [18] reported that BBR, PAL, and JAT were identified as active components in *RC* extract to lower the blood glucose and lipids, which were marked by a dose-dependent manner; when rats were given *RC* extract orally at a dose of 0.5 g/kg. day for 3 weeks, the blood glucose reduced by 58%. Yokozawa et al. [17] reported that COP, PAL, magnoflorine, and epiberberine might contribute to the protective effects of *RC* on oxidative stress through inhibition of cellular peroxynitrite generation.

The alkaloids isolated from *RC* may have beneficial effects on diabetic complications and DM due to the inhibitory activities against α-glucosidase or aldose reductase [49]. *RC* extract had the α-glucosidase inhibitory activity with the half maximal inhibitory (IC50) value at 3.528 mg mL(-1), which could be effective for treating DM, and the alkaloids were main components that inhibited α-glucosidase activity in *RC* extract [50]. Zhou et al. screened for α-glucosidase inhibitors, five components in the *RC* extract were found, and their structures were identified by electrospray ionization tandem mass spectrometry (ESI-MS) to be COP, epiberberine, JAT, BBR, and PAL [51]. Aldose reductase (AR) is the enzyme that leads to conversion of glucose to sorbitol, and its increased activity signifies in the development of long-term complications of DM [52]. Jung et al. [35] evaluated the inhibitory activities of the alkaloids from *RC* AR for the treatment of diabetic complications. Results showed that epiberberine, COP, and groenlandicine exhibited moderate inhibitory effects with IC(50) values of 100.1, 118.4, and 140.1 microM for rat lens aldose reductase (RLAR) and 168.1, 187.3, and 154.2 microM for human recombinant aldose reductase (HRAR), but BBR and PAL did not exhibit AR inhibitory effects at a higher concentration of 50 microg/ml, indicating that the presence of the dioxymethylene group in the D ring and the oxidized form of the dioxymethylene group in the A ring played important roles in inhibiting AR. *RC* and contained alkaloids therein had therapeutic effects on DM and its complications. Kwon et al. [53] showed the protective effect of *RC* on the cytotoxicity of pancreatic beta-cells; the action mechanism may relate to protecting apoptosis and necrosis through the inhibition of Deltapsim disruption, indicating that *RC* may be effective for preventing type 1 diabetes mellitus (T1DM).

RC extracts may be more effective than its single alkaloid; the mechanisms are related to the fact that different components may not only regulate targets in multiple pathways, and therefore enhancing pharmacological potency in a synergistic way, but also regulate the enzymes and transporters that are involved in hepatic and intestinal metabolism to improve oral drug bioavailability [54]. Fu et al. [46] compared the hypoglycemic activities of JAT, BBR, *RC* decoction, and compounds-mimic prescription (BBR-JAT) on blood glucose level in mice. Data suggested that *RC* decoction showed the most significant hypoglycemic activity. JAT also possessed the effect of decreasing blood glucose, which was less than that of BBR at the same dose. There was no significant difference between BBR-JAT and BBR ($P > 0.05$). The results showed that *RC* decoctionwas more effective than its single components BBR and JAT, indicating that other hypoglycemic components existed. Liu et al. [55] suggested that area under curve (AUC) and peak concentration (C_{max}) of BBR significantly increased in rats receiving *RC* extract compared with those receiving the pure BBR, indicating that *RC* extract showed better hypoglycemic activity than pure BBR. Liu et al. demonstrated the different metabolic interaction between the active components (BBR, COP, PAL, and JAT) of *RC* in human liver microsomes by HPLC. COP showed inhibition against the formation of the two metabolites of BBR with IC50 values of 6.5 and 8.3 μM, respectively, which indicated the strongest inhibition toward BBR metabolism. BBR suggested a weak inhibition against the production of COP metabolite with an IC50 value of 115 μM. PAL and JAT showed the weaker inhibitions against the formation of the metabolites of BBR and had little inhibitory effect on the formation of COP metabolite. BBR, COP, and JAT showed no inhibitory effect on the generation of PAL metabolite with an IC50 of more than 200 μM [55]. Zhu et al. [56] compared the hypoglycemic activity of *RC* alkaloids (BBR, JAP, PAL, and COP) in HepG2 cell through

measuring the glucose consumption and effect of cell vitality. Results indicated the alkaloids which ranged from 0.2 to 5 mg/L had the hypoglycemic activity, and BBR showed then cytotoxicity at the doge of 5 mg/L. Besides, composite alkaloids showed no cytotoxicity, but higher hypoglycemic potency compared with pure alkaloid, which may be achieved by synergistic interaction between alkaloids. Similar results were also demonstrated by another study [57]. Whether the hypoglycemic activity is best exerted synergically in a formula or multicomponent or independently as an active component remains to be investigated. The possible hypoglycemic effects and mechanisms of the other components of *RC* and the interactions among its various components are still needed to be demonstrated in future researches.

2.3. Pharmacokinetics of RC. The pharmacokinetic properties and potential herb-drug interactions found with *RC* alkaloids have been demonstrated by several studies. Generally, the systemic exposures of the alkaloids are extremely low after oral administration. The alkaloids may present their systemic effects through generated metabolites and/or the tissue distributed alkaloids themselves or through modulating effectors in the gut [58]. Yu et al. [59] investigated the pharmacokinetics of BBR, PAL, COP, epiberberine, and jatrorrhizine from *RC* in diabetic rats. *RC* extract (1.3 g/kg) was administered by oral gavage to the control rats and the diabetic rats induced by 6-week injection of streptozotocin. Blood samples (300 μL) were obtained from the ocular fundus vein before dosing and subsequently at selected intervals of 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h following administration. The concentrations of five types of alkaloids were determined. Compared to those in the control, there existed 170% to 330% increases in C_{max} and 150% to 350% increases in AUC_{0-24} of the five types of alkaloids, the bioavailability of the five protoberberine type alkaloids of *RC* extract enhanced in diabetic rats, which may potentially reach therapeutic concentrations and exert pharmacological effects in the body. Therefore, DM is responsible for the enhancements in the bioavailability of the five protoberberine type alkaloids of *RC* extract in rats, perhaps due to the pathological changes in absorption, distribution, metabolism, and excretion. In the previous study, Yu et al. showed that, after oral administration of *RC* extract, BBR, PAL, COP, epiberberine, and jatrorrhizine in 6-week streptozotocin- (STZ-) induced diabetic rats acquired a higher exposure. Subsequently, they demonstrated that the function and expression of intestinal P-glycoprotein (P-GP) were downregulated in STZ-induced diabetic rats, which contributed to the increased exposure of the five protoberberine alkaloids [60]. BBR, PAL, and jateorrhizine exhibited similar plasma concentration-time profiles under the same administration methods, which probably resulted from the similarity of their molecular structures, which contributed to their similar disposition process in vivo. A pharmacokinetic study suggested that C_{max} of BBR, PAL, and jatrorrhizine was 78.42 ± 12.19, 82.09 ± 17.44, and 55.35 ± 8.90 μg/L, respectively. All T_{max} of BBR, PAL, and jatrorrhizine was 0.75 ± 0.11 [61]. The quantification of three alkaloids (BBR, PAL, and COP) was investigated in the pharmacokinetics of the alkaloids from Xiexin Decoction in rats. A high-performance liquid chromatographic method was used. Results indicated that the linear ranges of the calibration curves were 1.6–160 ng/ml for all three alkaloids. The within-batch accuracy was 88.6–107.8% for BBR, 88.4–110.1% for PAL, and 90.4–108.3% for COP; the between-batch accuracy was 94.3–100.6% for BBR, 99.3–100.3% for COP, and 93.7–100.0% for PAL; the within-batch and between-batch precisions were <or=10.1% and <or=11.1%, respectively [62]. In a pharmacokinetic study, Xue et al. investigated the interaction of magnoflorine with the rest of compounds in *RC*. The rats were not only administered with magnoflorine orally (15, 30, and 60 mg/kg) and intravenously (10 mg/kg), but also administered with *RC* decoction (equivalent to 30 mg/kg of magnoflorine) intragastrically. Data suggested that magnoflorine possessed lower bioavailability and faster absorption and elimination. However, when magnoflorine was administered in *RC* decoction, pharmacokinetic parameters significantly altered ($P < 0.05$). Oral gavage of *RC* decoction decreased the absorption and elimination rates of magnoflorine, indicating that the pharmacokinetic interactions existed between magnoflorine and other components in *RC* [63]. Guo and Zhao [64] used different dosages of BBR group (40, 80, and 120 mg/kg, resp.) to treat diabetic mice, compared with gliclazide group (5 mg/kg). All the indicators showed no significant difference between groups ($P > 0.05$). When mice of each group were administrated drugs for 60 days, although hypoglycemic activity of BBR appeared slower than those of gliclazide, BBR improved the glucose tolerance and increased the level of insulin, which showed obvious dose-effect relationship.

3. The Clinical Practice of Using *RC*

3.1. Applicable Stage and Syndrome. Wu and Wei [65] observed efficacy of BBR in treating T2DM. Seventy-two cases were assigned to obese group and nonobese group; BBR (0.02 mg/kg) was administrated orally for 8 to 10 weeks. Results showed that insulin resistance and body mass index (BMI) of all cases improved after treatment, compared with before treatment ($P < 0.01$). BMI of obese group reduced more significantly than that of nonobese group ($P < 0.01$), indicating that BBR was more applicable to prediabetes and the early stage of T2DM characterized by insulin resistance and obesity. More researches were needed, because BBR was merely the component of *RC* and did not represent *RC* totally. Based on large amount of clinical practice and modern researches, stagnancy, heat, deficiency, and damage are thought of as four stages of T2DM [66, 67]. Stagnancy and heat stages mostly appear in the early and middle stage of T2DM. Deficiency stage is equivalent to traditional Xiaokezheng, and damage stage often accompanies various complications. Deficiency and damage stages appear in middle and late stages. *RC* is commonly used in the heat and deficiency stages of T2DM [68]. Tang and Shen [69] observed the hypoglycemic efficacy of BBR on different TCM syndromes of T2DM; 120 patients were assigned to four groups, including syndromes of yin deficiency and

excessive heat, damp-heat encumbering in the spleen, dual deficiency of qi and yin, and blood stasis in collaterals. BBR (0.5 g) was orally given three times daily for three months. Results indicated that BBR had better hypoglycemic effects on syndrome of damp-heat encumbering in the spleen than other groups; BMI decreased more significantly in group of damp-heat encumbering in the spleen ($P < 0.05$) than those of other groups ($P > 0.05$), which was consistent with properties of *RC*. The characteristics of splenic damp-heat syndrome commonly include bitter taste in the mouth, obesity, abdominal stuffiness and fullness, sticky fetid stool, yellow-greasy coating, and slippery and rapid pulse. [70] BBR could significantly improve the symptoms of splenic damp-heat syndrome of diabetic patients, as well as controlling the blood glucose effectively [71].

3.2. Reasonable Dose. The recommended dose of *RC* should be 15–45 g, even to a maximum dose of 60 g for alleviating the diabetic ketoacidosis (DKA) [72], whereas the routine dose in Chinese Pharmacopoeia (2010 edition) is 2–5 g is usually ineffective [73]. Liu [74] made a survey of the dose of *RC* in 1,321 effective formulas to treat T2DM (when the decrease percentage of fasting blood glucose (FBG) and postload plasma glucose (PBG) was >20% of those before treatment or the decrease percentage of Hemoglobin A1c (HbA1C) was >10% of that before treatment within 12 weeks, the formula was thought of as an effective formula, and other else was thought of as an ineffective formula), and the result showed that the common dose of *RC* to lower blood glucose was 15–45 g; the analysis on the correlation between dose of *RC* and level of FBG demonstrated that significant positive correlation existed, and the dose increased with the rise of FBG. The common dose of *RC* was 15 g when the level of FBG <7 mmol/L; 30 g when the level of FBG <10 mmol/L; and 30 g to 45 g when the level of FBG ≥10 mmol/L [68, 75, 76]. The dose of *RC* for treating T2DM was large; but the dose was small for treating the complications of T2DM [77]. Zhang et al. [78] observed the effects of Xinkai Kujiang Formula (XKF) with the different dose of *RC* on KKay mice with T2DM. XKF consisted of *RC* 20 g, Chaihu (*Radix Bupleuri*) 6 g, Huangqin (*Radix Scutellariae*) 10 g, Banxia (*Rhizoma Pinelliae*) 6 g, Wuweizi (*Fructus Schisandrae Chinensis*) 6 g, Dahuang (*Radix et Rhizoma Rhei*) 1 g, and Shengjiang (*Rhizoma Zingiberis Recens*) 3 g. The dose of *RC* was increased to 40 g in Xinkai Kujiang Jiawei Formula (XKJF) with doses of other herbs unchanging. Results showed that blood glucose decreased more significantly in XKJF group with dual doses of *RC* compared with that of XKF group ($P < 0.05$), indicating that the efficacy of XKF which lowered the blood glucose of KKay mice with T2DM was closely related to the dose of *RC*, and dose-effect relationship existed.

3.3. Variable Preparation Formulation. The therapeutic value of *RC* is mainly in the form of compound or polyherbal formulations and not as single herb. After referring to the documents, we found that several preparation formulations were mentioned to demonstrate hypoglycemic effects of *RC*, such as compound formula, decocting-free granules, BBR, total coptis alkaloids (TCA), pill, and tablet [17, 18, 65, 69, 75, 79, 80]. In general, decoction and decocting-free granules of *RC* are better than BBR and/or other alkaloids in terms of efficacy of lowering the blood glucose; the effect of decoction is better than that of pill and tablet; however the decoction is relatively unstable. Commonly speaking, compound formula decoction including *RC* is used for patients with high level of blood glucose, while pill and tablet are suitable for the control in stable level of blood glucose and long-term use, but this conclusion needs to be verified by more researches.

3.4. Toxicity/Side Effects and Solutions to Its Adverse Actions. According to the regulations of Singapore government in 1976, *RC* and BBR were forbidden to be used because it was deemed that a shortage of glucose 6 phosphate dehydrogenase (G6PD) could be caused after a pregnant woman or a newborn baby takes *RC* and BBR, which may lead to hemolytic jaundice of the newborn baby [81]. Liao [82] carried out a study where he provided *RC* decoction to 22 newborn babies in hospital, among which three were short of G6PD. According to his observation, the intake of *RC* would not cause hemolytic jaundice or any other side effects for either newborn babies that were short of G6PD or normal ones. Yi et al. [83] evaluated the toxicity of the *RC* and *RC* alkaloids (BBR, COP, PAL, and epiberberine). The cytotoxicity showed that the IC50 values of BBR, COP, PAL, and epiberberine in 3T3-L1 cells were 41.76, 56.48, 84.32, and 104.18 μg/mL, which in HepG2 cells were 48.17, 64.81, 112.80, and 120.58 μg/mL, respectively. In the acute toxicity assay, median lethal dose (LD50) values of four alkaloids were 713.57, 852.12, 1533.68, and 1360 mg/kg, respectively, which suggested that the toxicity of BBR was the maximum and PAL was the minimal. However, in the subchronic toxicity study, the currently recommended doses of *RC* alkaloids and *RC* consumed were relatively safe. There was also no abnormality in clinical signs, body and organ weights, hematological parameters, gross necropsy, and histopathology in mice after the oral administration of *RC* alkaloids and *RC* treatment. Linn et al. [84] carried out a retrospective analysis on the phenomena that *RC* and Huangbai (*Cortex Phellodendri Chinensis*) could cause hemolysis: they provided 20 patients with *RC* and Huangbai (*Cortex Phellodendri Chinensis*) for, respectively, 1,055 days and 1,252 days, demonstrating no organ toxicity or electrolyte disorder caused by *RC*. Therefore, they concluded that the use of *RC* within the dose range was safe and would not cause jaundice or kernicterus. Lee et al. [85] evaluated the no-observed-adverse-effect level (NOAEL) and the toxicity of *RC*, following repeat oral administration to rats for 13 weeks. *RC* was administered by oral gavage to groups of rats ($n = 10$/group) at dose levels of 0 (control), 25, 74, 222, 667, or 2000 mg/kg/day 5 times per week for 13 weeks, which suggested that the NOAEL of *RC* is determined to be 667 mg/kg/day for males and 2000 mg/kg/day for females. Qiu et al. [86] carried out a study on commonly used bitter-cold herbs and found that an intake of decocted *RC* liquid of the mice with a dose of more than 3 g·kg^{-1} could cause their death, the measured LD50 of *RC* being 4.89 g·kg^{-1}. In addition, they found that *RC* had a direct

effect to gastrointestinal tract and could cause loose stools and diarrhea. Later, they also found that bitter-cold herbs may do harm to the barrier function of gastric mucosa [87]. According to the animal experiment by Li and others [88], after the mice had taken a compatibility of *RC* and Huangqin (*Radix Scutellariae*) with a proportion of 1 : 0.5, the measured LD50 was equivalent to 20 times of that of an adult's daily dose of *RC*, and the measured LD50 under a combination of *RC* and Gancao (*Radix et Rhizoma Glycyrrhizae)* with the same proportion was equivalent to 42 times; when the proportion changed into 1 : 1 or 1 : 2, none of the mice died 7 days later. This proved that a compatibility of *RC* and other herbs such as Huangqin (*Radix Scutellariae*) or Gancao (*Radix et Rhizoma Glycyrrhizae*) could alleviate side effects of the mice and the optimal proportion should be 1 : 1 or 1 : 2. Chang et al. [89] retrospectively studied 116 patients with obese T2DM that were treated by the method of dispersing stagnation and clearing heat (including *RC*) without using hypoglycemic drugs; both of the 53 patients who were treated for one year and the 63 patients who were treated for two years showed very limited adverse reactions, and only 12 of them had got gastrointestinal reactions, including gastrointestinal discomforts and distention. These reactions alleviated when bitter-cold herbs reduced. If *RC* is taken over long period or used with large dose, it may cause "impairment of the stomach due to cold and bitterness," which mainly means that it may damage yang qi (the yang aspect of qi, particularly referring to that aspect of qi as functional activities) in the middle *jiao* (middle energizer, namely, the upper abdominal cavity, i.e., the portion between the diaphragm and the umbilicus housing the spleen, stomach, liver, and gallbladder), marked by diminished function of the spleen and stomach in digestion and absorption [90], thus resulting in discomforts of gastrointestinal tracts. After repeatedly clinical practices, we solve the contradiction of using *RC* by means of compatibility of medicinals. The compatibility of formulas and medicinals is a feature of TCM, which may greatly decrease the adverse reactions caused by *RC* [88]. In clinical practice, *RC* is commonly combined with some warm and acrid herbs, because warm and acrid medicine could restrain cold and bitterness; moreover, the compatibility of warmth & acridity and cold & bitterness could promote the function of the spleen and stomach and harmonize the middle energizer [91]. Ganjiang (*Rhizoma Zingiberis*) or Shengjiang (*Rhizoma Zingiberis Recens*) is commonly combined with *RC* in the clinical practice; the regular proportion of *RC* to Ganjiang (*Rhizoma Zingiberis*) is 6 : 1 and the regular proportion of *RC* to Shengjiang (*Rhizoma Zingiberis Recens*) is 4 : 1. When the patient had weak function of spleen and stomach, the dose of Ganjiang (*Rhizoma Zingiberis*) or Shengjiang (*Rhizoma Zingiberis Recens*) can be increased; the proportion of *RC* to the ginger may be 2 : 1, even 1 : 1 [68, 75, 76].

4. Summary

The rapidly increasing DM is becoming a predominant healthy problem, affecting 92.4 million persons in China. With increasing incidence of obesity, T2DM is likely to become even more prevalent in the future. It has a significant impact on the quality of life and the number of death as well as on the financial resources of public health care system. The treatment of T2DM and its complications mainly depend on western hypoglycemic drugs, and/or insulin, but more and more patients have been concerned about the potential toxicity and side effects, and they failed to delay the progression of diabetic complications; sometimes the clinical efficacy is far from satisfactory. Due to positive views of patients towards complementary and alternative medicine (CAM) therapies, and the increased availability of them, CAM therapies are increasingly and frequently used globally [92–94]; the commonly used therapies are traditional Chinese medicines, acupuncture, nutritional supplements and advice, spiritual healing, and relaxation techniques [94].

Natural plants, especially Chinese herbal medicines, have built up a characteristic medical system directed by traditional Chinese medical theory and provided rational means for various diseases including DM [12]. *Rhizoma coptidis*, a kind of classical heat-clearing and detoxifying herb, is playing an important role in treating T2DM, thus arousing strong interests in the mechanisms of its hypoglycemic activity. In this review, on one hand, we provided scientific evidence about the effective components, pharmacological researches, toxicity, and the randomized controlled trials (RCTs) on effectiveness and safety of *RC*. The modern investigation on *RC* pharmacological activity is actually developing and numerous scientific evidences are actually in progress. In recent years, some researches [95] found that diabetic patients have more Firmicutes and less Bacteroidetes than lean control; the change of gut microbiota caused activation of a network of inflammatory signal pathways via the Lipopolysaccharides (LPS) and CD14/toll-like receptor-4 (TLR4-) dependent pathway, which made the body in a state of low-grade inflammation; ultimately, T2DM came into being [96, 97]. *RC* was demonstrated to treat T2DM possibly via modulating the composition of gut microbiota (enrichment of beneficial microbiota and inhibition of harmful microbiota) [98]. However, almost all of the researches are made based on its single component BBR or others; *RC* that contains various active components may be more effective than its single component BBR and could provide multiple therapeutic effects. More clinical trials and animal experiments are still needed to study *RC*. Several researches on compound herbal medicines are not acceptable for western people; one way to change this situation is standardization; the need for adequate standards of herbal medicines to ensure quality, safety, and efficacy should be highlighted.

On the other hand, Chinese herbal medicines, instead of component, are often used by Chinese doctors; we introduced traditional Chinese medical knowledge to western people make them understand more traditional Chinese medical theory. *RC* is herb of bitter flavor and cold property. In TCM theory, bitter flavor is in direct opposition to sweet flavor, so the bitter flavor is an excellent approach to counteract sweet flavor [99]. *RC* was more applicable to prediabetes and the early stage of T2DM characterized by insulin resistance and obesity *RC* had better hypoglycemic effects on syndrome of damp-heat encumbering in the spleen, which may be related

to the significant improvement of the symptoms of splenic damp-heat syndrome, such as bitter taste in the mouth, obesity, abdominal stuffiness and fullness, sticky fetid stool, yellow-greasy coating, and slippery and rapid pulse. Several preparation formulations on *RC* were mentioned, including decoction, decoction-free granules, pill, and tablet, and the therapeutic value of *RC* in market is mainly in the form of compound or polyherbal formulations and not as single herb. The common dose of *RC* to lower blood glucose was 15–45 g. Besides, the dose of *RC* for treating T2DM was relatively large; but the dose was small for treating diabetic complications. Most of these conclusions were based on clinical experience, the lack of scientific and experimental evidence was a problem, or the grade of evidence was low; several limitations existed in this review. More clinical trials and animal experiments are required to provide stronger evidence.

Abbreviations

DM: Diabetes mellitus
TCM: Traditional Chinese medicine
RC: *Rhizoma coptidis*
T2DM: Type 2 diabetes mellitus
GLP-1: Glucagon-like protein-1
AMPK: Adenosine monophosphate-activated protein kinase pathway
InsR: Insulin receptor
PPARs: Peroxisome proliferator-activated receptors
InsR: Insulin receptor
LDLR: Low-density lipoprotein receptor
ERK: Extracellular signal-regulated kinase
HFD: High-fat diet-fed
BBR: Berberine
COP: Coptisine
PAL: Palmatine
JAT: Jatrorrhizine
HFHC: High-fat and high-cholesterol diet
HMGCR: 3-Hydroxy-3-methyl-glutaryl-CoA reductase
CYP7A1: Cholesterol 7-alpha hydroxy-lase
I/R: Ischemia and reperfusion
IL: Interleukin
TNF-α: Tumor necrosis factor-α
Rho: Rhodopsin
ROCK: Rho-kinase
ESI-MS: Electrospray ionization tandem mass spectrometry
IC50: Half maximal inhibitory
AR: Aldose reductase
RLAR: Rat lens aldose reductase
HRAR: Human recombinant aldose reductase
T1DM: Type 1 diabetes mellitus
AUC: Area under curve
C_{max}: Peak concentration
IC50: Half maximal inhibitory
STZ: Streptozotocin
P-GP: P-Glycoprotein
BMI: Body mass index
DKA: Diabetic ketoacidosis
FBG: Fasting blood glucose
PBG: Postload plasma glucose
HbA1C: Hemoglobin A1c
TCA: Total coptis alkaloids
G6PD: Glucose 6 phosphate dehydrogenase
LD50: Median lethal dose
NOAEL: No-observed-adverse-effect level
G6PD: Glucose 6 phosphate dehydrogenase
CAM: Complementary and alternative medicine
RCTs: Randomized controlled trials.

Conflict of Interests

No competing financial interests exist.

Authors' Contribution

Xiao-Lin Tong and Bing Pang proposed the paper's topic; Bing Pang and Xiao-Tong Yu wrote the paper, and the two of them contributed equally to this work and are both first coauthor; Qiang Zhou, Tian-Yu Zhao, and Han Wang consulted the references; and Xiao-Lin Tong and Cheng-Juan Gu revised the paper.

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Research Article

Toxicopathological Evaluation of Hydroethanol Extract of *Dianthus basuticus* in Wistar Rats

Anofi Omotayo Tom Ashafa and Mutiu Idowu Kazeem

Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba 9866, South Africa

Correspondence should be addressed to Anofi Omotayo Tom Ashafa; tom.ashafa@gmail.com

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Background. Dianthus basuticus is a commonly used medicinal plant in Basotho traditional medicine for the treatment of diabetes, but there is no report on its safety or toxicity. Therefore, we evaluated the toxicity profile of the hydroethanol whole plant extract of *Dianthus basuticus* in Wistar rats. *Methods.* Acute toxicity test was performed with single oral administration of 100–3200 mg/kg body weight of *D. basuticus* extract to rats and the animals were observed for 14 days for signs of toxicity. The subacute toxicity experiment was conducted by oral administration of graded doses (200, 400, and 800 mg/kg) of *D. basuticus* extract daily for 28 days. Behavioural changes as well as haematological, biochemical, and histological parameters were then evaluated. *Results.* There was no observable sign of toxicity in the acute toxicity test. There were significant decreases ($P < 0.05$) in the feed and water intake as well as total cholesterol and triglycerides of the *D. basuticus* extract-treated rats in subacute toxicity study. There were no treatment related differences in the haematological, biochemical, and histopathological evaluations. *Conclusions.* Administration of hydroethanol extract of *D. basuticus* may be safe at the dosages tested in this study but its continuous usage can cause anorexia.

1. Introduction

One of the widespread diseases in the world today that has defied cure is diabetes mellitus. International Diabetes Federation estimated that 382 million people are suffering from this disease and the number is projected to increase to 552 million in 2035 [1]. Despite the multifaceted approach (use of oral antidiabetic drugs, exercise, and lifestyle changes) taken in the management of this disease, diabetics continue to suffer from its complications. Oral hypoglycemic agents are also associated with several side effects ranging from hypoglycemia, weight gain, and chronic tissue damage [2]. This accounts for global increase in the usage of medicinal plants for the management of this disease.

The majority of the people from African descent use herbal remedies in one form or the other to manage health related problems such as diabetes [3]. Many of the users of plant derived medicines do so because of poverty, easy access, low cost, and perceived belief that all medicinal herbs, being natural, are generally safe and free from undesirable side effects while acting as an effective medicine [4]. However, very often, herbs may interact with medications that result in adverse conditions. Despite recent researches into the efficacy of herbal remedies, medicinal plants are still poorly understood due to lack of systemic nomenclature, good quality control and safety, and/or toxicity information on herbs [5]. Therefore, medicinal plants and their bioactive components should be put through thorough safety and toxicity tests.

Dianthus basuticus Burtt Davy belongs to the Caryophyllaceae family. It is distributed in the Eastern Cape, Gauteng, KwaZulu-Natal, Mpumalanga, and Free State provinces of South Africa. It is known as Lesotho Dianthus, Lesotho Carnation, Drakensberg Carnation, and grass of the road in English, Lesothose grootblom-wilde angelier in Afrikaans, or Hlokwa-la-tsela in Sesotho [6]. Among the Basotho, the plant is widely used in the management of diabetes, as immune modulator, and in increasing fertility of bulls [7]. It is also used in the treatment of chest pains, mumps, and infections. The antimicrobial and cytotoxicity investigations on this

plant revealed that extracts and fractions from *D. basuticus* inhibited a wide range of pathogenic bacteria at very low concentrations while hydroethanol and ethanol extracts were cytotoxic to brine shrimp nauplii [8].

Currently, there is immense reliance of the Basotho tribe on this species as an antidiabetic, yet, there is no information in the literature on the safety/toxicity of this valuable medicinal plant. The present study, therefore, investigated the effect of 28-day oral administration of *D. basuticus* whole plant extract on the biochemical, haematological, and histopathological parameters in Wistar rats.

2. Materials and Methods

2.1. Plant Collections. The plant material was collected in January 2013 from multiple population in the field around Qwaqwa within the Golden Gate Mountains (28° 28″ 111′ S and 28° 48″ 314′ E; altitude 11950 m). The species abundance was taken into consideration and collections were made in such a way that the existence of species was not threatened. Proper identification and authentication were done at the Bews Herbarium of the University of KwaZulu-Natal, Pietermaritzburg Campus, by Dr. C. J. Potgieter. Herbarium voucher with reference number (LamMed/01/2013/Qhb) was already deposited at the UFS-Qwaqwa Campus Herbarium.

2.2. Extract Preparation. 100 g of the dried powdered material was extracted in hydroethanol (50 : 50), with constant shaking on Labcon platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h. The extract was centrifuged (Hermle Laboratory Centrifuge, Lasec, South Africa) and later filtered using Whatman number 1 filter paper. The filtrate was concentrated using rotary evaporator under vacuum and later freeze-dried in a lyophilizer (Ilshin Lab. Co., Ltd., Seoul, Korea). The percentage yield of the extract was 18.71%.

2.3. Animals. Wistar rats of both sexes were obtained from the Animal House of the University of the Free State, South Africa, and were acclimatized for 1 week. They were housed in polypropylene cages under a 12 h light/dark cycle at 20–25°C and 50–60% relative humidity. All animals had access to standard rat chow (Epol Feeds, Westville, South Africa) and tap water *ad libitum*. Ethical approval for the study was obtained from the Interfaculty Ethics Committee of the University of the Free State, South Africa, with approval number NR 02/2013 and all experiments were performed according to the Guide for the Care and Use of Laboratory Animals [9].

2.4. Acute Toxicity Study. Acute oral toxicity was evaluated in rats in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines [10] with slight modification. Twenty (20) female animals weighing 180–200 g were divided into five groups (A–E) consisting of four animals each. Group A served as control and orally received 1 mL distilled water while groups B to E received 1 mL of 100, 400, 1600, and 3200 mg/kg body weight (b/w) of the extract. All animals were observed for clinical signs including mortality and moribundity, immediately after dosing and at 1, 2, 4, 8, and 12 h and then twice daily for 14 days. Abnormal findings were recorded with the time of onset and disappearance. Body weights and food consumption were measured daily. On the 14th day, all animals were sacrificed and organs of interest (lung, liver, heart, kidney, stomach, and intestine) were observed macroscopically. Since there was no death or any physically observed sign of toxicity, 200, 400, and 800 mg/kg b/w of the extract were selected for the subacute toxicity study.

2.5. Repeated Dose 28-Day Oral Toxicity Study. The study applied the OECD guidelines on repeated dose 28-day oral toxicity [11]. Forty (40) male Wistar rats were randomized into four groups of ten animals each. Group 1 (control) was orally administered distilled water. Groups 2 to 4 were orally treated with 200, 400, and 800 mg/kg body weight/day of *D. basuticus* hydroalcohol extract, respectively. The treatment continued for 28 days and the administration was done using metal oropharyngeal cannula. Observations were made twice daily for mortality and changes in general appearance or behaviour. The body weights were recorded every week, and the individual dose was adjusted for the body weight to maintain the target dose level for all rats. In addition, detailed clinical examination as well as measurement of food and water consumption was performed weekly.

2.5.1. Collection of Blood Sample and Isolation of Organs. The rats were humanely sacrificed on the 29th day by halothane euthanasia following fasting for 12–16 h. Aliquot of the blood was collected through cardiac puncture into sample bottles containing EDTA (BD Diagnostics, Preanalytical Systems, Midrand, USA) for haematological analysis while the remaining blood was kept in plain bottles from which serum was collected and stored for biochemical analysis. The rats were quickly dissected and the whole liver, kidneys, hearts, spleens, lungs, and the testes were excised, freed of fat, blotted with clean tissue paper, and then weighed. The organs to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat. Defined samples of the lung, heart, liver, kidney, and testes were placed in 10% neutral buffered formaldehyde for histopathological examination.

2.5.2. Determination of Haematological Parameters. Using Horiba ABX 80 Diagnostics system (ABX Pentra Montpellier, France), the following analyses were carried out on the whole blood: white blood cell (WBC), red blood cell (RBC), haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), and platelet (PLT).

2.5.3. Determination of Liver and Kidney Function Parameters. Serum was analyzed for total bilirubin (T-BIL), conjugated

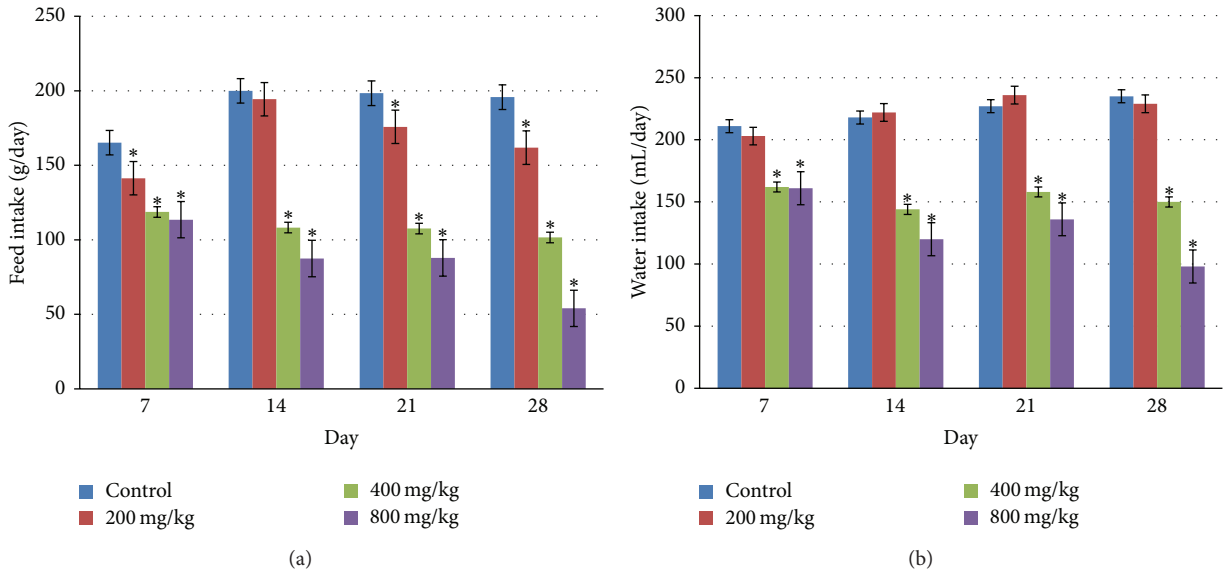

Figure 1: Effect of oral administration of hydroethanol extract of *Dianthus basuticus* on the consumption of (a) feed and (b) water during a 28-day toxicity study in rats. Data are expressed as mean ± SEM, *statistical significance at $P < 0.05$.

Table 1: Effect of administration of hydroethanol extract of *Dianthus basuticus* on some weight parameters of male Wistar rats.

Parameters	Dose (mg/kg body weight/day)			
	Control	200	400	800
Initial body weight (g)	257.10 ± 6.90	256.75 ± 3.98	240.81 ± 9.97	275.66 ± 4.22
Final body weight (g)	329.43 ± 6.22	328.66 ± 5.52	309.04 ± 2.94	317.85 ± 6.24
Relative liver weight (%)	3.20 ± 0.01	3.44 ± 0.02***	3.21 ± 0.02	3.03 ± 0.01***
Relative kidney weight (%)	0.63 ± 0.04	0.64 ± 0.02	0.58 ± 0.03**	0.59 ± 0.02
Relative lung weight (%)	0.87 ± 0.04	0.85 ± 0.04	0.89 ± 0.04	1.00 ± 0.05***
Relative spleen weight (%)	0.21 ± 0.02	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.02
Relative heart weight (%)	0.28 ± 0.02	0.31 ± 0.02	0.30 ± 0.03	0.31 ± 0.01
Relative testis weight (%)	1.07 ± 0.06	1.11 ± 0.03	1.22 ± 0.05***	1.15 ± 0.08***

Values are presented as mean ± SEM ($n = 10$).
**Statistically different from the control at $P < 0.01$.
***Statistically different from the control at $P < 0.001$.

bilirubin (C-BIL), total protein (TP), albumin (ALB), globulin (GLB), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (UR), uric acid (UA), and creatinine (CRE) using a BS-200 automatic biochemistry analyzer (Mindray Co., Ltd.) while sodium (Na), potassium (K), and calcium (Ca) were analyzed using Roche electrolyte analyzer (AVL9181; Roche, Germany).

2.5.4. Determination of Serum Lipid Profile. Total cholesterol (TC), triacylglycerol (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were evaluated using the methods of Allain et al. [12], Tietz [13], Grove [14], and Bergmenyer [15] as described in the Quimica Clinica Aplicada assay kits.

2.5.5. Histopathological Examination. The organs (lungs, heart, kidney, liver, and testis) were fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70%, 90%, and 95% v/v), cleaned in xylene, and embedded in paraffin wax [16]. Tissue sections were prepared and stained with hematoxylin and eosin. The photomicrographs were taken at ×400 using the Leitz, DIALUX research microscope.

2.6. Statistical Analysis. Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, La Jolla, CA 92037, USA). Data were expressed as means of ten replicates ± SEM and were subjected to analysis of variance (ANOVA) followed by Bonferroni post hoc test. Statistical significance was considered at $P < 0.05$.

3. Results

3.1. Acute Toxicity Study. After 14 days, no death or signs of toxicity were observed in all the groups of rats treated with

Table 2: Effect of administration of hydroethanol extract of *Dianthus basuticus* on some haematological parameters of male Wistar rats.

Parameters	Dose (mg/kg body weight/day)			
	Control	200	400	800
White blood cell ($\times 10^3/\mu$L)	7.77 ± 1.83	8.00 ± 0.98	6.87 ± 2.23	8.55 ± 2.47
Red blood cell ($\times 10^6/\mu$L)	8.45 ± 0.23	8.91 ± 0.41	8.69 ± 0.31	8.84 ± 0.54
Haematocrit (%)	0.46 ± 0.01	0.46 ± 0.02	0.47 ± 0.02	0.47 ± 0.02
Haemoglobin (g/dL)	15.83 ± 0.15	15.93 ± 0.64	16.10 ± 0.70	16.35 ± 0.64
MCV (fL)	54.67 ± 0.58	54.00 ± 0.00	54.33 ± 1.15	52.50 ± 0.71***
MCH (pg)	18.67 ± 0.58	18.00 ± 0.00	18.67 ± 0.58	18.30 ± 0.42
MCHC (g/dL)	34.67 ± 0.57	34.00 ± 0.00	34.00 ± 1.00	35.00 ± 0.0
RDW (fL)	19.20 ± 1.11	19.37 ± 0.49	19.77 ± 1.00	19.70 ± 1.56
Neutrophils (%)	1.76 ± 0.47	1.72 ± 0.65	1.69 ± 0.68	1.32 ± 0.25
Lymphocytes (%)	5.36 ± 1.36	5.72 ± 0.33	5.26 ± 1.47	6.55 ± 1.96*
Monocytes (%)	0.32 ± 0.15	0.46 ± 0.11	0.37 ± 0.11	0.36 ± 0.08
Eosinophils (%)	0.13 ± 0.04	0.11 ± 0.07	0.16 ± 0.04	0.31 ± 0.13
Basophils (%)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.00
Platelets ($\times 10^3/\mu$L)	914.67 ± 76.56	881.33 ± 70.63	872.00 ± 59.92	1006.50 ± 43.13***

Values are presented as mean ± SEM ($n = 10$).
*Statistically different from the control at $P < 0.05$.
***Statistically different from the control at $P < 0.001$.

Table 3: Liver and kidney function parameters of male Wistar rats administered with hydroethanol extract of *Dianthus basuticus*.

Parameters	Dose (mg/kg body weight/day)			
	Control	200	400	800
Total bilirubin (μmol/L)	5.00 ± 1.00	5.67 ± 2.08	4.00 ± 1.73	7.00 ± 1.41
Conjugated bilirubin (μmol/L)	2.33 ± 0.58	3.00 ± 0.00	1.67 ± 0.58	3.50 ± 0.71
Total protein (g/L)	62.33 ± 4.16	64.00 ± 4.36	65.67 ± 0.68	65.33 ± 0.58
Albumin (g/L)	33.00 ± 0.50	33.33 ± 0.45	33.33 ± 0.06	35.00 ± 0.28
Globulin (g/L)	29.33 ± 4.16	30.67 ± 3.21	32.33 ± 0.57	34.00 ± 0.72
Alkaline phosphatase (U/L)	277.67 ± 26.91	287.67 ± 21.72	314.00 ± 20.48***	276.00 ± 4.24
γ-Glutamyl transferase (U/L)	3.00 ± 3.46	3.00 ± 2.00	1.33 ± 0.57	4.00 ± 1.41
Aspartate aminotransferase (U/L)	179.00 ± 23.07	168.33 ± 4.04	145.67 ± 13.57***	187.50 ± 6.36
Alanine aminotransferase (U/L)	81.67 ± 7.09	75.33 ± 6.43	75.00 ± 5.29	84.00 ± 5.66
Sodium (mmol/L)	144.00 ± 1.00	145.67 ± 1.53	145.67 ± 1.53	142.50 ± 2.12
Potassium (mmol/L)	5.10 ± 0.20	4.93 ± 0.45	4.53 ± 0.31	5.40 ± 0.14
Calcium (mmol/L)	2.48 ± 0.01	2.49 ± 0.06	2.54 ± 0.09	2.46 ± 0.01
Creatinine (mmol/L)	43.00 ± 2.00	36.00 ± 1.53	46.00 ± 5.00	43.00 ± 5.66
Chloride (mmol/L)	103.00 ± 1.00	105.67 ± 1.15	104.33 ± 1.52	105.50 ± 2.12
Urea (mmol/L)	5.90 ± 0.44	7.20 ± 1.08	5.83 ± 0.61	5.60 ± 0.71
Uric acid (mmol/L)	0.09 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.10 ± 0.00

Values are presented as mean ± SEM ($n = 10$).
***Statistically different from the control at $P < 0.001$.

different doses (100, 400, 1600, and 3200 mg/kg) of the plant extract.

3.2. Subacute Toxicity

3.2.1. Clinical Signs and Mortality. There was no mortality attributed with the administration of *D. basuticus* extract during the period of study. One animal died in the 400 mg/kg/day group on d 16 while another one died in the 200 mg/kg body weight group on d 24 of the experiment. The death of these animals may be due to gavage accident as the body weights and food intake of these animals before death did not reduce when compared to other animals in the same group.

3.2.2. Food and Water Intake. The weekly mean food and water intake of the rats administered different doses of *D. basuticus* extract is shown in Figure 1. Throughout the duration of the experiment, there were significant reductions ($P < 0.05$) in the food intake between all the extract-treated groups compared to the control. However, only the rats in

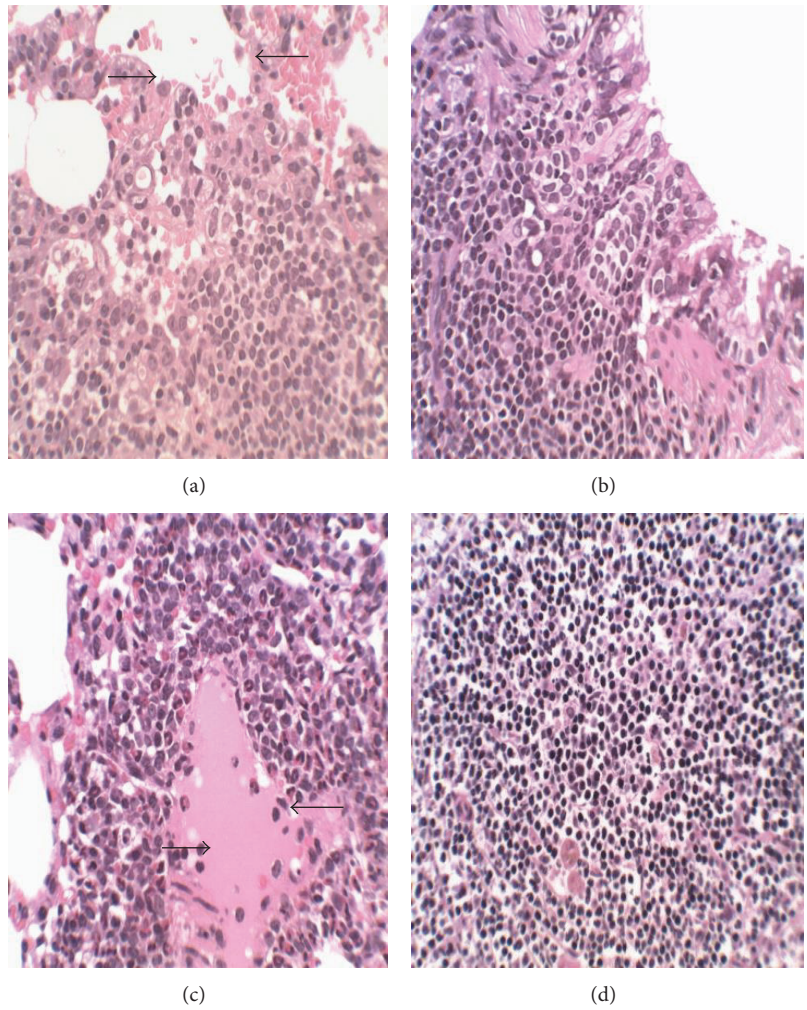

Figure 2: Photomicrograph of section of the lungs of rats following 28-day administration of (a) distilled water, (b) 200 mg/kg, (c) 400 mg/kg, and (d) 800 mg/kg body weight/day of hydroethanol extract of *Dianthus basuticus* (haematoxylin and eosin, ×400).

the 400 and 800 mg/kg groups exhibited significant decrease ($P < 0.05$) in their water intake when compared with control.

3.2.3. Body and Organ Weights. There was no significant difference ($P > 0.05$) between the body weights of the extract-treated groups compared to the control (Table 1). The relative liver weight increased significantly ($P < 0.001$) in the 200 and 800 mg/kg groups while there was significant decrease ($P < 0.01$) in the relative kidney weight of the 400 mg/kg group compared to the control. Significant elevation ($P < 0.001$) was also witnessed in the relative testis weight by the 400 and 800 mg/kg groups while the relative lung weight only rose in the 800 mg/kg group.

3.2.4. Haematological Parameters. Table 2 showed that there was significant decrease ($P < 0.001$) in the MCV as well as increase in the lymphocytes ($P < 0.05$) and platelets ($P < 0.001$) of the animals in the 800 mg/kg group when compared to the control while other haematological parameters were not significantly affected.

3.2.5. Liver and Kidney Functions Parameters. The result of serum liver and kidney function parameters of rats administered hydroethanol extract of *D. basuticus* is presented in Table 3. Animals in the 400 mg/kg group witnessed significant increase ($P < 0.001$) in alkaline phosphatase (ALP) and decrease ($P < 0.001$) in aspartate aminotransferase (AST) compared to the control animals. All other parameters tested were not significantly different in all the groups compared to control.

3.2.6. Lipid Profile. At all the doses tested, there were significant reductions ($P < 0.001$) in the total cholesterol level of the rats compared to the control (Table 4). Animals in the 200 and 400 mg/kg groups witnessed significant increase ($P < 0.001$) in the triglyceride level while it reduces ($P < 0.001$) in the 800 mg/kg group. However, administration of 200 mg/kg

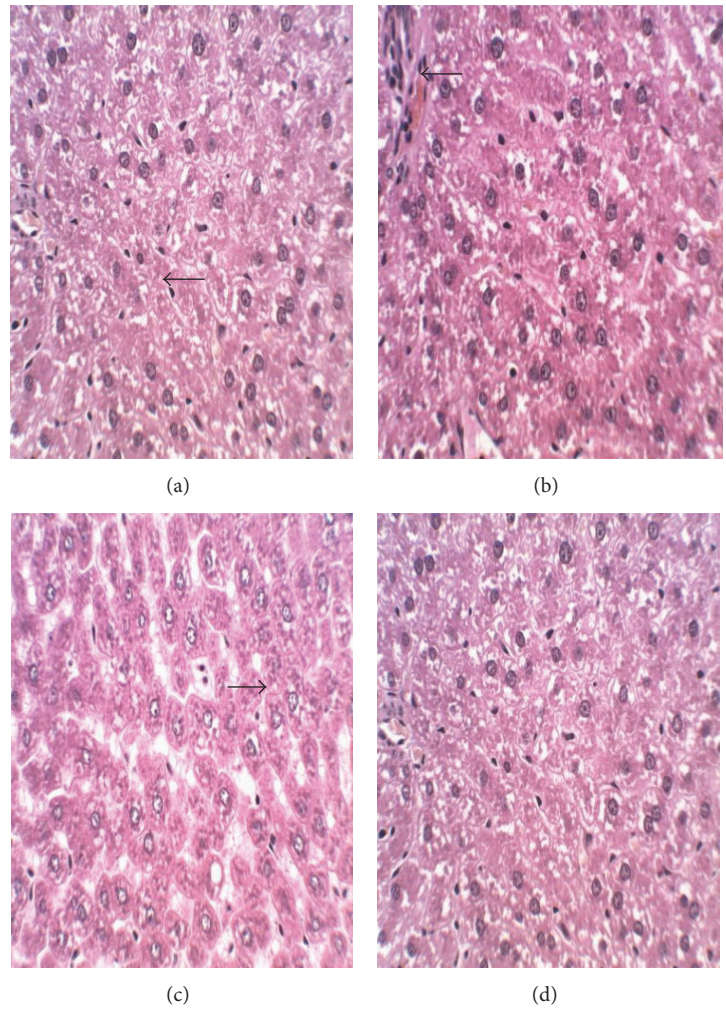

Figure 3: Photomicrograph of section of the liver of rats following 28-day administration of (a) distilled water, (b) 200 mg/kg, (c) 400 mg/kg, and (d) 800 mg/kg body weight/day of hydroethanol extract of *Dianthus basuticus* (haematoxylin and eosin, ×400).

Table 4: Serum lipid profile of male Wistar rats administered with hydroethanol extract of *Dianthus basuticus*.

Parameters	Dose (mg/kg body weight/day)			
	Control	200	400	800
Total cholesterol (mmol/L)	1.46 ± 0.17	1.20 ± 0.07***	1.37 ± 0.15*	1.20 ± 0.04***
Triglycerides (mmol/L)	1.41 ± 0.13	1.60 ± 0.09***	1.62 ± 0.12***	0.88 ± 0.01***
HDL-C (mmol/L)	0.96 ± 0.03	1.07 ± 0.07**	0.99 ± 0.06	1.03 ± 0.06
LDL-C (mmol/L)	0.27 ± 0.06	0.27 ± 0.03	0.30 ± 0.01	0.20 ± 0.00
Atherogenic index	0.25 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.22 ± 0.01

Values are presented as mean ± SEM ($n = 10$).
*Statistically different from the control at $P < 0.05$.
**Statistically different from the control at $P < 0.01$.
***Statistically different from the control at $P < 0.001$.

of the extract to rats caused significant elevation ($P < 0.01$) in their HDL-C concentration compared to the control.

3.2.7. Histopathological Examination. No gross abnormalities related to the administration of the extract were observed in any of the euthanized animals at the conclusion of the experiment. In the lungs of both control and treated animals, peribronchiolar infiltration of lymphocytes and granulocytes was observed which indicated mild inflammation (Figure 2). There was appearance of swollen glycogen in the liver of the extract-treated rats (Figure 3). Degeneration of testicular tubules, which hitherto indicates sperm formation, was also

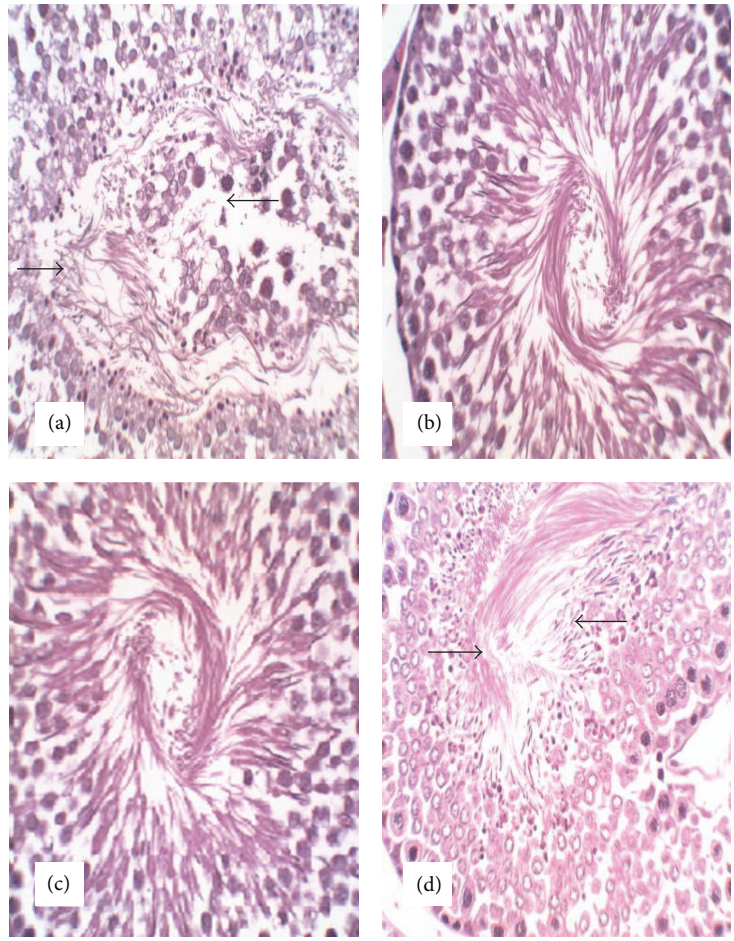

Figure 4: Photomicrograph of section of the testis of rats following 28-day administration of (a) distilled water, (b) 200 mg/kg, (c) 400 mg/kg, and (d) 800 mg/kg body weight/day of hydroethanol extract of *Dianthus basuticus* (haematoxylin and eosin, ×400).

noted in all the groups tested (Figure 4). However, there were no specific changes visible in the kidneys and hearts of the extract-treated groups compared to the control animals (Figures 5 and 6).

4. Discussion

The dose-dependent reduction in food and water consumption of *D. basuticus* extract-treated rats could be an indication that the extract decreases the sense of taste and appetite of the animals. Food and water are essential for life and are required for the growth and development of all organisms. However, the reduction in both the food and water intake did not produce concomitant decrease in the body weight of the animals. Change in the body weights is one of the first critical signs of toxicity [17]. The mean body weights of animals in all experimental groups increased with the duration of the study and were not significantly different from one another. The weight gained by the animals during the experimental period may be an indication that the extract did not hamper the growth of the animals [18]. However, the significant changes in the relative weight of the liver (200 and 800 mg/kg), testis (400 and 800 mg/kg), kidney (400 mg/kg), and lung (800 mg/kg) of the rats may not be regarded as adverse effect because they were not dose-dependent and are not correlated with pathological organ lesions [19].

Haematopoietic system is one of the important parameters used to determine the physiological and pathological status of mammals, as it provides information on the reaction of the body to injury [20]. Though there was significant increase in the platelets counts of the animals in the 800 mg/kg group, this value is still within the physiological range (837–1455 $\times 10^3/\mu$L) for Wistar rats [21] and this increase may indicate stimulatory effect on erythropoietin [22]. The observed increase in the lymphocytes level of the 800 mg/kg group may suggest boosting of the immune system of the animals since lymphocytes are the main effector cells of the immune system [22]. However, the reduction in the MCV level may not be regarded as toxic effect because its value in conjunction with that of MCH, MCHC, and RDW relates to the integrity of individual red blood cell [23] while these parameters and red blood cell count were not affected in this study.

Alkaline phosphatase is a marker enzyme of the plasma membrane as well as the endoplasmic reticulum and is present in the cells lining the biliary duct of the liver [24]. Therefore, increase in serum ALP activities may indicate

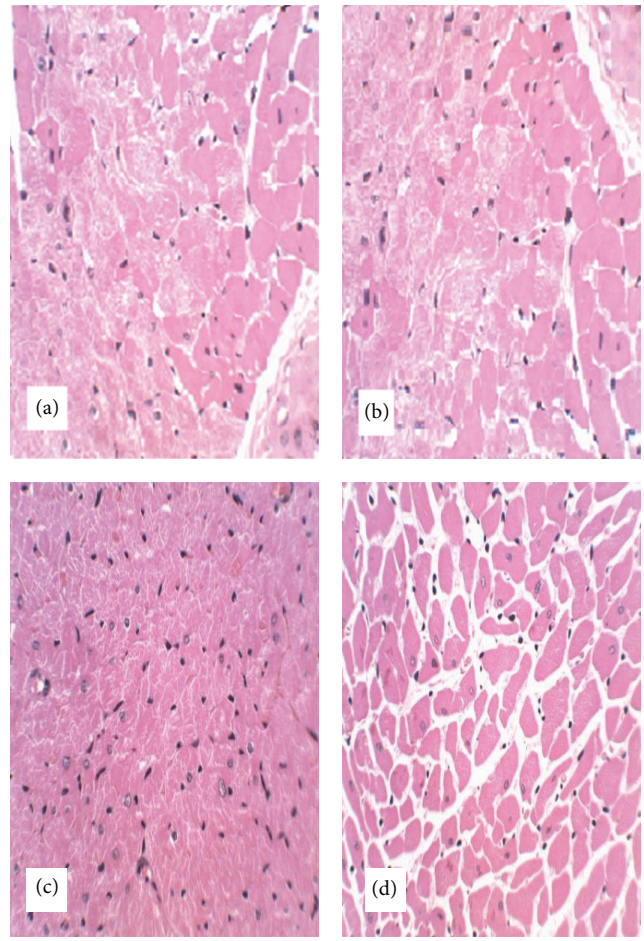

Figure 5: Photomicrograph of section of the heart of rats following 28-day administration of (a) distilled water, (b) 200 mg/kg, (c) 400 mg/kg, and (d) 800 mg/kg body weight/day of hydroethanol extract of *Dianthus basuticus* (haematoxylin and eosin, ×400).

alteration in the permeability of the plasma membrane and cholestatic diseases such as gall stone [25], while alterations in the activity of serum AST may produce consequential effects on the metabolism of amino acids and its biochemical regulation. The significant change in the concentrations of serums ALP and AST in the 400 mg/kg group appears to be biologically irrelevant as there was no dose-response relationship because rats in the highest dose group were not affected. This alteration was not correlated with any pathological lesion in the liver and all other liver function parameters were not affected. It is worthy of note that there was no alteration in all the kidney function parameters (creatinine, urea, uric acid as well as sodium, potassium, and chloride ion) analysed in this study which may be an indication of safety [26].

Alterations in the concentration of lipids like TC, HDL-C, and triglycerides can provide information on the status of lipid metabolism as well as predisposition of the animals to atherosclerosis [27]. The reduction in TC level of animals in all the treated groups may be associated with impairment in the β-oxidation of fatty acids [28]. Elevation in triglyceride levels in the 200 and 400 mg/kg groups and its decrease in the 800 mg/kg group may be an indication of addition and depletion of the energy store, respectively, in the animals [29]. However, this may not be toxicologically significant due to the inconsistency in the trend among the dosages. HDL-C is an antiatherogenic factor which is important in the transport of cholesterol from cells to the liver where it is catabolized [30]. Increase in the HDL-C level of all the treated groups which is significant only in the 200 mg/kg group may suggest that there was continuous export of excess cholesterol to the liver for excretion into the bile, thereby reducing the risk of atherosclerosis or coronary artery diseases [31]. This result implies that this extract may be useful in the management of heart related diseases.

There were no treatment related microscopic changes in all the organs observed in this study. The peribronchiolar infiltration of lymphocytes found in the lungs of these animals may possibly be due to inadequate air inspired by these animals since it is found in both the treated and control groups [32]. All morphological changes observed in the liver and testis were randomly distributed between extract-treated and control animals, and the incidences were within the range of normal background lesions. It can therefore be inferred that all histological changes observed were mild and are not considered to be indication of toxicity of the extract.

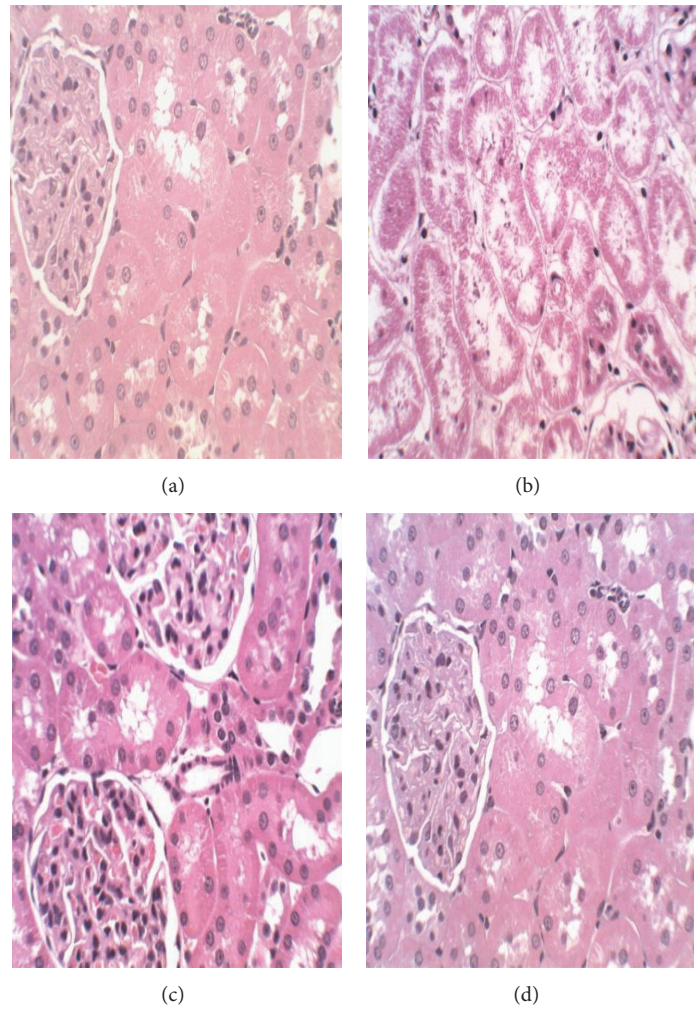

Figure 6: Photomicrograph of section of the kidney of rats following 28-day administration of (a) distilled water, (b) 200 mg/kg, (c) 400 mg/kg, and (d) 800 mg/kg body weight/day of hydroethanol extract of *Dianthus basuticus* (haematoxylin and eosin, ×400).

5. Conclusions

These results demonstrate that hydroethanol extract of *Dianthus basuticus* can cause alterations in food and water consumption but did not produce any consistent change in haematological, biochemical, and histopathological parameters of rats. It can therefore be concluded that the administration of this extract at the dosages studied (200–800 mg/kg body weight) may be safe but caution should be taken in its long-term usage as it could lead to anorexia.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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Review Article

Innovative Thoughts on Treating Diabetes from the Perspective of Traditional Chinese Medicine

Bing Pang,[1] Qiang Zhou,[2] Tian-Yu Zhao,[1] Li-Sha He,[1] Jing Guo,[1] Hong-Dong Chen,[1] Lin-Hua Zhao,[3] and Xiao-Lin Tong[1]

[1]*Department of Endocrinology, Guang'anmen Hospital of China Academy of Chinese Medical Sciences, Beijing 100054, China*
[2]*Department of Digestion, Beijing Hospital of Traditional Chinese Medicine, Capital University of Medicine Sciences, Beijing 100010, China*
[3]*Laboratory of Molecular Biology, Guang'anmen Hospital of China Academy of Chinese Medical Sciences, Beijing 100054, China*

Correspondence should be addressed to Lin-Hua Zhao; melonzhao@163.com and Xiao-Lin Tong; xiaolintong66@sina.com

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The rapidly increasing incidence of diabetes mellitus (DM) is becoming a major public health issue. As one of the important parts in complementary and alternative therapies, traditional Chinese medicine (TCM) is promising in treating DM. In this review, we summarize new thoughts on treating DM that aim to improve the clinical efficacy of TCM from the perspectives of principle, methods, formula, herbs, and doses. Our approach is as follows: principle: we use a combination of symptoms, syndromes, and diseases as a new mode for treating diabetes; methods: emphasizing heat-clearing in the early and middle stage of T2DM and invigorating blood circulation throughout the whole process of T2DM are two innovative methods to treat T2DM; formulas and herbs: choosing formulas and herbs based on the combination of TCM theory and current medicine. We will emphasize four strategies to help doctors choose formulas and herbs, including treatment based on syndrome differentiation, choosing herbs of bitter and sour flavors to counteract sweet flavor, choosing formulas and herbs aimed at main symptoms, and using modern pharmacological achievements in clinical practice; dose: reasonable drug dose plays an important role in the treatment of DM and a close relationship exists between dose and clinical efficacy.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder caused by either absolute deficiency in insulin secretion or reduction in the biological effectiveness of insulin. The global prevalence of DM among adults aged 20–79 years was 8.3% in 2013 [1]. As one of the largest developing countries, China has the biggest population of patients with DM with 92.4 million, which account for 9.7% of the adult population. In addition, 148.2 million adults (15.5%) have prediabetes [2]. DM has a significant impact on the quality of life and life expectancy of people as well as on the economic burden on the health care system. Therefore, it represents a major public health issue [3]. Type 2 diabetes mellitus (T2DM) is the predominant form of DM and accounts for 90–95% of the diabetic populations, due to an increased number of elderly patients and a greater prevalence of obesity and sedentary lifestyles [4, 5]. Management of T2DM is still a challenge and the standard therapy for T2DM includes balanced diet, appropriate exercise, use of oral hypoglycemic drugs, and/or subcutaneous insulin injections [6]. Although considerable progress has been made regarding hypoglycemic drugs and insulin, Western medicine still has some limitations. Traditional Chinese medicine (TCM) has a long history of more than 2000 years in treating DM [7, 8], and there are several advantages in treating DM with TCM, including lower rate of toxicity and/or side effects, holistic regulation of metabolic problems, reversal of risk factors leading to T2DM, and delaying diabetic complications. Due to the differences in etiology, pathogenesis, diagnosis, and interventions between traditional Xiaoke disease and T2DM, several new therapeutic thoughts have been recently proposed. In this review, we summarized these thoughts based on principle, method, formula, herbs, and dose through literature analysis in both

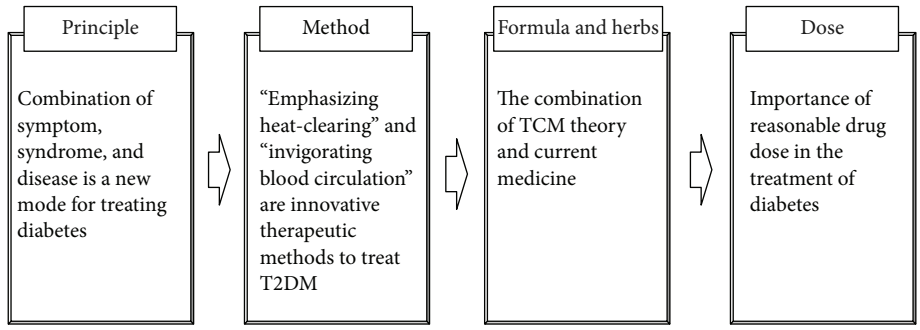

Figure 1: The scheme figure of the innovative thoughts in the treatment of diabetes.

English and Chinese search engines to guide clinicians in treating T2DM. The scheme figure of the innovative thoughts in the treatment of diabetes is shown in Figure 1.

2. Principle: Combination of Symptom, Syndrome, and Disease Is a New Mode for Treating Diabetes

The "combination of symptoms, syndrome, and disease" has been widely used in the treatment of several chronic difficult diseases [9–11]. The mode of combining symptoms, syndromes, and diseases is shown in Figure 2. Syndromes, also known as "zheng" or "pattern," are the abstraction and generalization of the pathological changes at a certain stage of a disease, which shows the essence of a disease more deeply and completely [12]. Syndrome differentiation is diagnosed through comprehensive consideration of symptoms and signs (tongue appearance and pulse feeling included) and has implications for determining the cause, location, and nature of the disease and the patient's physical condition, as well as the trend of development [13]. As an example for syndrome differentiation, one T2DM patient with obesity, reddened complexion, stuffiness and fullness in the abdomen, red tongue, yellow-greasy coating, and slippery pulse may suffer from typical phlegm and heat stasis syndrome, while the other T2DM patient may suffer from losing weight, fatigue, excessive sweating, dry mouth, insomnia, red tongue, thin coating, and vacuous and rapid pulse and may be differentiated with the syndrome of dual deficiency of qi and yin. The condition was specific to the individual and appropriate treatment was suggested. Syndrome differentiation is the most remarkable characteristic in TCM, and all diagnostic and therapeutic methods of TCM are derived from this principle.

However, syndrome differentiation has several limitations. It regulates the patient's physical condition with a holistic approach to health, but the need to relieve the patient's most painful symptoms is not met in the short term. Moreover, several diseases are found before the appearance of signs and symptoms, which leads to "no syndrome may differentiate." According to these reasons, more attention should be paid to alleviate the main symptoms. A symptom is a characteristic sign of a particular disease and is a (bodily or mental) phenomenon, circumstance, or change in condition arising from and accompanying a disease or another pathological condition [14], which includes the subjective perception of patients, as well as objective indicators of diseases obtained from testing methods. In ancient China, physicians treated diseases mainly by directly improving symptoms. Some herbal classics described herb efficacies by alleviating the main symptoms; for example, Chuanwu (*Radix Aconiti Praeparata*) may alleviate pain, Banxia (*Rhizoma Pinelliae*) may alleviate nausea and vomiting, Walengzi (*Concha Arcae*) may relieve gastric hyperacidity, and so on. There are several advantages in aiming at main symptoms. First, it is an effective way to relieve the most painful symptoms directly. For example, some diabetic patients also have erectile dysfunction (ED), which may be the most painful symptoms to male patients. Chuanxiong (*Rhizoma Chuanxiong*) and Wugong (*Scolopendra*) were first considered to improve this symptom directly, and subsequently other formulas and herbs were added to constitute a complete prescription. Secondly, difficult diseases always have a complicated etiology and pathogenesis, which results in difficulties with syndrome differentiation; "treating aimed at main symptoms" has the advantage of simplifying the differentiated process and reversing the trends of acute disease directly, thus achieving great clinical efficacy. Thirdly, it could solve the problem of "no syndrome may differentiate"; patients who did not show obvious symptoms in the clinic were found to have abnormal blood lipid indicators and can be treated with herbs such as Shanzha (*Fructus Crataegi*), Hongqu (*Red konjac powder*), and Wuguchong (*Oriental latrine fly larvina*) aimed at hyperlipidemia.

As mentioned above, the characteristics of a syndrome are relatively widespread and abstract, which is easy to conceal the difference [9–11]. The following example may help to explain the shortage of syndrome differentiation. Tuberculosis, lung cancer, diabetes, and chronic nephritis all have a similar syndrome of dual deficiency of qi and yin, but the pathogenesis and prognosis of the above diseases are different, thereby indicating that they should be treated with the same TCM method of boosting qi and nourishing yin; however, this treatment may have insufficient effects on the diseases. This is why syndrome differentiation has strong effects on improving the syndrome but poor effects on treating diseases. Thus, more attention has to be paid to treating diseases. A disease is a condition of poor (more or

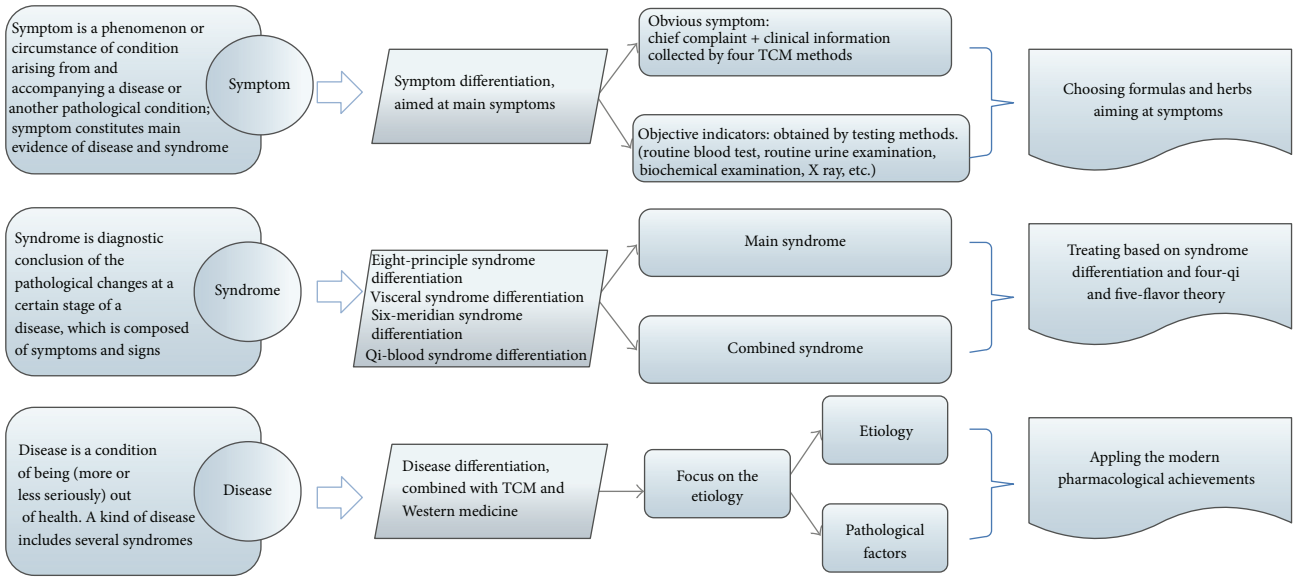

Figure 2: The mode of combining symptom, syndrome, and disease.

less seriously) health [9–11]. Disease differentiation provides the main direction for treatment and improves the specificity of the treatment. A lot of the attention should be paid to pathological characteristics of the disease as well as objective indicators such as X-rays and ultra sound. For example, when diabetes, lung cancer, and tuberculosis were found to have similar syndrome of dual deficiency of qi and yin, based on the principle of boosting qi and nourishing yin, the treatment of diabetes may lower the blood glucose, while the treatment for lung cancer may help fight the tumor, and the treatment in tuberculosis is for eliminating *M. tuberculosis*.

Currently, the combination of symptoms, syndromes, and diseases has become a common mode in the diagnosis and treatment of TCM [9–11], which focuses on "treating aimed at main symptoms directly," "highlighting chemical or biochemical indicators," "choosing the formulas that treat both TCM syndromes and diseases in Western medicine," "emphasizing etiology, pathogenesis, and diagnosis of diseases in Western medicine," and so on. It is an important way for TCM merging with modern clinical treatment. The following example may help to understand the mode of combination of symptoms, syndromes, and diseases, and the details are in Figure 3.

3. Method: Two Important and Innovative Therapeutic Methods

3.1. Differences Exist between Modern Clinical Features of T2DM and the "Three Excess and One Loss" of Traditional Xiaoke Disease. In traditional Chinese medicine (TCM), DM may fall under the categories of "Xiaoke disease" and others. It is characterized by excessive drinking, excessive food consumption, excessive urination, and weight loss. All of these symptoms are commonly referred to as "three excess and one loss." The main pathogenesis lies in yin deficiency leading to endogenous dryness-heat in the body, and blood stasis and phlegm retention are often present. If prolonged yin deficiency impairs yang, dual deficiency of qi and yin as well as dual deficiency of yin and yang will occur. Therefore, the main TCM therapeutic methods for Xiaoke disease are invigorating qi, nourishing yin, clearing away the heat, and promoting fluid production [3, 15, 16]. Famous formulas including Yuye Tang, Bai Hu Jia Renshen Tang, and Jin Gui Shen Qi Wan are widely used [3, 4, 15, 16]. In recent years, several studies have also demonstrated that the distinctive symptoms of T2DM are the "three excess and one loss" [17]. However, modern clinics have found several new features of patients with T2DM, which are as follows. First, 50% of patients with T2DM are without any symptoms, while the diabetic symptoms are not typical in 80% of patients [18]. Clark et al. [19] showed that patients who controlled the blood glucose poorly presented with diabetic symptoms that are defined by the American Diabetes Association (ADA), while patients who controlled their blood glucose well during the early stage of T2DM had no symptoms. Su and Yang [20] proposed that the symptoms of "three excess and one loss" were only manifested in patients with moderate to severe degrees of T2DM. In ancient times, the diagnosis of Xiaoke disease was primarily based on the symptoms of patients, Xiaoke disease could only be diagnosed when these distinctive symptoms appeared, and there was no intervention with Western hypoglycemic drugs. Nowadays, it is often physical examination that leads to the diagnosis of T2DM before the appearance of the "three excess and one loss," even in prediabetes. The examination of blood glucose is convenient and easy. The early interventions by Western hypoglycemic drugs are common, and the therapeutic methods achieve continuous optimization. Secondly, overweight or obese patients are the main population that suffers from T2DM. In European and American countries, approximately

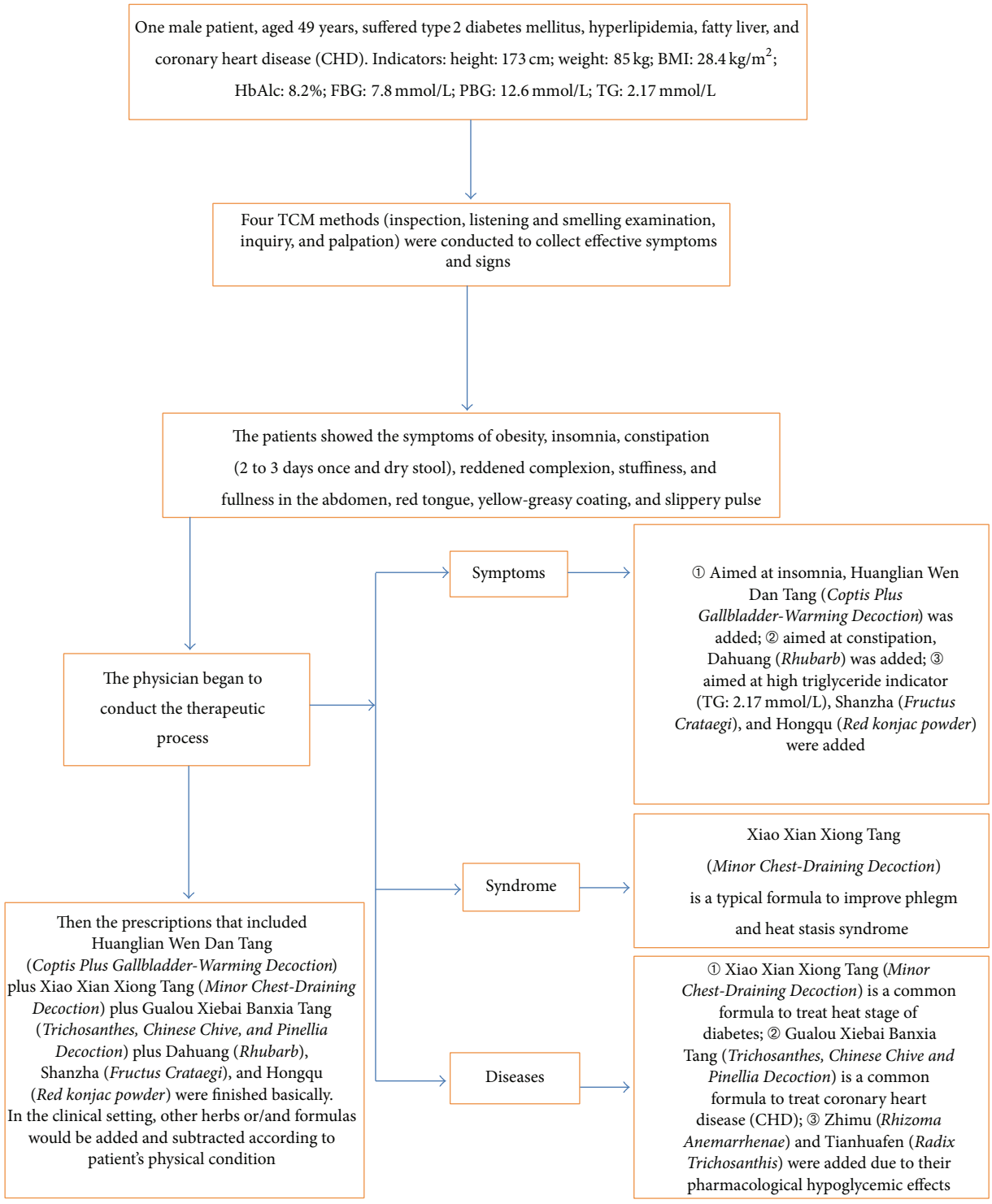

Figure 3: The clinical application of the new mode.

85% of T2DM patients are overweight or obese, and only 15% of them are normal or thin [21], which is similar to the situation in China. Sixty percent of patients with T2DM also have dyslipidemia. Hyperglycemia, hyperlipidemia, obesity, and fatty liver always occur in combination and cause diseases. Thirdly, patients with the sthenia syndrome outnumber the cases with asthenia syndromes, and internal heat is the core pathogenesis of obese T2DM in the early and middle stages of T2DM [8, 22, 23]. Due to the changes in the etiology, pathogenesis, diagnosis, and interventions between traditional Xiaoke disease and T2DM, new therapeutic methods have been proposed to adapt to the clinical need.

3.2. Emphasizing Heat-Clearing in the Early and Middle Stages of T2DM. According to the above, the basic pathogenesis in the early and middle stages of T2DM is associated with "heat"; some scholars have also proposed a concept of "toxin," such as "glucose toxin" (too much sugar), "lipid toxin" (too much fat), or too many "inflammatory actors," which refers to the excessive harmful substances in the body of type 2 diabetic patients due to overintake of sweet and greasy food [24, 25]; therefore, "heat" and "toxin" are considered important factors leading to DM. Huang Lian (*Rhizoma Coptidis*) is the classical heat-clearing and detoxifying herb for DM, and berberine (BBR) is an important active component of Huang Lian [26]. Yin et al. [27] investigated the clinical efficacy and safety of BBR in a pilot study. Thirty-six adults with newly diagnosed T2DM were randomly assigned to BBR or metformin treatment (500 mg three times a day) in a 3-month study. Results showed that BBR significantly lowered hemoglobin A1c (HbA1C), fasting blood glucose (FBG), postload plasma glucose (PBG), and TG in patients with T2DM ($P < 0.05$ or $P < 0.01$). Forty-eight adults with poorly controlled T2DM were treated with supplemental BBR for 3 months in a second study. There was a significant decrease in the level of blood glucose and lipids, indicating that the hypoglycemic effect of BBR was similar to that of metformin. Green tea has the property of cold and lowers the fire [28], and some studies have provided evidence that drinking tea could improve insulin resistance and ameliorate the potential risk for T2DM [29, 30]. Current medicine has generally accepted that DM is usually associated with chronic subclinical inflammation [31]. The role of inflammation in the pathogenesis of T2DM and its vascular complications has been confirmed by several studies [32]. Traditional Chinese herbs and formulas usually exert the hypoglycemic effects by controlling inflammation; some heat-clearing and detoxifying herbs and formulas especially possess anti-inflammatory effects. Many studies have shown that heat-clearing herbs could control the blood glucose by inhibiting inflammation, such as Huang Lian (*Rhizoma Coptidis*), GeGen (*Radix Puerariae*), ZhiMu (*Rhizoma Anemarrhenae*), and Tian Hua Fen (*Radix Trichosanthis*) [4, 32]. Huang-Lian-Jie-Du-Tang (HLJDT) is the classical heat-clearing and detoxifying formula used for diabetes [33]. Current medicine has shown that it exhibits anti-inflammatory effects in BALB/c mice and carrageenan-induced mice by inhibiting the production or expression of malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), prostaglandin E (2) (PGE2), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) to lower the blood glucose [34–37]. The method of "Kaiyu Qingre (dissipate stagnation of qi and clear away the heat)" has been proposed according to evidence-based medicine; in one research on observing the Chinese herbal medicine on obese type 2 diabetic patients, Kaiyu Qingre Jiangzhuo formula (KQJF) was given to the treatment group, while metformin was given to the control group. The results showed that there was no significant difference statistically between two groups on lowering the blood glucose ($P > 0.05$) [38]. It is the first evidence of Chinese herbal medicine on lowering the blood glucose in the clinic [8].

3.3. Invigorating Blood Circulation throughout the Entire T2DM Process. There are different degrees of vascular lesions in 50% of patients with newly diagnosed T2DM, although some patients' symptoms are atypical [39]. T2DM subclinical vascular lesions are caused by abnormal glycolipid metabolism, oxidative stress, inflammatory factors, insulin resistance, and so on, which exist throughout the entire T2DM process and gradually lead to diabetic complications. Complicated lesions may involve many organs, such as heart, brain, kidneys, retina, nervous system, and skin [40]. Vascular lesions may be considered collateral damage in TCM; the pathogenesis of collateral damage changes from collateral qi stagnation to collateral blood stasis, then to collateral blockage, and finally to collateral damage [41–43]. Sublingual collateral vessels are generally observed to determine the degree of collateral damage, and the observation includes the following two aspects: the body and the color of collateral vessels [42]. Treatment should be aimed at improving the blood circulation and removing obstruction in vessels [41, 42]. Luotong (modified Di Dang Tang, mainly composed of Dahuang (*Rhubarb*), Shuizhi (*Hirudo*), and Taoren (*Semen Persicae*)) is widely applied to activate blood and unblock the collaterals. In the clinical setting, combined therapies of hypoglycemia and Luotong could slow down the progression from impaired glucose tolerance (IGT) to diabetes [42]. Tang-Luo-Ning (TLN, mainly composed of Huangqi (*Radix Astragali*), Danshen (*Radix et Rhizoma Salviae Miltiorrhizae*), and Chishao (*Radix Paeoniae Rubra*)) is used to activate blood and unblock the collaterals. Animal experiments have shown that treatment with TLN may be helpful in delaying the progression of diabetic peripheral neuropathy (DPN) by exerting a neural protection effect [44]. Tong Xin Luo (TXL) has been used in patients with diabetic nephropathy (DN) and has been registered in the State Food and Drug Administration of China. TXL showed positive effects on decreasing the 24-hour urine albumin excretion ratio (24 h UAER) and blood urea nitrogen (BUN). In the treatment of early DN, TXL could improve renal microcirculation, reduce Cys-C and UAER, and delay the progression of renal damage. The mechanism may be related to inhibition of TGF-β1-induced epithelial-to-mesenchymal transition in DN [45]. A type of aqueous extract of Huangqi (*Radix Astragali*), Danggui (*Angelica sinensis*), and Sanqi (*Panax notoginseng*) with the therapeutic efficacy of nourishing the blood and invigorating the blood is effective in preventing diabetic retinopathy (DR) by inhibiting leukocyte adherent to the vascular wall, attenuated vascular leakage, and formation of acellular capillaries [46].

4. Formula and Herbs: The Combination of TCM Theory and Current Medicine

4.1. Treatment Based on Syndrome Differentiation. The first guideline for DM was published in 2007, named *Guideline of Prevention and Treatment of Diabetes by TCM*. With the unification of TCM terminology, diabetes-related terminology became gradually normalized and standardized [8]. Generally speaking, the stagnation stage that represents

the body is in a state of congestion and stagnation during the early period of DM, which could be differentiated into syndromes of qi stagnation due to liver depression and spleen and stomach congestion. Xiaoyao Powder or Houpo Sanwu Tang is recommended to dissipate the stagnation and remove the congestion. The heat stage represents the development of diseases and could be seen more as the sthenia syndrome during the early or middle periods of T2DM. Based on the comprehensive and systemic review, peer review, validation sessions, and analysis of literature, the TCM clinical guidelines were finally formulated, and the heat stage was differentiated into six common clinical syndromes. Heat-clearing and fire-draining are important therapeutic methods. Da Chaihu Tang is commonly recommended for liver and stomach stagnated heat syndromes, Bai Hu Tang for lung and stomach exuberant heat syndromes, Dahuang Huanglian Xie Xin Tang for stomach and intestine excessive heat syndromes, Gegen Qin Lian Tang for intestinal damp and heat syndromes, Xiao Xian Xiong Tang for phlegm and heat stasis syndromes, and San Huang Tang plus Wu Wei Xiao Du Yin for intense heat toxin syndrome. The deficiency stage represents the further development of the disease and could be seen in more syndromes of asthenia and sthenia in complex middle or late periods of diabetes. The deficiency stage can be differentiated into five syndromes, including deficiency of body liquid due to excessive heat, effulgent fire due to yin deficiency, dual deficiency of qi and yin, spleen deficiency and stomach congestion, and cold and heat in complexity. The key points of treatment in this stage are supplementing the deficiency and eliminating the excess. Bai Hu Jia Renshen Tang, Zhi Bai Dihuang Wan, Sheng Mai Yin plus Zeng Ye Tang, Banxia Xie Xin Tang, or Wumei Wan is recommended. The damage stage represents the end of the disease. At this stage, the functions of the *zang-fu* organs become gradually weaker, and some pathological factors accumulate, such as phlegm, turbid, stasis, or toxin. The treatment should be based on regulating *yin* and *yang*. The damage stage could be differentiated into liver-kidney yin deficiency, dual deficiency of yin and yang, and spleen-kidney yang deficiency syndromes. Qi Ju Dihuang Wan, Jin Gui Shen Qi Wan, and Fuzi Li Zhong Wan are commonly used [22]. The details are shown in Table 1.

4.2. Choosing Herbs of Bitter and Sour Flavors to Counteract Sweet Flavor. According to TCM theory, four-qi and five-flavor theory are one of the basic concepts, and the method of "bitter and sour flavors to counteract sweet flavor" is a great approach to lower blood glucose levels. Bitter flavor is in direct opposition to sweet flavor, and sour flavor can neutralize sweet flavor [47]. Herbs with bitter and sour flavors are excellent when used to treat hyperglycemia. Herbs with a bitter flavor are based on San Huang Tang, Longdancao (*Radix et Rhizoma Gentianae*), Kushen (*Radix Sophorae Flavescentis*), Kuding (*Herba Corydalis Bungeanae*), and Shanzhizi (*Fructus Gardeniae*) and also could be considered, which generally include bitter flavor and cold property. Herbs of sour flavor are represented by Fructus Mume formula, Shanzhuyu (*Fructus Corni*), Suanzaoren (*Semen Ziziphi Spinosae*), and Shiliupi (*Pericarpium Granati*) and should also be considered [48].

4.3. Choosing Formulas and Herbs Aimed at Main Symptoms. Alleviating the main symptoms is important in treating T2DM and its complications; thus, the selection of formulas and herbs should be based on the main symptoms. For example, vomiting is the most troublesome problem for diabetics with severe gastroparesis (DGP), Xiao-Banxia-Tang combined with Suye Huanglian Yin is commonly used to relieve nausea and vomiting [49–51]. Proteinuria and edema are obvious symptoms for DN, which could be improved by Liuwei Dihuang Decoction [52, 53] and large amounts of Huangqi (*Radix Astragali*), Danshen (*Salvia miltiorrhiza*), and Fuling (*Poria*) [54, 55]. DPN patients with acral numbness and pain could be treated with Huangqi Guizhi Wu Wu Decoction [56] and large amounts of Chuanwu (*Radix Aconiti Praeparata*) [57] to improve symptoms and increase nerve conduction velocities (NCVs). The details showed in Table 3.

4.4. Applying Modern Pharmacological Achievements. With the development of modern pharmacological products, the effective components provide evidence for herbs or formulas to treat diseases [58]. There are several herbs that possess definite hypoglycemic effects and are often used in the traditional Chinese formulas for T2DM and its complications, including Huanglian (*Rhizoma Coptidis*), Huangqin (*Radix Scutellariae*), Renshen (*Radix et Rhizoma Ginseng*), Zhimu (*Rhizoma Anemarrhenae*), and Tianhuafen (*Radix Trichosanthis*) [4, 16]. Details are shown in Table 2. Some herbs have great effects on improving other indicators, Weilingxian (*Radix et Rhizoma Clematidis*) may lower the blood uric acid [59], Wuweizi (*Fructus Schisandrae Chinensis*) may lower the aminotransferase [60], and Yinchen (*Herba Artemisiae Scopariae*) and Huzhang (*Rhizoma Polygoni Cuspidati*) may improve fatty liver [61, 62]. In the clinical setting, the application of pharmacological products plays an important role in the treatment of DM.

5. Importance of Drug Dose in the Treatment of Diabetes

The therapeutic efficacy of TCM may be not only determined by syndrome differentiation, formula compatibility, medicinal properties and quality, water decoction, and administration method but also closely related to the applicable drug dose. As the saying goes, "the secret of traditional Chinese medicine is in the dose," the dose of herbs has always been difficult to study [63].

According to traditional concept, Chinese herbal medicines are only considered supplementary treatment for lowering the blood glucose. However, we have confirmed that Chinese herbal medicine possesses independent antihyperglycemic effects based on large scales of randomized controlled trials (RCTs), and adverse events were less common than with metformin [38]. The key point to lowering the blood glucose independently is dose. In our previous study, we demonstrated the relationship

Table 1: Classical formulas and Chinese herbs recommended for T2DM treatment.

Stage	Syndrome	Formula	Efficacy	Components
Stagnation	Qi stagnation due to liver depression	Xiaoyao Powder	Soothing the liver, dissipating stagnation of qi	*Radix Bupleuri*, *Radix Angelicae Sinensis*, *Yam*, *Atractylodes*, *Poria cocos*, *Glycyrrhiza*, *Herba Menthae*, *Rhizoma Zingiberis Recens.*
	Spleen and stomach congestion	Houpo Sanwu Tang	Moving qi, removing food stagnation	*Officinal Magnolia Bark*, *Rhubarb*, *Gardenia.*
Heat	Liver and stomach stagnated heat	Da Chaihu Tang	Clearing liver heat, draining stomach fire	*Radix Bupleuri*, *Radix Scutellariae*, *Rhubarb*, *Gardenia, Yam*, *Rhizoma Pinelliae*, *Rhizoma Zingiberis Recens*, *Fructus Jujubae.*
	Lung and stomach exuberant heat	Bai Hu Tang	Clearing lung heat, engendering fluids to quench thirst	*Gypsum Fibrosum*, *Rhizoma Anemarrhenae*, *Oryza sativa* L., *Glycyrrhiza.*
	Stomach and intestine excessive heat	Dahuang Huanglian Xie Xin Tang	Draining stomach and intestine fire	*Rhubarb*, *Rhizoma Coptidis*, *Radix Scutellariae.*
	Intestinal damp and heat	Gegen Qin Lian Tang	Clearing heat and draining dampness	*Pueraria*, *Rhizoma Coptidis*, *Radix Scutellariae*, *Glycyrrhiza.*
	Phlegm and heat stasis	Xiao Xian Xiong Tang	Clearing heat and dissolving phlegm	*Rhizoma Coptidis*, *Rhizoma Pinelliae*, *Semen Trichosanthis.*
	Intense heat toxin	San Huang Tang plus Wu Wei Xiao Du Yin	Draining fire and resolving toxins	*Rhizoma Coptidis*, *Radix Scutellariae*, *Rhubarb*, *Flos Lonicerae Japonicae*, *Flos Chrysanthemi Indici*, *Herba Taraxaci*, *Herba Violae*, *Herba Begoniae Fimbristipulatae.*
Deficiency	Deficiency of body liquid due to excessive heat	Bai Hu plus Renshen Tang	Clearing lung heat, promoting fluid production	*Gypsum Fibrosum*, *Rhizoma Anemarrhenae*, *Oryza Sativa L.*, *Glycyrrhiza*, *Ginseng.*
	Effulgent fire due to yin deficiency	Zhi Bai Dihuang Wan	Enriching yin, clearing the fire	*Rhizoma Anemarrhenae*, *Cortex Phellodendri*, *Radix Rehmanniae*, *Radix Asparragi Officinalis*, *Rhizoma Dioscoreae*, *Poria cocos*, *Rhizoma Alismatis*, *Cortex moutan*.
	Dual deficiency of qi and yin	Sheng Mai Yin plus Zeng Ye Tang	Boosting qi and nourishing yin	*Ginseng*, *Radix Ophiopogonis*, *Fructus Schisandrae Chinensis*, *Radix scrophulariae*, *Radix Rehmanniae*.
	Spleen deficiency and stomach congestion	Banxia Xie Xin Tang	Dispersing stagnation with bitter-acrid medicinals	*Rhizoma Pinelliae*, *Zingiberis*, *Ginseng*, *Rhizoma Coptidis*, *Radix Scutellariae*, *Fructus Jujubae*, *Glycyrrhiza*.
	Cold and heat in complexity	Wumei Wan	Clearing the upper and warming the lower	*Fructus Mume*, *Herba Asari*, *Zingiberis*, *Rhizoma Coptidis*, *Radix Angelicae Sinensis*, *Typhonii Gigantei*, *Fructus Zanthoxyli*, *Ramulus Cinnamomi*, *Ginseng*, *Cortex Phellodendri*.
Damage	Liver-kidney yin deficiency	Qi Ju Dihuang Wan	Enriching and nourishing the liver and kidney	*Fructus Lycii*, *Flos Chrysanthemi*, *Radix Rehmanniae*, *Radix Asparragi Officinalis*, *Rhizoma Dioscoreae*, *Poria cocos*, *Rhizoma Alismatis*, *Cortex moutan*.
	Dual deficiency of yin and yang	Jin Gui Shen Qi Wan	Enriching yin and supplementing yang	*Typhonii Gigantei*, *Cortex Cinnamomi*, *Radix Rehmanniae*, *Radix Asparragi Officinalis*, *Rhizoma Dioscoreae*, *Poria cocos*, *Alisma*, *Cortex moutan*.
	Spleen-kidney yang deficiency	Fuzi Li Zhong Wan	Warming and supplementing the spleen and kidney	*Typhonii Gigantei*, *Zingiberis*, *Ginseng*, *Atractylodes*, *Glycyrrhiza.*

Table 2: Classifications of function of herbal medicines possessing hypoglycemic efficacy.

TCM efficacies	Herbal medicines
Clearing heat	Huanglian (*Rhizoma Coptidis*), Tianhuafen (*Radix trichosanthis*), Zhimu (*Rhizoma Anemarrhenae*), Huangbai (*Cortex Phellodendri*), Gegen (*Radix Puerariae*), Kugua (*Fructus Balsampear*), Shigao (*Gypsum Fibrosum*), Huangqin (*Radix Scutellariae*), Zhizi (*Fructus Coini*), Digupi (*Cortex Lycii Radicis*), Lugen (*Rhizoma Phragmitis*)
Nourishing yin (promoting body fluids production)	Dihuang (*Radix Rehmanniae*), Shanzhuyu (*Radix Asparragi Officinalis*), Wumei (*Fructus Mume*), Yuzhu (*Rhizoma Polygonati Odorati*), Maidong (*Radix Ophiopogonis*), Gouqizi (*Fructus Lycii*), Nvzhenzi (*Fructus Ligustri Lucidi*), Wuweizi (*Fructus Schisandrae*), Shihu (*Herba Dendrobii*), Shengmuli (*Concha Ostreae*), Xuanshen (*Radix Scrophulariae*)
Invigorating qi (fortifying the spleen)	Huangqi (*Radix Astragali seu Hedysari*), Renshen (*Radix Ginseng*), Huangjing (*Rhizoma polygonati*), Cangzhu (*Rhizoma Atractylodis*), Shanyao (*Rhizoma Dioscoreae*), Yiyiren (*Semen Coicis*)
Activating stasis	Danshen (*Radix Salviae Miltiorrhizae*), Sanqi (*Radix Notoginseng*), Guijianyu (*Ramulus Euonymi*), Chishao (*Radix Paeoniae Rubra*), Shuizhi (*Hirudo*), Chuanxiong (*Rhizoma Ligustici Chuanxiong*), Danggui (*Radix Angelicae Sinensis*), Taoren (*Semen Persicae*)
Warming yang	Tusizi (*Semen Cuscutae*), Yinyanghuo (*Herba Epimedii*), Dongchongxiacao (*Cordyceps*), Bajitian (*Radix Morindae oficinalis*), Roucongrong (*Herba Cistanches*), Dasuan (*Allii Sativi Bulbus*), Buguzhi (*Fructus Psoraleae*), Fuzi (*Radix Aconiti Lateralis Praeparata*)
Draining water	Zexie (*Rhizoma Alismatis*), Fuling (*Poria cocos*), Yumixu (*Stigma Maydis*), Dongguapi (*Exocarpium Benincasae*)

Table 3: Chinese herbal formulas mentioned in the review.

Formulas	Components
Yuye Tang	Shanyao (*Rhizoma Dioscoreae*), Huangqi (*Radix Astragali seu Hedysari*), Zhimu (*Rhizoma Anemarrhenae*), Jineijin (*Endothelium Corneum Gigeriae Galli*), Gegen (*Radix Puerariae*), Wumei (*Fructus Mume*), Tianhuafen (*Radix Trichosanthis*)
Xiao Banxia Tang	Banxia (*Rhizoma Pinelliae*), Shengjiang (*Rhizoma Zingiberis Recens*)
Suye Huanglian Yin	Huanglian (*Rhizoma Coptidis*), Zisuye (*Folium Perillae*)
Huangqi Guizhi Wu Wu Tang	Huang Qi (*Radix Astragali seu Hedysari*), Gui Zhi (*Ramulus Cinnamomi*), Shanyao (*Rhizoma Dioscoreae*), Sheng Jiang (*Rhizoma Zingiberis Recens*), Da Zao (*Fructus Jujubae*)
Di Dang Tang	Dahuang (*Rhubarb*), Shuizhi (*Hirudo*), Taoren (*Semen Persicae*), Mangchong (*Tabanus*)

between dose and effect through RCTs. One hundred and eighty-seven T2DM patients were randomly allocated to receive high (HD, $n = 44$), moderate (MD, $n = 52$), and low doses (LD, $n = 50$) of Gegen Qin Lian Decoction or the placebo ($n = 41$) for 12 weeks. Patients that received the HD or MD showed significant difference in adjusted mean changes from baseline of HbA1c and FBG compared with the LD and placebo groups. The dose-effect relationship is obvious [64]. Huanglian is commonly used in the heat and deficiency stages of T2DM [22]. Liu made a survey of the dose of Huanglian in 1,321 effective formulas (when the decreased percentage of FBG and PBG was >20% of those before treatment or the decreased percentage of HbAlc was >10% of that before treatment within 12 weeks, the formula was thought of as an effective formula, and other else was thought of as an ineffective formula) to treat T2DM, and the result showed that commonly recommended dose of Huanglianwas 15 g when FBG < 7 mmol/L, 30 g when FBG < 10 mmol/L, and 30 g to 45 g when FBG was ≥10 mmol/L [48]. There is a positive correlation between the dose of Huanglian and the decrease of blood glucose.

Chuanwu (*Radix Aconiti Praeparata*) is commonly used in the treatment of DPN with severe acral pain, tingling, and cold. The recommended dose of Chuanwu (*Radix Aconiti Praeparata*) should be "15–60 g," even to a maximum dose of 120 g for alleviating the pain, whereas the routine dose of "1.5–3 g" in Chinese Pharmacopoeia (2010 edition) is usually ineffective. The decocted time of Chuanwu (*Radix Aconiti Praeparata*) should be more than 60 mins, and medicinal compatibility with Gancao (*Radix et Rhizoma Glycyrrhizae*) or Baimi (*Mel*) is also necessary to resolve toxins [57]. Banxia (*Rhizoma Pinelliae*) and Shengjiang (*Rhizoma Zingiberis Recens*) are often used for treating DGP nausea and vomiting. The routine doses of Banxia (*Rhizoma Pinelliae*) and Shengjiang (*Rhizoma Zingiberis Recens*) are "3–9 g" and "3–9 g," respectively, whereas the recommended dose of Banxia (*Rhizoma Pinelliae*) should be "15–60 g," and the dose of Shengjiang (*Rhizoma Zingiberis Recens*) should be "15–30 g" [50, 65, 66].

6. Discussion

With the increasing incidence of obesity, T2DM is likely to become even more prevalent in the future. It has a significant impact on the quality of life and the number

of deaths as well as on the financial resources of the public health care system. Currently, CAM therapies are widespread in both developing and developed countries. Due to positive views of patients regarding CAM therapies and the increased availability of them, they are frequently used for T2DM globally [67]. The commonly used CAM therapies include Chinese herbal medicines, acupuncture, nutritional supplements and advice, spiritual healing, and relaxation techniques [7]. Recently, treating obese T2DM with acupuncture has become popular, and a lot of progress has been made to indicate that acupuncture is safe and effective [68]. Chinese herbal medicine contains various active ingredients, which could provide multiple therapeutic effects on multiple targets, such as enhancement of insulin sensitivity, stimulation of insulin secretion, or reduction of carbohydrate absorption [16]. Chinese herbal medicines could also help treat the diabetic complications by ameliorating abnormalities related to blood viscosity, microcirculation, and oxidative stress [69]. In the light of recent studies, it is not difficult to find that the etiology, pathogenesis, and therapeutic strategies of diabetes have been changed recently. With the development of modern diagnosis and treatment on DM, the thoughts of highlighting the combination of symptoms, syndromes, and diseases, reunderstanding the etiology and pathogenesis of diabetes, emphasizing heat-clearing and invigorating blood circulation, and choosing formula and herbs are based on the combination of TCM theory and current medicine, and paying attention to dosage has been gradually and widely accepted; only by adopting these thoughts, the clinical efficacy of Chinese formulas and herbs on DM may be improved. We have confirmed the effects of formulas and herbs on regulating metabolic problems from integrated perspectives. For example, obese diabetes patients have hyperglycemia along with fatty liver, hyperlipidemia, hypertension, hyperuricemia, and other metabolic disorders. Western medicine has not found an effective way to treat the metabolic syndrome; each abnormality has been treated separately. Here, we take advantage of TCM with a holistic approach. Furthermore, formulas and herbs may also reverse risk factors leading to diabetes. In one study, we observed that the Chinese herbal formula Tianqi Jiang Tang Capsule reduced progression from impaired glucose tolerance (IGT) to diabetes. After a 12-month treatment, results demonstrated that Tianqi significantly decreased the incidence of T2DM in subjects with IGT by 32.1% compared with placebo [70]. There are also diverse Chinese patent drugs commonly used for treating DM clinically, including Xiaoke Wan, Jiangtangjia Pian, YuquanWan, and Tangmaikang Keli, which also play an important role [4]. In the clinical setting, a large amount of clinical experience has been accumulated, and these innovative thoughts have been gradually accepted and promoted the development of TCM. The emergence of evidence-based medicine (EBM) has provided objective efficacy assessment of TCM with new thoughts and methods [71]; well-designed, large-scale, high-quality multicenter RCTs are still required to provide stronger evidence in the future. With continuous efforts, TCM will undoubtedly play a more important role in fighting T2DM.

Abbreviations

DM: Diabetes mellitus
T2DM: Type 2 diabetes mellitus
TCM: Traditional Chinese medicine
BBR: Berberine
HbAlC: Hemoglobin A1c
FBG: Fasting blood glucose
PBG: Postprandial blood glucose
CHO: Cholesterol
TG: Triglyceride
HDL-C: High-density lipoprotein
LDL-C: Low-density lipoprotein
BMI: Body mass index
Cr: Creatinine
BUN: Blood urea nitrogen
RCTs: Randomized controlled trials.

Conflict of Interests

No competing financial interests existed.

Authors' Contribution

Xiao-Lin Tong and Lin-Hua Zhao proposed the paper topic; the two of them contributed equally to this work and are both co-corresponding authors; Bing Pang and Qiang Zhou wrote the paper; the two of them contributed equally to this work and are both co-first authors; Tian-Yu Zhao consulted the references; Li-Sha He and Hong-Dong Chen drew the figures; Jing Guo and Lin-Hua Zhao revised the paper.

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Review Article

The Effects of Chinese Medicine on Activation of Wnt/β-Catenin Signal Pathway under High Glucose Condition

Wei Liu, Xiaochun Liang, and Dan Yang

Department of Traditional Chinese Medicine, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, No. 1 Shuaifuyuan, Dongcheng District, Beijing 100730, China

Correspondence should be addressed to Xiaochun Liang; liangxc@vip.sina.com

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Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia and a series of complications. The Wnt/β-catenin signaling pathway is a complex protein interaction network, which is also a key regulator of cell proliferation and differentiation. Many scholars have found that high glucose can activate the Wnt signaling pathway. However, the effects of activation of this pathway in the presence of high glucose levels during the progression of diabetes still remained unclear. Here, we provide a review of the study on the effects of high glucose state on the Wnt/β-catenin signal pathway and the influence of Chinese medicine on it.

1. Introduction

Diabetes mellitus (DM) is a heavy burden for patients worldwide, and the number of affected individuals is growing significantly. By 2030, it is estimated that it will affect almost 552 million [1]. High glucose is the initiating factor of DM and a series of complications, the mechanisms of which are intricate and complex. The Wnt/β-catenin signaling pathway is a complex protein interaction network, which is also a key regulator of cell proliferation and differentiation. Many scholars have focused on its important role during embryonic development [2], the proliferation and differentiation of osteoblasts [3, 4], and tumorigenesis [5, 6]. It has also been reported that Wnt/β-catenin signaling can be promoted by the highly conserved gene *Fezf2* to stimulate neuronal differentiation during forebrain development [7]. Recent investigations have highlighted the role of the Wnt signaling pathway in metabolic homeostasis and its implication in diabetes as well as other metabolic diseases. It has been confirmed in recent years that high glucose can activate this pathway [8]. This paper provides a review of studies on the effects of the Wnt/β-catenin signal pathway in the presence of high glucose levels and the influence of Chinese medicine on it.

2. Wnt/β-Catenin Signaling Pathway

The Wnt signal pathway mainly includes the following: extracellular factor (Wnt), transmembrane receptors (frizzled), cytoplasmic protein (β-catenin), nuclear transcription factor (TCFS/LEF), and a series of proteins [9]. Many recent studies have shown that the Wnt/β-catenin signaling pathway is closely related to diabetic nephropathy [10–12], diabetic myocardium [13], and diabetic retinopathy [14, 15].

Wnts are secreted glycoproteins expressed in the developing somites and surrounding tissues that function as extracellular signals to be part of a signaling cascade in a wide group of organisms. They are a family of factors involved in the embryonic development and regulate many processes including embryonic patterning, fate specification, axon guidance, synaptogenesis, stem cell-like renewal, cell specification, proliferation, migration, adhesion, survival, differentiation, and apoptosis. Signaling regulated by Wnt ligand binding plays an important and often essential role in the processes during growth and development. According to the different modes of Wnt protein mediated signal transduction, the Wnt signal transduction pathway can be divided into the canonical Wnt signaling pathway, the noncanonical Wnt signaling pathway, the planar cell polarity (PCP), and the protein kinase A

pathway [16]. It is known that there are 19 members of the Wnt protein family [11]. Feigenson et al. [17] used Wnt-3a cultured oligodendrocyte precursor cells containing medium and discovered that the myelin basic protein was associated with reduced myelin formation, thus suggesting that Wnt-3a inhibited the differentiation from oligodendrocyte precursor cells into immature oligodendrocytes through the Wnt/β-catenin signaling pathway.

The canonical pathway resulted in β-catenin accumulation through interaction between Wnt protein and cytoplasmic proteins, which interacted with transcription factors and attached to the DNA sequence of -YCTTTGWW to regulate its downstream gene transcription. The noncanonical pathway improves the levels of intracellular calcium by activating different elements of heterotrimeric G proteins to activate the Wnt/Ca2 + channels [18]. Among them, β-catenin is the key factor of the canonical Wnt signaling pathway, which is a cytosolic protein that is degraded by the ubiquitin proteasome system by glycogen synthase kinase 3 beta (Gsk-3β) phosphorylation. It forms a complex after combining with LEF/TCF and Smad4, thereby promoting the latter into the nucleus and regulating the expression of specific target genes. It is the main effector molecule cytoplasm within the classical Wnt approach and is also associated with the intercellular junctions and cadherin [19, 20]. The concentration of β-catenin in the cytoplasm determines the activation and inhibition of the canonical Wnt pathway [21]. Inhibition of Gsk-3β phosphorylation can result in β-catenin accumulation in the nucleus and activation of the Wnt pathway in the cytoplasm. Therefore, increased expression of β-catenin suggests the activation of Wnt/β-catenin signaling pathway, and inhibitors of Gsk-3β are viewed as an activator of Wnt/β-catenin signal pathway [22, 23].

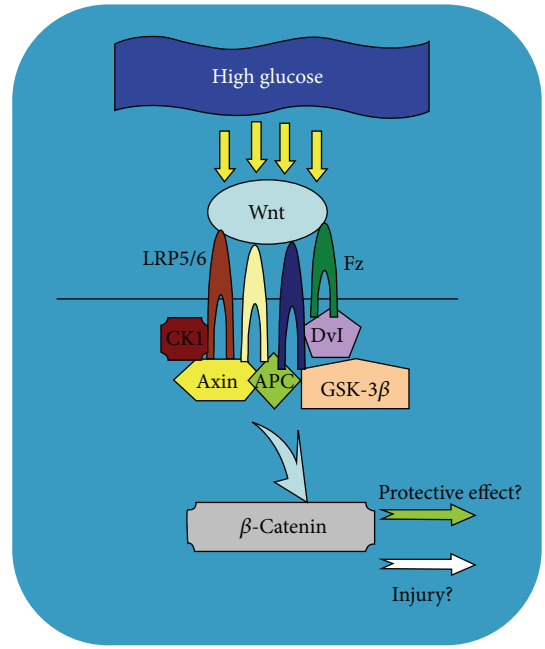

Figure 1: Wnt/β-catenin signaling pathway in high glucose condition.

3. Activation of Wnt/β-Catenin Signal Pathway in the Presence of High Glucose Levels

Many scholars have confirmed that high glucose levels can activate the Wnt/β-catenin signaling pathway, but the activation of this pathway is a protective response of organization or an injury is still controversial. The possible relationship between high glucose and the activation of this pathway is shown in Figure 1. The study by Chong et al. [24] showed that the key enzyme GSK-3β in the Wnt/β-catenin signal pathway was phosphorylation inhibited at high glucose levels, and the activation pathway can inhibit the degradation of apoptosis nuclear DNA, thus playing a protective role in a variety of vascular endothelial cells. Yang et al. [25] reported that, in diabetic rat models induced by STZ, the expression of Wnt/β-catenin signaling pathway is upregulated, and β-catenin as the core of the upstream gene APC and downstream gene c-Myc was expressed and upregulated in the islet regeneration process to promote the regeneration of damaged pancreatic islet cell. Sun et al. [26] used a special puncher to create a round hole on both sides of the middle of the type 1 diabetic rat dorsal spine to create skin defects under aseptic conditions. Then, they divided the rats into diabetes, lithium chloride (pathway activator), and epidermal growth factor groups to observe the wound healing and to detect the expression of β-catenin. The results showed that the activation of Wnt/β-catenin signaling pathway can promote the healing of diabetic wounds. Some scholars showed that in mouse podocytes early diabetic podocyte injury was caused by upregulation of transient receptor potential cation channel 6 (TRPC6), which is regulated by the canonical Wnt signalling pathway. This indicates that the Wnt/β-catenin signalling pathway may potentially be active in pathogenesis of TRPC6-mediated diabetic podocyte injury [27].

On the other hand, Huo et al. [28] used different concentrations of glucose on rat peritoneal mesothelial cells and immunohistochemistry to detect the β-catenin protein expression on cells. They also used RT-PCR assay for the detection of β-catenin mRNA. The results showed that high glucose can induce the increased expression of β-catenin in rat peritoneal mesothelial cells, indicating that high glucose levels can upregulate the expression of β-catenin induced peritoneal injury leading to fibrosis. The research of Hwang et al. [11] and Rooney et al. [29] showed that Wnt/β-catenin signal pathway was closely related to the occurrence of diabetic nephropathy renal interstitial fibrosis and that blocking or inhibiting this pathway might be a new target for treatment of diabetic nephropathy. Yan et al. [30] reported that, in the process of diabetic nephropathy, activation of the canonical Wnt pathway may be involved in the high glucose mediated transdifferentiation process of renal tubular epithelial cells leading to renal interstitial fibrosis. Liu and Lai [31] cultured SD rat glomerular podocytes in different glucose concentrations, detecting nephrin and activation of β-catenin and Wnt-1 expression by indirect immunofluorescence and Western blot analysis, and results showed that, after 12 h of

high glucose exposure, podocyte Wnt-1 and activation of β-catenin expression began to increase and reached its peak at 24 h. The Wnt/β-catenin signaling pathway may be involved in the phenotypic transformation of podocytes induced by high glucose levels. García-Jiménez et al. [32] reported that the enhanced expression of the Wnt/β-catenin signal pathway in tumor cells at high glucose levels may be one reason for certain cancers in DM patients. Li et al. [14] set up the DM rat model induced by STZ, with Evans blue detection of retinal vascular permeability, immunohistochemistry, and Western blot analysis for detection of the rat retina and trypsin digested retinal microvascular β-catenin protein. Results showed that, after 12 W, the expression of retinal and microvascular β-catenin protein increased significantly, and the extent of this increase is associated with the duration of DM, thereby suggesting that the occurrence of β-catenin protein may be involved in early diabetic retinopathy. However, the research was only limited to the detection of β-catenin protein and still could not fully explain the role of Wnt/β-catenin pathway. Portal-Núñez et al. [33] also observed the STZ induced DM rat models with the parathyroid treatment group as the control, using the gene chip technology and rt-PCR analysis of Wnt/β-catenin signal pathway change and analysis of β-catenin protein expression by immunohistochemical method change. The results showed that β-catenin expression in both osteoblasts and bone cells in DM rats decreased significantly, which was not due to a decrease in bone cell activity. In addition, they also found that, in the osteoblast related β-catenin signal transduction process, transfer of β-catenin to the nucleus was significantly reduced, thus leading to failure of intracellular signal transduction. It proved that dysregulation of the Wnt/β-catenin pathway was involved in the occurrence and development of DM osteoporosis. Wang et al. [34] detected the expression of β-catenin and its downstream target gene WISP-1 in Wnt signal pathway in STZ induced DM rats myocardial tissue by immunohistochemical method. The results showed that both increased, thereby suggesting that the activation of the pathway was involved in DM induced myocardial injury. There were also reports that the protein and mRNA levels of Wnt2, β-catenin, and c-Myc were progressively increased 4, 8, and 12 weeks following DM. However, the expression of the endogenous Wnt inhibitor Dickkopf-1 was increased after STZ injection and then decreased as diabetic cardiomyopathy developed. Jia et al. [9] found that the expression of Wnt and cytoplasmic β-catenin was upregulated in proximal tubular epithelial cells under DN conditions both in vitro and in vivo. Injection of LRP5 and LRP6 antibodies suppressed activation of the Wnt pathway and decreased the formation of extracellular matrix in DN animal models, suggesting that Wnt/β-catenin signaling might be involved in tubular-interstitial fibrosis in DN. It was also reported that Wnt/β-catenin/GSK3β signaling pathway is activated in the development of diabetic cardiomyopathy [35]. Qi et al. [36] found that lithium chloride-induced Wnt signaling activation downstream of the pigment epithelium-derived factor (PEDF) interaction site attenuated the inhibitory effect of PEDF and rescued the wound-healing deficiency in diabetic mice. These results suggest that elevated circulating PEDF levels contribute to impaired wound healing in the process of angiogenesis and vasculogenesis through the inhibition of Wnt/β-catenin signaling. In addition, some researchers have found that enhanced proliferation, accompanied by increased aerobic glycolysis, was detected in colorectal epithelium of patients with diabetes. β-Catenin accumulation with altered phosphorylation correlated with the proliferative changes [37].

Besides, as is known to all, T2DM is often associated with atherosclerosis. Nowadays, a lot of data have demonstrated that β-catenin activation is a key component of arteriosclerotic physiology, particularly in diabetic arteriosclerosis [38]. Gaudio et al. [39] found that an established modulator of the canonical Wnt signalling named sclerostin may protect against progression of vascular complications in diabetic patients, possibly by attenuating upregulation of β-catenin activity in the vascular cells.

Mechanisms of activated Wnt/β-catenin signaling in DM are very complex and still unclear now. At present, researches about the effects of this signaling pathway on DM and a series of complications were focused on the activity of the Wnt/β-catenin signaling pathway involved in the regulation of morphological changes and pathogenesis in cells, its effect on high glucose induced cells apoptosis via the promotion of caspase-3 and poly (ADP-ribose) polymerase cleavage, its influence on diabetic wound healing, and its effect on the regulation of bone and vascular and other metabolic processes. It has been suggested that sustained Wnt/β-catenin expression is essential for its protective role against cellular damage, while abnormal activation of Wnt/β-catenin results in adverse effects and promotes the progression of DM.

4. Effects of Chinese Medicine on the Pathway

Chinese medicine has multitarget and multiangle effects, which has caused wide attention. Lv et al. [40] used the high-sugar levels combined with different concentrations of Rhein in serum-free medium cultured human mesangial cells in vitro and analyzed cell proliferation by MTT method and the expression of Wnt/β-catenin gene in glomerular mesangial cells and the effects of Rhein on it by RT-PCR method. The results showed that, in the basic state, mesangial cells express a certain amount of Wnt/β-catenin. After glucose stimulation, the expression of the Wnt/β-catenin gene was increased, thereby suggesting that Rhein may inhibit the high glucose induced proliferation of mesangial cells by down-regulation of Wnt/β-catenin gene expression. Huang et al. [41] divided the human proximal tubular epithelial cells into normal glucose group, high glucose group, and high glucose + tanshinone IIA intervention group. Immunohistochemistry and Western blot analysis were used to observe the protein expression of β-catenin, epithelial cell marker protein of E-cadherin, and mesenchymal cell marker protein α-SMA, and RT-PCR was used to detect the mRNA expression of β-catenin, epithelial cell marker protein of E-cadherin. The results showed that the final concentration of 100 μmol/L tanshinone IIA can be significantly reduced by the ectopic expression of β-catenin, and the expression of β-catenin in

nuclear protein and mRNA decreased significantly at this concentration, thereby indicating that the Wnt/β-catenin signaling pathway is involved in the high glucose induced transdifferentiation of renal tubular epithelial cells. Tanshinone IIA could inhibit this process by downregulating the expression of the Wnt/β-catenin signaling pathway activity and protecting the kidneys. Deng and Fang [42] randomly divided STZ rats into the diabetic nephropathy model group, the *Astragalus* group, the diabetic nephropathy model group + losartan treatment group, and the diabetic nephropathy model group + *Astragalus* combined with losartan treatment group. Immunohistochemistry and FQ-PCR methods were used to detect protein expression as well as expression of Wnt4, β-catenin, and TGF-β1 mRNA in renal interstitium. The results showed that *Astragalus* can protect the kidney by downregulating the expression of Wnt4, β-catenin, and TGF-β1 in renal interstitium. Duan et al. [43] observed the effects of the Yishen Capsule (mainly composed of *Astragalus*, *Angelica*, Gorgon fruit, oriental water plantain rhizome, *Rhodiola*, etc.) on diabetic nephropathy rats induced by STZ. They found that, at the end of 12 W, the expression of Wnt pathway inhibition factor-secreted frizzled related protein-1 in renal tubular-interstital cells increased compared with the control group, and β-catenin expression appeared in the cytoplasm or (and) nucleus. PCR results showed increases in the two mRNA expression levels. After Yishen Capsule treatment, the secreted frizzled related protein-1 in tubulointerstital cells further increased compared to the control, while β-catenin mRNA expression was reduced, which indicated that the Yishen Capsule could upregulate the Wnt pathway inhibiting factor, which played a role in renal protection in diabetic nephropathy rats.

In addition, the study by Lange et al. [44] found that the Wnt/β-catenin signaling pathway was closely related to the neural stem cell proliferation and differentiation. There were also reports showing that dysregulation of the Wnt/β-catenin signaling pathway may be responsible for inefficient myelin repair after human nervous system lesions [45]. Osakada et al. [46] found that the Wnt signaling pathway could promote the regeneration in the retina of adult mammals through animal experiments. Meanwhile, scholars observing the differentiation of nerve stem cells with β-catenin siRNA found that β-catenin played a key role in promoting the differentiation from neural stem cells into neurons in high-pressure oxygen in vitro [47]. Scholars explored the effect of total glucosides of peony (TGP) on Wnt/β-catenin signal transduction pathway expression in kidney of diabetic rats. They found that Wnt-1 and β-catenin expression increased in kidney of high-fat high-sugar induced type 2 diabetic rats. Compared with diabetic group, the level of serum creatinine, blood urea nitrogen, 24 h urine protein, mean glomerular area, and mean glomerular volume were decreased, renal histopathology was improved, and expression of Wnt-1 and β-catenin mRNA and protein was reduced in TGP group. These results showed Wnt/β-catenin abnormal activation in kidney of type 2 diabetic rats; TGP can improve kidney damage in diabetic rats and delay the development of diabetic nephropathy by inhibiting the Wnt/β-catenin signaling pathway [48].

5. Summary and Prospect

With the improvement of living standards, DM as a metabolic syndrome has a significant influence in the global scope, not to mention its peculiar set of complications. Many scholars have been exploring its pathogenesis, but that does not encompass the whole picture. The Wnt/β-catenin signaling pathway as a protein interaction network can be activated by high glucose levels. However, the relationship between the pathway and DM as well as the appearance of its complications is still unclear and needs future investigation to better clarify the accurate role. Currently, the study of the activated Wnt/β-catenin signaling pathway at high glucose levels is relatively more concentrated in animal experiments in vitro, and most of these experimental methods are used to detect the expression of the pathway's upstream or downstream protein and mRNA. Some parts of the comprehensive and systemic study of the pathway are still poorly understood. Regulation of specific genes on this pathway and the exact mechanism are also unclear. In addition, studies of the activation of this pathway under high glucose conditions are mainly focused on diabetic nephropathy, diabetic retinopathy, diabetic osteoporosis, diabetic cardiomyopathy, and so on. However, the effects of this pathway on diabetic peripheral neuropathy are still unknown, thereby suggesting that there are still parts of the activation of the Wnt/β-catenin pathway at high glucose levels that are worth exploring. Further investigation into the role of Wnt signaling during DM will functionally find novel therapeutic target for DM.

At the same time, there are few studies on the effects of traditional Chinese medicine on regulating this pathway at high glucose levels. We look forward to these studies, as long as they reveal the mechanism of diabetes and its complications from a new angle, make full use of the multidirection, multitarget role of Chinese medicine, and explore the exact effective components from the numerous complexes. The goal is to develop more effective new measures and methods for treatment of diabetes and its complications as well as its prevention so that we can relieve the physical and mental suffering of diabetic patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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