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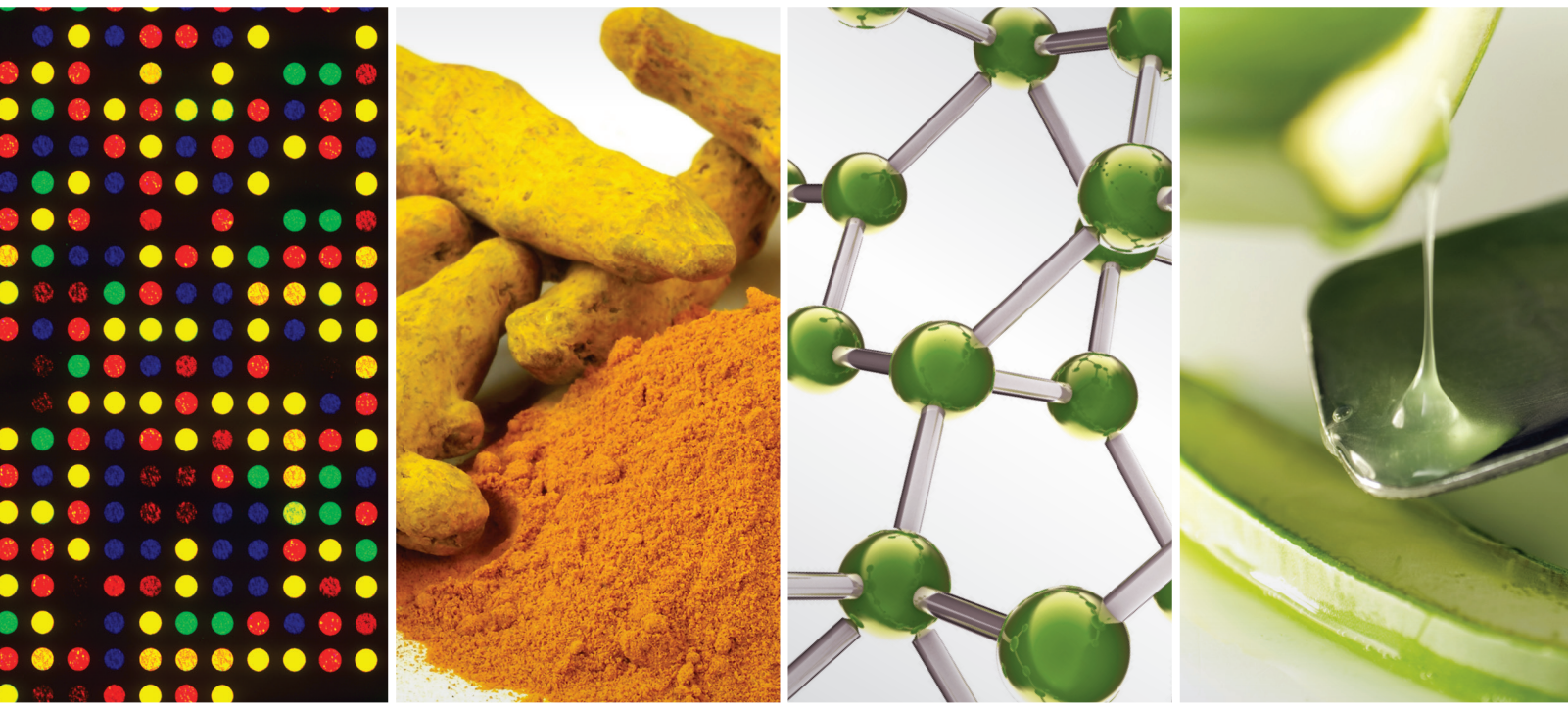
Herb-Drug Interactions: Systematic Review, Mechanisms, and Therapies

Guest Editors: Zhong Zuo, Min Huang, Isadore Kanfer, Moses S. S. Chow, and William C. S. Cho



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Editorial Board

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Editorial

Herb-Drug Interactions: Systematic Review, Mechanisms, and Therapies

Zhong Zuo,[1] Min Huang,[2] Isadore Kanfer,[3] Moses S. S. Chow,[4] and William C. S. Cho[5]

[1]*School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong*
[2]*School of Pharmaceutical Sciences, Sun Yat-sen University, Guang Zhou 510006, China*
[3]*Faculty of Pharmacy, Rhodes University, Grahamstown 6140, South Africa*
[4]*Center for Advanced Drug Research and Evaluation, College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766, USA*
[5]*Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong*

Correspondence should be addressed to Zhong Zuo; joanzuo@cuhk.edu.hk

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Since the use of herbs in daily life has become quite prevalent, issues of the safety of coadministration of such products together with Western medicines should be brought into attention. Although the pharmacokinetics and pharmacodynamics of Western medicines are well-known, the activities of any coadministered herbal products have not been well studied due to their complex components and variability. Most reports on drug-drug or herb-drug interactions focus more on pharmacokinetics than on the pharmacodynamics. However, both effects cannot be ignored in practice, especially for interactions that may occur between a single component Western medicine and a multicomponent herbal product. Herb-drug interactions are essential considerations that need to be addressed by undertaking high quality scientific research and conducting thorough systematic literature reviews.

Since our call for submission in January 2014, this special issue on has attracted over forty papers worldwide, ranging from reviews and preclinical research studies to clinical investigations. The ten final accepted articles cover the topics of (1) systematic reviews on the herb-drug interactions of clinically well-known narrow therapeutic index drugs, (2) recent method development and mechanistic studies on herb-drug interactions, and (3) clinical outcomes for commonly seen combination use of herbs and drugs. The five review papers from S. Mogami and T. Hattori, B. Ge et al., Y. K. Fong et al., S. Chen et al., and D. S. Kiefer et al. provided comprehensive updates on the interactions between herbs and Western drugs in the therapeutic areas ranging from oncology, gastrointestinal, and cardiovascular to central nervous system, among which the article entitled "*Interaction of carbamazepine with herbs, dietary supplement, and food: a systematic review*" was featured on http://www.mdlinx.com/ and selected as number 2 on the nursing site at http://www.mdlinx.com/nursing/news-article.cfm/4784813. Other three articles from Q. Chang et al., A. C. Müller et al., and F. Qiu et al., demonstrated the current advanced approaches for the clinical investigations of herb-drug interactions with emphasis on simultaneous monitoring of both pharmacokinetic and pharmacodynamic changes. The two articles by G. Wu and L. Huang et al. illustrated the current innovative mechanistic approaches in studying herb-drug interactions.

We are of the opinion that the current special issue not only highlights the most updated research tools available in the investigation of herb-drug interaction, but also provides some essential skills for the healthcare researchers and practitioners to solve some relevant issues they may encounter in this field.

Zhong Zuo
Min Huang
Isadore Kanfer
Moses S. S. Chow
William C. S. Cho

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Research Article

Analgesia Synergism of Essential Oil from Pericarp of *Zanthoxylum schinifolium* and Verapamil

Gao Wu[1] and Hanbin Wu[2]

[1] *Department of Pharmacy, The 411st Hospital of PLA, Shanghai 200081, China*
[2] *Clinical Pharmacy, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China*

Correspondence should be addressed to Hanbin Wu; hanbinwu2000@outlook.com

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Objective. To evaluate the synergistic analgesic effect of essential oil of *Zanthoxylum schinifolium* Sieb. et Zucc. (EOZ) and verapamil (Ver). *Method*. The qualitative and quantitative composition of EOZ were determined with gas chromatography/Mass spectrometer. The interaction between EOZ and Ver in antinociceptive activity was evaluated by using acetic acid-induced writhing, hot plate, and tail flick tests in mice and in isolated toad sciatic nerve test. *Results*. Linalool, limonene, and sabinene are the major components of EOZ. EOZ (middle-dose: 40 mg·kg^{-1}, high-dose: 80 mg·kg^{-1}) and EOZ + Ver (Each dose group) have remarkable analgesic effects on pain in mice induced by acetic acid-induced writhing, hot plate, and tail flick tests. Low-dose EOZ (20 mg·kg^{-1}) had no analgesic action, but when it is combined with Ver it has shown significant antinociception. Verapamil has a faint analgesic effect but was not able to inhibit action potential transmission in toad sciatic nerve. EOZ (0.2%) and EOZ + Ver (0.2% + 0.05%) also inhibited action potential transmission in toad sciatic nerve. Combination of EOZ with Ver had a greater analgesic effect and inhibition of nerve action potential transmission compared to its components EOZ and Ver. *Conclusion*. The combination of EOZ with Ver produces a synergistic analgesic effect.

1. Introduction

There are many different names for the *Zanthoxylum* genus in China, with the most popular name being "huajiao" (flower pepper), which refers to the group of related species. The two most commercially popular species are *bungeanum* (red huajiao) and *schinifolium* (green huajiao). The pericarps of several *Zanthoxylum* species are used in China and other East Asian countries as a kind of spice and in traditional Chinese medicine for their therapeutic properties [1]. It is effective for the treatment of inflammatory diseases, epigastric pain, stomachache, toothache, ascariosis, diarrhea, and dysentery. In addition, the pericarps are also used as antimicrobials, insect repellents, antioxidants, and feeding deterrents. These functions are due to the pericarp essential oil [2, 3]. It has antioxidant, blood lipid regulating, antiplatelet, antithrombotic, and antihypertensive properties, which provide protection from stress-induced myocardial injury, as well as anti-inflammatory, analgesic, immunomodulatory, and antitumor functions [4]. Those effects are attributed to the monoterpenes, which are the major chemical components of the essential oils. Limonene and linalool are monoterpene prevalent in the essential oils. Fresh huajiao has a very high content of essential oil, up to 11%. A total of 120 aroma compounds for each species have been found. In the essential oils, linalyl acetate (15%), linalool (13%), and limonene (12%) are the major components of red huajiao, whereas linalool (29%), limonene (14%), and sabinene (13%) are the major components of green huajiao [5–8]. As previously reported linalool and limonene produce antinociceptive activities in several behavioral assays. Green huajiao presented a higher antinociceptive effect than red huajiao, which has higher linalool and limonene content compared to red huajiao [9–11].

Pain is an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage. Calcium plays an important role in the transmission of pain signals in the central nervous system [12]. At the presynaptic nerve terminal, voltage-gated calcium channels (VGCCs) open in response to action potentials to allow an influx of

calcium ions. The influx, in turn, leads to the release of various neurotransmitters that diffuse across the synaptic cleft to the postsynaptic membrane and bind to their specific receptors. The binding of morphine to μ-opioid receptors leads to the inhibition of neurons concerned with the transmission of pain. The μ-opioid receptor does so by blocking VGCCs, opening inwardly rectifying potassium channels and inhibiting the activity of adenylyl cyclase [13]. The release of pain-producing neurotransmitters like substance P from the presynaptic terminals in the spinal cord is thereby decreased, leading to relief from pain [14]. A number of studies have shown an increase in the analgesic response to opioids like morphine, when coadministered with L-type calcium channel blockers (CCBs) [15–18].

Experiments show that the essential oil of *Zanthoxylum* (EOZ) inhibits the contraction of uterine smooth of rat and colon smooth of rabbit, mainly by blocking calcium channels and consequently decreasing the influx of extracellular calcium and release of intracellular calcium [19, 20]. Therefore, the present study was undertaken to examine whether EOZ and the calcium channel blocker verapamil have a synergistic analgesic effect.

2. Materials and Methods

2.1. Plant Material and Reference Drugs. The pericarps of *Zanthoxylum* (green huajiao) were purchased at Shanghai Hongqiao Chinese Herbal Medicine Co., Ltd. The plant material was identified as the pericarp of *Zanthoxylum schinifolium* Sieb. et Zucc.: 1% lidocaine (Huarui Pharmacy, Wuxi, Jiangsu, China), Aspirin (Shanghai Pharmaceutical Co., Ltd. Xinyi Pharmaceutical Factory, Shanghai, China), verapamil (Sigma Pharmaceutical Industrial Co.), and morphine hydrochloride injection (Shenyang first pharmaceutical factory, Shenyang, China).

2.2. Animals. Kunming mice (25–30 g) were purchased from Shanghai Experimental Animal Center (Shanghai, China) and raised in our specific pathogen-free and air-conditioned animal facility. All of the experiments were performed with the sciatic nerves dissected from wild adult toads (Bufo bufo gargarizans Cantor) weighing 60–90 g.

2.3. Preparation and Analysis of Essential Oil of Zanthoxylum bungeanum. Essential oil of *Zanthoxylum schinifolium* (EOZ) was extracted using a modified Clevenger apparatus by the hydrodistillation technique. The obtained EOZ was dried over sodium sulfate and used as the basic material. EOZ was stored in hermetically sealed glass receptacles with rubber stoppers, covered with aluminum foil to protect the contents from light, and kept under refrigeration at 4°C until use.

The components of the EOZ were analyzed using GC/MS-QP 2010 (Tokyo, Japan), with an autoinjector (AOC-20i) and autosampler (AOC-20s). Sample was eluted with Helium gas. Components were separated with capillary column (Rtx-1 MS Prepared by Restek Corporation USA) having 30 m × 0.250 mm and 0.25 micrometer thickness. Electronimpact ionization mode was with energy 70 eV, ion source temperature 200°C, interface temperature 250°C with 28.8 KPa pressure, and 1.8 min solvent cut time. Injector temperature was 250°C and operated in split mode with 2 mL/min. The column was programmed at a temperature of 40°C for 3 min initially and then changed to 150°C at the rate of 15°C/min and kept constant for 15 min. The column temperature was increased to 250°C at a rate of 5°C per min and was maintained for 3 min. Mass spectra were acquired in the range of 20 to 400 m/z. A series of normal alkanes was also injected under same analytical conditions with that of the essential oil for the calculation of retention indices. Components of the essential oil were identified by comparing the mass spectra obtained with those of standard mass spectra from the NIST library (NIST 08). Relative concentration of the components was calculated from the peak areas of the total ion chromatograms.

2.4. Evaluation of Antinociceptive Activity of EOZ

2.4.1. Acetic Acid-Induced Writhing Test. The method previously described was used to evaluate the antinociceptive activity [21]. Kunming mice, of either sex, were injected with 0.6% acetic acid in 0.9% normal saline (0.2 mL) by intraperitoneal injection. The number of writhings within 15 min was then recorded, and the writhing number was regarded as the pain threshold. The selection of qualified mice involved choosing those in which the writhing number within 15 min was 11~59, those in which the writhing number was more than 60 times or less than 10 times were abandoned. Mouse button on the standard body followed by the writhing number, and then per ten mice with similar writhing number as a group, were randomly assigned to nine different experimental groups: the control group (0.9% normal saline, NS, 60 mg·kg^{-1}, p.o.), EOZ different groups (at the doses of 20 mg·kg^{-1}, 40 mg·kg^{-1}, and 80 mg·kg^{-1}, p.o.), Ver group (5 mg·kg^{-1}, p.o.), combined EOZ + Ver different groups (at the doses of 20 mg·kg^{-1} + 5mg·kg^{-1}, 40 mg·kg^{-1} + 5 mg·kg^{-1}, and 80 mg·kg^{-1} + 5 mg·kg^{-1}, p.o.), and reference Asp group (aspirin, 200 mg·kg^{-1}, p.o.). A week later, experimentation began, and all test drugs were given orally to the separate groups of mice prior to acetic acid injection. The mice were observed and counted for the number of abdominal constrictions and stretchings in a period of 0–20 min. The responses of the mice in the treated groups were compared with those animals in the control group. The percentage of inhibition of the number of writhings was calculated.

2.4.2. Hot Plate Test. Kunming female mice were used [22]. The hot plate was an electrically heated iron surface of water bath kept at a constant temperature of 55.0 ± 0.5°C After 30 min of treatment (except only 15 min for morphine) with all test drugs, the control group (0.9% normal saline, NS, 60 mg·kg^{-1}, p.o.), EOZ different groups (at the doses of 20 mg·kg^{-1}, 40 mg·kg^{-1}, and 80 mg·kg^{-1}, p.o.), Ver group (5 mg·kg^{-1}, p.o.), combined EOZ + Ver different groups (at the doses of 20 mg·kg^{-1} + 5mg·kg^{-1}, 40 mg·kg^{-1} + 5 mg·kg^{-1},

and 80 mg·kg^{-1} + 5 mg·kg^{-1}, p.o.), and reference Mor group (morphine, 10 mg·kg^{-1}, i.p.) mice (n = 10 per group) were placed on the heated surface, with Plexiglas walls to constrain their locomotion on the plate. The latency to a discomfort reaction (licking of the paws or jumping) was recorded at 15, 30, 60, 90, and 120 min after drug treatment; the reaction time of 0 min was the start of the test. A cut-off time of 60 s was chosen to indicate complete analgesia and to avoid tissue injury. Only mice that showed a nociceptive response within 15 s were used in the experiments.

2.4.3. Tail Flick Test. This test was applied as described above [21]. The lower two-thirds of the tail was immersed in a beaker containing hot water kept at 50 ± 0.5°C. The time in seconds until the tail was withdrawn from the water was defined as the reaction time. The reaction time was then measured 0, 15, 30, 60, 90, and 120 min after 30 min of treatment (except only 15 min for morphine) with all test drugs (NS, EOZ, Ver, EOZ + Ver, and Mor); the reaction time of 0 min was the start of the test. Mice (n = 10 per group) showing a pretreatment reaction time greater than 5 s in the tail flick test were not used in the experiment. A cut-off time of 20 s was set to avoid tissue damage.

2.4.4. Effect on Isolated Toad Sciatic Nerve. The toads were rapidly decapitated and killed [23], exposed both sides of the sciatic nerve lumbar plexus nerves to the legs of blunt isolated and immediately maintained in Ringer solution (RS). Fifty isolated toad sciatic nerves were selected and randomly assigned to 5 groups of 10 each: control group (RS), EOZ + Verapamil compound group (EOZ + Ver, 0.2% EOZ + Ver 0.05%), EOZ group (EOZ, 0.2%), verapamil group (Ver, 0.05%), and lidocaine group (Lid, 1% lidocaine). Compound nerve action potential (CNAP) was recorded via an extracellular recording technique with a BL-420F Acquisition System (Taimeng Technology, Chengdu, China). After 30 minutes of stabilization in RS, segments of nerve measuring 5 cm were placed in a Plexiglas nerve chamber. The space between the electrodes was fixed during the entire procedure. The stimulating voltage was set to produce a maximal CNAP using single square pulses of supra maximal strength and 0.5 milliseconds in duration. Then put the drug's cotton balls (cotton balls as rare as possible, the same size) on the nerves (between the stimulation side and recording-side), and the added liquid is about 0.05 mL. At 1, 5, and 10 min after dosing, the waveform changes and action potential wave disappearance time were observed and recorded.

2.5. Statistical Analysis. The same subject was observed using repeated-measure design. In this design, each subject serves as its own control. Results are presented as mean ± SEM, with (N) indicating the number of subjects. All analyses were performed using the SPSS 13.0 statistical software (SPSS, Chicago, IL). Statistically significant differences between groups were calculated by the application of analysis of variance (ANOVA) followed by Bonferroni's test. The independent t-test was used for comparison between 2 groups. P values less than 0.05 ($P < 0.05$) were used as the significance level.

Table 1: Main constituents of essential oil from *Zanthoxylum schinifolium* Sieb. et Zucc. determined by GS-MS.

Retention (min)	Compound name	Relative content (%)
7.356	.alpha.-Pinene	1.03
8.475	.beta.-Phellandrene	1.26
8.856	Sabinene	9.16
9.157	.beta.-Myrcene	3.87
9.835	.alpha.-Terpinene	1.05
10.158	Eucalyptol	1.64
10.262	d-Limonene	15.34
10.751	1,8-Cineole	1.05
11.122	.gamma.-Terpinene	1.02
12.671	Cis-sabinene hydrate	1.18
12.304	Linalool	32.54
13.154	Linalyl acetate	1.06
14.796	Terpineol	2.56
19.255	4-Terpinenol	1.78
22.038	.alpha.-Caryophyllene	1.41
22.847	Germacrene	1.32
23.469	.gamma.-Cadinene	1.03
	Different compounds	Trace (<1%)

3. Results

3.1. Analysis of Essential Oil of Zanthoxylum bungeanum (EOZ). The percentage yield of essential oil based on the dried pericarp was 2.82% (v : w). Composition of essential oil has been summarized in Table 1. Linalool, d-limonene, and sabinene are the major constituents of the essential oil.

3.2. Acetic Acid-Induced Writhing Test. All oral administrations of test drugs except EOZ low-dose group caused a significant reduction in the number of writhing episodes induced by acetic acid compared to the control ($P < 0.05$ and $P < 0.01$). The combined EOZ + Ver groups resulted in 52.8%, 34.9, and 13.4% reduction of writhing episodes number compared to the relevant sole EOZ groups, respectively. It showed that low-dose EOZ group had no significant difference with control group in the number of writhing episodes (>0.05), but it is combined with Ver; writhing number could be reduced by 52.8% and was similar to the reference group (aspirin group), which is more obvious than that of the middle and high dose of EOZ (34.9 and 13.4% resp.). The analgesic effect was weaker in the sole EOZ group whose combined EOZ + Ver group resulted in an increase greater degree of synergistic analgesic effect. The results are provided in Table 2.

3.3. Hot Plate Test. There was no significant difference between each treatment group's reaction time at 15 min after administration and the reaction time before administration, but there was an increasing trend, except in the reference Mor group. All drug groups (EOZ + Ver, EOZ) except Ver

Table 2: Effect of turning body induced by acetic acid in mice ($x \pm s$, $n = 10$).

Group	Dosage (mg/kg)	潜伏期 latency of stomach ache/min	扭体次数 amount of turning body/15 min
NS		2.8 ± 0.9	43.5 ± 13.8
EOZ low-dose	20 mg	3.0 ± 1.6③	40.7 ± 15.3③
EOZ middle-dose	40 mg	3.6 ± 1.8③	29.5 ± 5.6②
EOZ high-dose	80 mg	4.2 ± 1.7①	18.6 ± 6.4②
Ver	5 mg	3.9 ± 2.0③	19.6 ± 5.2②
EOZ + Ver low-dose	20 mg + 5 mg	4.3 ± 2.2①	19.2 ± 5.5②④
EOZ + Ver middle-dose	40 mg + 5 mg	4.5 ± 2.8①	18.3 ± 4.6②④
EOZ + Ver high-dose	80 mg + 5 mg	5.1 ± 3.1①	16.1 ± 4.8②④
Asp	200 mg	3.7 ± 1.3①	18.6 ± 5.5②

Values are presented as the mean ± S.E.M. ($n = 10$).
Compared with NS group: ①: $P < 0.05$, ②: $P < 0.01$, ③: $P > 0.05$; compared with relevant EOZ group: ④: $P < 0.01$.

Table 3: Analegsia effect of the pain induced by hot-plate in mice ($x \pm s$, $n = 10$).

Group	Dosage (mg/kg)	Pain threshold of preadmin/s	Pain threshold of proadmin/s				
			15 min	30 min	60 min	90 min	120 min
NS		19.6 ± 5.8	19.3 ± 5.5	19.9 ± 5.3	20.3 ± 5.1	20.5 ± 5.5	19.8 ± 5.4
EOZ low-dose	20 mg	19.8 ± 4.7	20.5 ± 5.8③	20.9 ± 6.1③	20.8 ± 5.8③	20.8 ± 6.2③	20.5 ± 5.6③
EOZ middle-dose	40 mg	19.7 ± 4.8	23.9 ± 6.5③	31.4 ± 6.3①	32.2 ± 7.1①	34.8 ± 7.2①	34.6 ± 6.9①
EOZ high-dose	80 mg	19.7 ± 5.3	24.5 ± 5.6③	35.2 ± 5.3②	37.5 ± 6.1②	38.1 ± 5.6②	37.8 ± 5.3②
Ver	5 mg	20.3 ± 3.2	20.6 ± 5.2③	20.9 ± 6.1③	19.8 ± 6.4	21.5 ± 6.2③	21.6 ± 6.6③
EOZ + Ver low-dose	20 mg + 5 mg	20.5 ± 4.1	25.8 ± 6.6③	32.2 ± 5.7②④	33.5 ± 5.2②④	34.8 ± 5.9②④	35.5 ± 6.2②④
EOZ + Ver middle-dose	40 mg + 5 mg	19.6 ± 5.1	25.4 ± 5.2③	37.4 ± 5.3②④	40.6 ± 6.1②④	40.7 ± 6.1②④	41.5 ± 7.0②④
EOZ + Ver high-dose	80 mg + 5 mg	19.8 ± 4.8	26.2 ± 8.2③	41.2 ± 6.8②④	45.2 ± 6.8②④	46.7 ± 6.5②④	45.8 ± 7.2②④
Mor	10 mg	19.8 ± 4.6	45.3 ± 6.7②	46.8 ± 7.2②	45.3 ± 7.1②	24.9 ± 5.6③	21.7 ± 4.9③

Compared with NS group: ①: $P < 0.05$, ②: $P < 0.01$, ③: $P > 0.05$; compared with relevant EOZ group: ④: $P < 0.01$.

group and low-dose EOZ group considerably increased the animal's reaction time to the heat stimulus after 30 min. This indicates that their analgesic effects of EOZ come into play at about 30 min or so and are maintained for at least 120 min; however, Mor was only maintained for 60 min. Although the Ver and low-dose EOZ produced no significant increase in the reaction time throughout the observation period, the analgesic effect of low-dose combined EOZ + Ver was significantly ($P < 0.01$) greater than that of the relevant low-dose EOZ after 30 min. The analgesic effect of all EOZ + Ver groups were significantly ($P < 0.01$) greater than that of the relevant sole EOZ groups, respectively, indicating that EOZ and Ver have a synergistic analgesic effect for the hot plate test. The results are provided in Table 3.

3.4. Tail Flick Test. After treatment administration, there was no significant difference in the tail flick latency (TFL) between each treatment group's reaction time at 15 min, except in the reference Mor group. All treatment groups except the Ver group and low-dose EOZ group significantly ($P < 0.001$ and $P < 0.05$, resp.) increased the tail flick latency in 30 min to 120 min observation period, as compared with the control group. All combined EOZ + Ver groups showed a significant ($P < 0.05$) increase in the reaction time when compared with the relevant sole EOZ group, respectively. This indicates that EOZ and Ver have a synergistic analgesic effect for the tail flick test. The results are provided in Table 4.

3.5. Isolated Toad Sciatic Nerve Test. No significant differences were found in the baseline values of nerve action potential amplitude among the 5 groups. The negative amplitude after drug administration continuously declined in the combined EOZ + Ver group and the EOZ group, as well as in the Lid group. In contrast, the negative amplitude in the RS group and Ver group remained stable. The conduction blockade induced by EOZ + Ver and EOZ had a faster onset (amplitude begins to decrease) and the action potential disappeared faster as compared with lidocaine (onset: 1 minute, 1 minute versus 10 minutes and disappear: 3 minutes, 5 minutes versus 22 minutes). For both groups, the strength of conduction blockade was greater than that of the Lid group (the negative amplitude decreased baseline 46% and 27% versus 3%). The disappearance of the action potential of the combined EOZ + Ver group was faster than

Table 4: Effect of the tail-curling latencies of mice in the warm water tail-flick test ($x \pm s$, $n = 10$).

Group	Dosage (mg/kg)	Basal TFL/s	TFL of proadmin/s				
			15 min	30 min	60 min	90 min	120 min
NS		3.42 ± 0.52	3.48 ± 0.61	3.51 ± 0.72	3.62 ± 0.89	3.46 ± 0.61	3.48 ± 0.63
EOZ low-dose	20 mg	3.52 ± 0.68	3.72 ± 0.79③	4.16 ± 0.97③	4.25 ± 0.92③	4.20 ± 0.91③	4.25 ± 0.92③
EOZ middle-dose	40 mg	3.45 ± 0.62	4.15 ± 0.67③	4.85 ± 0.78①	5.01 ± 0.88①	5.12 ± 0.75①	5.12 ± 0.85①
EOZ high-dose	80 mg	3.50 ± 0.58	4.14 ± 0.85③	5.23 ± 0.82②	5.41 ± 0.76②	5.42 ± 0.83②	5.41 ± 0.86②
Ver	5 mg	3.49 ± 0.78	3.50 ± 0.75③	3.51 ± 0.82③	3.41 ± 0.84③	3.54 ± 8.6③	3.48 ± 0.85③
EOZ + Ver low-dose	20 mg + 5 mg	3.38 ± 0.72	4.56 ± 0.76③	6.85 ± 0.78②④	7.22 ± 0.75②④	7.34 ± 0.85②④	6.46 ± 0.78②④
EOZ + Ver middle-dose	40 mg + 5 mg	3.32 ± 0.65	4.12 ± 0.78③	9.23 ± 0.66②④	9.54 ± 0.78②④	9.68 ± 0.82②④	9.57 ± 0.65②④
EOZ + Ver high-dose	80 mg + 5 mg	3.47 ± 0.60	4.11 ± 0.73③	11.2 ± 0.78②④	12.4 ± 0.85②④	13.1 ± 0.87②④	12.5 ± 0.91②④
Mor	10 mg	3.56 ± 0.63	13.68 ± 0.86②	12.62 ± 0.76②	11.42 ± 0.79②	8.49 ± 0.88②	5.52 ± 0.82②

Compared with NS group: ①: $P < 0.05$, ②: $P < 0.01$, ③: $P > 0.05$; compared with EOZ group: ④: $P < 0.05$.

Table 5: Effect of amplitude of action potential (AP) of sciatic nerve in toads ($x \pm s$, $n = 5$).

Group	Dosage (g/mL)	Preadmin/mV	Proadmin/mV			Time of AP vanishing
			1 min	5 min	10 min	min
RS		4.35 ± 1.25	4.26 ± 1.32	4.30 ± 1.16	4.33 ± 1.53	exist
EOZ	0.2%	4.39 ± 1.43	3.21 ± 0.85①③	0	0	4.98 ± 0.92③
Ver	0.05%	4.42 ± 1.02	4.39 ± 1.32	4.43 ± 1.06	4.29 ± 1.34	exist
EOZ + Ver	0.2% + 0.05%	4.45 ± 1.03	2.36 ± 1.02②③	0	0	2.62 ± 0.58③⑤
Lid	1%	4.53 ± 1.12	4.52 ± 1.35	4.50 ± 1.65①③	4.38 ± 1.65①③	22.89 ± 6.52③

Compared with preadministration: ①: $P < 0.05$, ②: $P < 0.01$; compared with NS group: ③: $P < 0.05$, ④: $P < 0.01$; compared with EOZ group: ⑤: $P < 0.05$.

those of the EOZ group, suggesting that the combination of EOZ and Ver had a synergistic effect in the isolated toad sciatic nerve test. The results are provided in Table 5.

4. Discussion

The percentage yield of essential oil based on the dried pericarp was 2.82% (v : w). Linalool, d-limonene, and sabinene are the major constituents of the essential oil. The results are consistent with those reported [5].

In the present study, our results demonstrated that Ver showed an analgesic effect only in writhing reaction; in the hot plate test and tail-flick test, Ver alone did not produce an analgesic effect. However, when verapamil was combined with EOZ, its analgesic effect is obviously enhanced as shown by the three animal experiment results. Low-dose EOZ had no analgesic action, but when it is combined with Ver it has shown significant antinociception which was similar to middle and high dose combined EOZ + Ver. It indicated that Ver could not only enhance the analgesic action of EOZ, but also reduce its dosage. In other words, the analgesic potency of combined EOZ + Ver groups significantly improved compared to the relevant sole EOZ groups, respectively, in the three animal experiments. Results showed that the EOZ and Ver analgesics have a synergistic effect. Calcium is a coupling factor necessary in presynaptic membrane excitability and neurotransmitter release; the release of neurotransmitters induced by noxious stimuli is related to electrical activity of VGCCs on the membrane of synaptic endplate. Experiments with VGCC antagonists revealed that L-, N-, and P/Q-, but not T-type channels, are involved in nociception, and potentiation of opioid-induced antinociception was more frequently seen with L-type antagonists [24]. The L-type CCB verapamil potentiates morphine analgesia through a peripheral mechanism. Earlier studies have shown that intrathecal administration of Ver did not show any antinociception; however, when intrathecally administered, Ver combined with ineffective or moderately effective doses of intrathecally administered morphine produced significant antinociception. These interactions were synergistic [25]. The study demonstrated that intrathecally administered L-type calcium channel blockers diltiazem or Ver produced both somatic and visceral antinociception and motor block dose dependently. Further, Ver evoked antinociception in the mouse hot-plate test, and further experiments showed that these effects might be due to the agonistic activity of verapamil at μ-, δ-, and κ 3-receptor subtypes. Interestingly, some of the CCBs (diltiazem and Ver) also increase morphine levels in the brain when coadministered together, as compared to morphine alone, after systemic administration [26]. Thus, Ver can have a synergistic effect on the analgesic effect of opioids. EOZ inhibits the contraction of smooth muscle mainly by blocking calcium channels, and its calcium antagonism mechanism is not exactly the same as that of Ver [19, 20]. The results of the present study indicate that Ver increases the analgesic effect of EOZ. The mechanism may be different from the analgesia synergism of Ver and opioids, and they prevent Ca^{2+} influx in different ways, which create a synergistic analgesic effect.

The present study showed that EOZ (middle and high dose) and combined EOZ + Ver possess significant antinociceptive effects as evaluated in the acetic acid-induced writhing test, hot plate test, and tail-flick test, and they inhibit the induction of action potentials in toad sciatic nerve. Ver has a weak analgesic effect and no effect in sciatic nerve action potential block. The analgesic effects and inhibition of nerve action potential conduction of combination of EOZ and Ver are greater than both component EOZ and verapamil. The acetic acid-induced writhing model is a chemical stimulus widely used for the evaluation of peripheral antinociceptive activity. In this model, acetic acid indirectly induces the release of endogenous mediators, stimulating the peripheral nociceptors and sensitive neurons that were sensitive to the inflammatory mediators. The results of this study revealed that the analgesic potency of combined EOZ + Ver groups were significantly improved than the relevant sole EOZ groups, respectively, in acetic acid-induced writhing test, similar to the reference drug aspirin (200 mg/kg). Therefore, one possible mechanism of antinociceptive activity of EOZ could be a blockade of the effect or the release of endogenous substances. EOZ has been reported to inhibit the contractions of smooth muscle [19, 20]. The antinociceptive action of EOZ observed in this study may not be involved with the inhibition of smooth muscle contractions by EOZ. The tonic inhibition of smooth muscle contractions produced by EOZ may be responsible for the inhibition of contortions but is not related to its analgesic activity. However, the results of this test do not ascertain whether the antinociceptive effect was mediated by a central or peripheral process.

To evaluate possible participation of the central analgesic system in the antinociceptive activity of EOZ, the hot plate test and tail-flick test were employed [27]. In the tail immersion test, which consists of a thermal stimulus, an increase in the reaction time is generally considered to be an important parameter for evaluating central antinociceptive activity. The hot plate test is predominantly a spinal reflex or behavioral reaction and used to test supraspinal analgesia in compounds. Both are considered to be supraspinally integrated responses. It is, therefore, selective for centrally acting analgesic drugs like morphine. EOZ was found to have antinociceptive activity in the hot plate test and tail immersion test in middle and high dose. These tests also revealed that the antinociceptive effect of EOZ on mice remained present for at least up to 120 min after administration in middle and high dose. However, morphine, a well-known opioid agonist, produced a profound antinociceptive effect to the hot plate test in the period of 60 min. The analgesic effect of all combined EOZ + Ver groups were significantly ($P < 0.01$) greater than that of the relevant sole EOZ groups, respectively, indicating that EOZ and Ver have a synergistic analgesic effect for the hot plate test and tail immersion test. In other words, the analgesic potency of EOZ was strengthened after the addition of Ver. The antinociceptive effects of EOZ involve supraspinal as well as spinal components, as demonstrated by the use of the hot plate and tail immersion tests, respectively. Therefore, taking all these data together, we believe that the antinociceptive activity of EOZ is most likely to be mediated peripherally and centrally.

The major result of isolated toad sciatic nerve test was that EOZ reversibly inhibited compound nerve action potential of the toad sciatic nerve, and Ver has synergistic analgesic effect on EOZ. The mechanism is not only related to blocking calcium influx, but may also be related to Na^+ channel blockers. Early study shows EOZ has a local anesthesia; analgesic effect of EOZ is due to the effect of its local anesthesia. The target of local anesthetic is usually a Na^+ channel on the inner side of the nerve cell membrane. Local anesthetics inhibit the transmission of nerve impulses in nerve fibers by blocking Na^+ channels, producing a local anesthetic effect, and have no relation with Ca^{2+} opioid receptors. According to the theory of neural electrophysiology, formation of nerve action potential does not directly involve Ca^{2+}. The nerve fiber action potential opens a large number of Na^+ channels on the cell membrane, leading to a Na^+ influx. Lidocaine reduces the internal flow of Na^+ by blocking Na^+ channels, thus inhibiting the action potential of sciatic nerve. The local anesthetic effect of EOZ has previously been observed. Thus we presume that the analgesic effect of EOZ may be related to the blocking of Na^+ channels on nerve cell membranes, which reduces the amplitude of the action potential due to the influx of Na^+ and produces an anesthesia effect. The analgesia synergism of the combination of EOZ with Ver is probably caused by the blocking of nerve impulses from the different way. At the presynaptic nerve terminal, VGCCs open in response to action potentials to allow an influx of calcium ions. The influx is a graded process, varying in a linear manner with the frequency of action potentials. An inhibition of the postsynaptic current was observed with L-type CCB (Ver) after electrical stimulation of dorsal nerve root [28]. Ver blocks the extracellular Ca^{2+} influx, reduces neurotransmitter release, decreases impulse conduction in nerve cells, and enhances the analgesic effect of EOZ. The results of the present study indicate that the onset and disappearance of toad sciatic nerve action potentially inhibited by EOZ and combined EOZ + Ver are fast and strong as compared to lidocaine. The mechanism may be the reason that effective components of EOZ are smaller molecules and more lipophilic chemical characteristics compared with lidocaine; these characteristics are conducive to molecules of EOZ penetrating effectively in nerve cells. The details of these mechanisms need further investigation.

5. Conclusion

Our result pertaining to the composition of essential oil has shown that linalool, limonene, and sabinene are the major components of essential oil from *Zanthoxylum schinifolium* Sieb. et Zucc. (EOZ). A synergistic interaction was observed between EOZ and verapamil in acetic acid-induced writhing test, hot plate test, tail flick test, and isolated toad sciatic nerve test. The results from this study can be extrapolated to clinical settings and additionally confirmed in different experimental models of pain, suggesting that this combination could be useful to treat diseases associated with pain in human beings. Further studies are necessary for the analgesic mechanism of EOZ and verapamil synergistic effect.

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

Ginseng and Anticancer Drug Combination to Improve Cancer Chemotherapy: A Critical Review

Shihong Chen,[1,2] Zhijun Wang,[2] Ying Huang,[3] Stephen A. O'Barr,[3] Rebecca A. Wong,[2] Steven Yeung,[2] and Moses Sing Sum Chow[2]

[1] *Department of Pharmacy, Xiamen Medical College, Xiamen, Fujian 361008, China*
[2] *Center for Advancement of Drug Research and Evaluation, College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766, USA*
[3] *Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766, USA*

Correspondence should be addressed to Moses Sing Sum Chow; msschow@westernu.edu

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Ginseng, a well-known herb, is often used in combination with anticancer drugs to enhance chemotherapy. Its wide usage as well as many documentations are often cited to support its clinical benefit of such combination therapy. However the literature based on objective evidence to make such recommendation is still lacking. The present review critically evaluated relevant studies reported in English and Chinese literature on such combination. Based on our review, we found good evidence from *in vitro* and *in vivo* animal studies showing enhanced antitumor effect when ginseng is used in combination with some anticancer drugs. However, there is insufficient clinical evidence of such benefit as very few clinical studies are available. Future research should focus on clinically relevant studies of such combination to validate the utility of ginseng in cancer.

1. Introduction

The concept of herb-herb or herb-drug combination to enhance therapeutic benefit has been utilized and practiced in China according to Chinese medicine principles for more than 2000 years [1]. Recently, herbs in combinations with anticancer drugs have been found to be capable of resensitizing the chemoresistance developed from repeated use of the anticancer drug [2]. Thus, the use of herb-drug combination to enhance therapeutic effect is of great interest, especially in cancer chemotherapy.

Among many herbs touted to improve cancer treatment, none has probably enjoyed as much worldwide reputation and interest as ginseng. Ginseng is widely used and is included in the pharmacopoeias in China, Japan, Germany, France, Austria, and the United Kingdom. In Asian countries and Western Europe, it is widely available as an over-the-counter drug and also commonly used as an adjuvant for cancer therapy [3, 4]. In the US, ginseng is one of the most frequently purchased herbs; it is available and used as a dietary or an herbal supplement but not as a drug approved by the Food and Drug Administration [5]. It is consumed regularly by more than 6 million Americans [6], as the second top-selling herbal supplement (US $62 million in annual sales in 2000 and about US $83 million in 2010) [7, 8]. In 2002, a national survey of men and women in the US has estimated that 4-5% of those aged 45–64 years had used ginseng [9].

Although ginseng consumption is not limited for in cancer patients, its benefit in cancer appears to be well accepted. Common reasons for the use of ginseng by cancer patients are to improve clinical outcomes, enhance quality of life, treat cancer-related symptoms, reduce adverse effects of chemotherapy, and potentially enhance the effects of chemotherapeutic agents. In addition, ginseng may exert a chemopreventive action: an epidemiological study has shown that patients taking ginseng had a 50% lower risk of cancer recurrence compared to patients not taking ginseng [10].

In view of its wide usage and potential benefit when used in combination with anticancer drugs, the present paper intends to critically review the evidence of such

benefit as well as potential mechanisms involved. Although numerous reviews on ginseng-drug interaction have been already published [47, 48], no article has critically reviewed ginseng-anticancer drug combination for improvement of chemotherapy.

Both English and Chinese publications on ginseng and anticancer drug combination to improve cancer chemotherapy were searched from the Medline database (1990~2013) and China Academic Journals Database (1983~2013), respectively. All articles from *in vitro*, *in vivo* animal models, and human studies on the combination of ginseng or its active components with chemotherapeutic agent for anticancer effect were included. Because there are several types of ginseng with different active components, their general properties are briefly discussed to provide relevant background information before reviewing the specific combination in order to provide better understanding of the rationale of such combination.

2. Different Types of Ginseng and Its Preparation

Ginseng is a perennial herb that belongs to the Araliaceae family and *Panax* genus [49]. The root is the preferred part of the plant due to the presence of active components (see below), and the species most commonly used are *Panax ginseng* C.A. Meyer (Asian ginseng or Korean ginseng) and *Panax quinquefolius* (American ginseng). *Panax ginseng* C.A. Meyer is usually cultivated in China and Korea and has been used as a medicinal herb in China, Japan, and Korea for thousands of years. Its commonly claimed health benefits include immunity enhancement, stress relief, and prevention of aging. *Panax quinquefolius,* originally grown in United States and Canada, has been used by Native Americans for hundreds of years. So far, majority of research on ginseng has been on *Panax ginseng* C.A. Meyer [5, 17].

Panax ginseng C.A. Meyer is usually harvested after 4 to 6 years of cultivation and is classified into three types based on processing methods: (1) fresh (less than 4 years old, consumed in its fresh state), (2) white ginseng (4 to 6 years old, typically air or oven dried after peeling), (3) red ginseng (6 years old, steamed prior to drying, without peeling). These processing methods are intended to improve efficacy, safety, and preservation [50]. Recently, a new heat-processed ginseng, called Sun ginseng (SG), has been prepared by steaming with white ginseng at high temperature and pressure. Sun ginseng has been reported to contain more unique ginsenosides than the red ginseng. A preparation containing Sun ginseng extract with specific standardization is now available as functional food in Korea [21, 22, 24].

Many ginseng products are available on the market as fresh slices, juice, extract (tincture or boiled), powder, tea, tablet, capsule, and other forms. Two-year-old fresh ginseng is also used as an ingredient in Korean chicken-ginseng soup (boiled chicken with young ginseng root), *samketang* [51]. The traditional Chinese ginseng preparation widely used clinically in China is Shengmai which consists of red ginseng, lilyturf root, and magnolia vine fruit [28, 40].

The quality of ginseng is believed to vary with the age at harvest. When ginseng is harvested at the time of 5 to 6 years, it is considered the "best" with ginsenoside content at its highest [52]. According to several laboratory investigations, the quality of commercially available ginseng products can vary considerably. Negative trial results may be due to poor product quality rather than lack of efficacy [35]. Thus, evaluation of study results must take product quality control into consideration.

3. Active Components of Ginseng Relevant to Anticancer Effect

Ginseng contains various active components including ginsenosides, polysaccharides, flavonoids, volatile oils, amino acid, and vitamins. Of these active components, ginsenosides and ginseng polysaccharides appear to be responsible for the anticancer effect [8].

Ginsenosides are the main pharmacologically active ingredients responsible for the four major actions of ginseng: vasorelaxation, antioxidation, anti-inflammation, and anticancer effect. Ginsenosides, being amphipathic in nature, are steroidal saponins that contain four transring rigid steroid skeleton. They differ from each other mainly by the number, type, and location of their sugar moieties. Thus far, more than 40 different ginsenosides have been identified and isolated. Ginsenosides can be classified into three groups based on the chemical structure of aglycones: (1) protopanaxadiol group (PPD) or diols, for example, Rb1, Rb2, Rb3, Rc, Rd, Rg3, and Rh2; (2) protopanaxatriol group (PPT) or triols, for example, Re, Rf, Rg1, Rg2, and Rh1; (3) oleanane group: only Ro (0.6% of all ginsenosides) [53, 54]. The total percentage of ginsenosides (w/w) can vary from 1.9% to 8.1% in ginseng root preparations [17]. Red ginseng can possess higher activity than white ginseng, due to the presence of unique ginsenosides (Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, and F4) produced during steaming method [15, 18, 24]. The relative amounts of ginsenosides may also be used to differentiate *Panax* species. For example, American ginseng has little or no Rf, and *Panax* ginseng has higher levels of Rg1 but lower levels of Rb1 (or higher ratio of Rg1/Rb1) compared to those of American ginseng [5, 49, 55, 56]. Ginsenosides are also used as marker compounds for ginseng quality control, of which Rg1, Rc, Rd, Re, Rb1, and Rb2 are quantitatively the most important and prevalent. According to a Ginseng Evaluation Program led by the American Botanical Council of Austin, Texas, Rb1, Rb2, Rc, Rd, Re, and Rg1 account for >90% of the total ginsenoside content of the *Panax* ginseng root, whereas, Rb1, Rb3, Rc, Rd, Re, and Rg1 make up more than 70% of total ginsenoside content in American ginseng [8]. Each ginsenoside may differ in pharmacology and mechanisms due to its different chemical structure.

Researchers are now focusing on using purified individual ginsenosides to reveal the specific mechanism of action instead of using whole ginseng root extracts. The most commonly studied ginsenosides are Rb1, Re, Rg1, Rg3, and Rh1 [5]. The relevant ginsenosides (Rb1, Rg1, Rg3, and

Rh1) for anticancer activity and corresponding molecular mechanisms are listed in Table 1.

Besides ginsenosides, ginseng polysaccharides also possess antitumor effect through modulation of innate immunity. Ginseng polysaccharides (present in 15% of ginseng root), including neutral and acidic polysaccharides, are water-soluble. It has been reported that *Panax* ginseng polysaccharides contain starch-like polysaccharide and pectin and can be fractionated into two neutral (WGPN and WGPA-N) and six acidic fractions (WGPA-1-RG, WGPA-2-RG, WGPA-1-HG, WGPA-2-HG, WGPA-3-HG, and WGPA-4-HG) by a combination of ethanol precipitation, ion exchange, and gel permeation chromatographies [57]. Many immunological studies have been performed with crude polysaccharide fractions, which are usually prepared by ethanol precipitation after extracting ginseng root with hot water. These polysaccharides have been reported to exert antitumor activity by regulating the immune response of the host organism. Using lymphocyte proliferation assays, both polysaccharides have been found to be potent B and T cell stimulators [57]. The acidic polysaccharides (10,000–150,000 MW), being readily soluble in water, are thought to be more active than neutral ones [27, 31, 32]. Ginseng pectin has also been shown to inhibit the actions of galectin-3, a β-galactoside-binding protein associated with cancer progression [58]. Nonsaponin constituents (immunomodulating polysaccharides) and the harmonizing constituents still remain to be explored.

4. Effects of Ginseng in Combination with Anticancer Drugs

4.1. In Vitro Studies. A number of *in vitro* studies have shown an enhanced anticancer effect when the ginseng extract or its active component is combined with a chemotherapeutic agent (see Table 2). One specific effect is increasing the cytotoxicity of chemotherapeutic agents.

Ginseng extracts, including ginsenosides, have been found to enhance the cytotoxicity of several chemotherapeutic agents such as 5-fluorouracil (5-FU, an antimetabolite), irinotecan (a plant alkaloid), mitomycin C (an antibiotics), docetaxel (a taxane agent belonging to a plant alkaloid), cisplatin (an alkylating agent), and others at the concentration range of 0.1–300 μg/mL (see Table 2). One ginsenoside, Rg3, has been found to inhibit growth of various human tumor cells, such as prostate cancer cells (LNCaP, PC-3, and DU145), Lewis lung cancer cells, colon cancer cells (SW620 and HCT116), and B16 melanoma cells. Ginseng has been also found to enhance the cytotoxicity of docetaxel, cisplatin, and doxorubicin at low doses [21, 22]. Inhibition of nuclear factor-kappa (NF-κB) may be one of the potential mechanisms of the observed effect. NF-κB mediates tumor promotion, angiogenesis, metastasis, and resistance to chemotherapeutics through the expression of genes participating in malignant transformation and tumor promotion. Kim and coworkers have found that Rg3 can suppress the expression of several antiapoptosis genes (Bcl-2, Cox-2, c-Fos, c-Jun, cyclin D1, etc.) via inhibiting NF-κB and thus enhancing the susceptibility of colon cancer cells to docetaxel and other chemotherapeutics [21, 22].

Also, panaxadiol, a pseudoaglycone of diol-type ginsenoside, has been found to enhance the anticancer effects of some anticancer drugs through the regulation of cell cycle transition and the induction of apoptotic cells. Apoptosis, highly regulatory process of programmed death involving the caspase protease family, is considered to be a key factor. Apoptosis may play an important role in the panaxadiol enhanced antiproliferative effects of irinotecan on human colorectal cancer cells as well as when used in combination with 5-FU [18, 20].

Furthermore, the synergistic inhibitory effect of *Panax* ginseng when combined with 5-FU has been observed in human gastric cancer cell line BGC823 [16]. This appears to involve NO which has been found to directly suppress the growth of BGC823 cells by inducing G0/G1 phase arrest through the regulation of Akt signaling pathway. Ginsenosides may increase NO production by inducing endothelial nitric oxide synthase (eNOS) phosphorylation via the ER-mediated PI3-kinase/Akt pathway [16].

One major difficulty in cancer chemotherapy is the development of broad anticancer drug resistance by tumor cells. This phenomenon has been termed multidrug resistance (MDR) [8]. The overexpression of P-glycoprotein (Pgp) or the multidrug resistance-associated proteins (MRPs) confer MDR to cancer cells. Ginseng extracts may induce chemosensitization of conventional anticancer agents via downregulation of MDR-1 expression (Pgp inhibition) [26, 28]. Choi et al. found that protopanaxatriol ginsenosides can potentially reverse Pgp-mediated MDR by increasing the intracellular accumulation of drugs through competitive inhibition of Pgp [26]. In addition, Shengmai can enhance the sensitivity of cancer cells (human lung carcinoma A549, gastric carcinoma SGC-7901, breast carcinoma MCF-7, and hepatocellular carcinoma HepG-2) to various anticancer drug such as gemcitabine (an antimetabolite), cisplatin, paclitaxel (a taxane agent belonging to a plant alkaloid), and epirubicin (an antibiotics) via downregulation of the mRNA level of MDR-1 [28].

Another benefit of ginseng when used with the anticancer drug is a potential reduction in drug induced toxicity. Baek et al. have found ginsenosides Rh4 and Rk3, the active principles of Sun ginseng (SG), to significantly reduce the cisplatin-induced nephrotoxicity in LLC-PK1 cells in a dose-dependent manner. The mechanisms of function and structure-activity relationships with other ginsenosides remain to be investigated [24]. Ginsenoside Rd may also ameliorate cisplatin-induced renal injury, a process in which apoptosis may play a central role [25].

A critical concern in the above *in vitro* studies is the relatively high concentration of ginseng extract/active components used (up to 300 μg/mL). Such a high concentration is likely not achievable *in vivo*, as the C_{max} of some ginsenosides following oral administration in rats has shown to be less than 0.7 μg/mL [59, 60]. Verification of *in vitro* benefit from lower concentrations of ginseng or from *in vivo* studies will be essential.

Table 1: Anticancer activities of commonly studied relevant ginsenosides [5, 8, 11–14].

Ginsenoside	Anticancer activity	Molecular mechanism
Rb1	Weakly antiproliferative; antiangiogenic	(i) Inhibit capillary genesis (ii) Inhibit TNF-a release (iii) Protect against oxidative stress (iv) Inhibit tube-like structure formation of endothelial cells by regulating pigment epithelium-derived factor (PEDF) through estrogen receptor-β [13, 14]
Rb3	No antiproliferative activity	Inhibit TNF-α release
Rg1	Antiproliferative	(i) Inhibit oncogenes c-myc, c-fos (ii) Downregulate nucleophosmin.
Rg3	Antiproliferative, apoptotic, antiangiogenic, antimetastatic, anti-invasive, and cell cycle regulation, [8, 11, 12]	(i) Regulate mitochondrial cytochrome C, poly ADP ribose polymerase (PARP) and C9 (ii) Inhibit MMP-2 and 9 (iii) Inhibit adhesion of metastatic cells to basement membrane (iv) Inhibit MDR (most potent among all ginsenosides)
Rh1	Causes differentiation of teratocarcinoma cells, strongly apoptotic	(i) Bind to steroid receptor (ii) Inhibit TNF-α (iii) Inhibit phosphorylation of JAK1, STAT1, STAT3, and ERK

4.2. In Vivo Animal Studies. A number of positive benefits have been delineated with ginseng and anticancer drug combinations. They include increase of drug exposure, inhibition of the angiogenesis and metastasis, survival benefit, reduction of side effects of anticancer drugs, and therapeutic improvement (see Table 3).

A pharmacokinetic interaction leading to enhancement of certain anticancer drugs has been reported. After pretreatment with 3.0 mg/kg *Panax* ginseng extract orally twice daily for ten consecutive days, the elimination half-life of 5-FU has been shown to significantly increase by approximately 58.8% (79.17 *versus* 125.72). The increase in $t_{1/2}$ caused by *Panax* ginseng extract can result in a higher drug exposure of 5-FU, which may lead to a longer drug effect [16]. The specific mechanism however is not known. Ginseng may increase the exposure of other drugs including docetaxel. *In vitro* studies using human liver microsomes have suggested that ginseng as well as its various ginsenosides, at clinically relevant concentrations, can moderately inhibit CYP1A1, CYP1A2, CYP1B1, CYP2D6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 [61].

Angiogenesis, the process of pathological vascular ingrowth critical for tumor expansion, is now known to play an important role in both growth and metastasis of some cancers. Ginsenoside Rg3 has been found to inhibit tumor angiogenesis. Combined therapy with Rg3 and low-dose gemcitabine or cyclophosphamide has been found to produce significant antiangiogenic effect without overt toxicity. The combined therapy has been shown to decrease vascular endothelial growth factor (VEGF) expression and microvascular density as well as blood flow in tumors (by color Doppler flow imaging) and peak systolic velocity when compared with the control mice. The combined therapy may have selectively enhanced the damage or cytotoxic effects of chemotherapy on newly formed blood vessels while simultaneously reduced Ki-67, VEGF, bcl-2, and P53 gene expression which may partially be responsible for their antiangiogenic and antitumor effects [11, 34, 36].

Survival benefit has been reported with the combination of ginseng or its active components with anticancer agents. In one study, combination treatment with paclitaxel (5 or 15 mg/kg) and acidic polysaccharide (25 mg/kg) has resulted in a 28.6 or 42.8% increase in 30-day life span of ICR mice bearing sarcoma 180 tumor cells, compared to paclitaxel treatment alone [27]. In another study, up to 53% of the BALB/c mice treated with combination of cyclophosphamide and an acidic polysaccharide (25 mg/kg) have shown an increase in survival rate compared with only 10% with cyclophosphamide alone [32]. At least 3 positive survival studies have been reported with ginsenoside Rg3 alone or in combination with anticancer drug: (1): treatment with Rg3, cyclophosphamide, or their combination in athymic mice bearing human ovarian cancer SKOV-3 has been found to improve survival 23.72, 25.90, and 27.12 days, respectively, compared to 13.6 days with the control [34]. (2): combination with gemcitabine has been found to increase survival rate (100%) compared with control or gemcitabine (60% or 70%) in 18 days after treatments [11]. (3): in 50% mice that survived cancer cell implantation, cyclophosphamide (low-dose), Rg3 alone, and their combination treatment groups result in 70, 77, and 95 days compared to only 29 days survival in the control group. The Rg3 anticaner drug combination treatment has shown to induce the longest survival [36]. The mechanism of such benefit however is not clear.

The combination of ginseng and various anticancer drugs have been found to lessen the reduction of weight loss, nausea/vomiting, diaphragm muscle toxicity, immunosuppression, and liver and renal function deterioration (see Table 3). Ginseng extract may decrease the side effect of weight loss from anticancer drugs by increasing the protein and RNA contents of muscles and liver in rats [11, 16, 31, 36, 62]. In addition, Ge and coworkers have found the effect of Shengmai (Chinese herbal preparation consisting of red ginseng, lilyturf root, and magnolia vine fruit) to be capable of protecting diaphragm muscles from doxorubicin induced toxicity which appears to be correlated with a decrease in

Table 2: *In vitro* studies of ginseng in combination with other anticancer drugs.

Ginseng products	Source	Cells	Conc.	Anticancer drugs	Direct action (cytotoxicity)	Indirect action	Reference
Panax ginseng extracts (RG and WG)	Tongrentang Pharmacy, Beijing, China; WG compared with RG: lower in Rg1, Rb1 and Rd; higher in Rg3.	HCT-116	100~300 μg/mL	5-FU	HCT-116 (+): RG > WG	(1) Apoptosis induction: RG (−) (2) Cell cycle arrest: RG (G1 phase)	[15]
Panax ginseng extracts	Tongrentang Pharmacy, Beijing, China, major ginsenosides Rg1, Rb1, Rd	BIU-87, A549, SW480, BGC823	0.1–100 μg/mL	5-FU	BGC823 (+) SW480 (−) A549 (−) BIU-873 (−)	None	[16]
Ginsenoside Panaxadiol	National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China	HCT-116, SW-480 HCT-116	10 μM 5~25 μM	Irinotecan 5-FU	HCT-116 (+) SW-480 (+) HCT-116 (+)	(1) Apoptosis induction (2) Cell cycle arrest: G1 phase [17]/S phase [14]	[18] [19]
Ginsenoside Panaxytriol	Red *Panax* ginseng, Nikkan Korai Ninjin, Kobe, Japan	MK-1	1~12.5 μg/mL	mitomycin C	MK-1 (+)	Increase cellular drug accumulation (by decreasing membrane fluidity)	[20]
Ginsenoside Rg3	Korea ginseng: Sun ginseng	SW620, HCT116	25~100 μM	Docetaxel cisplatin doxorubicin paclitaxel	SW620 (+) HCT116 (+)	(1) Apoptosis induction (2) Cell cycle arrest: G0/G1 phase	[21]
		LNCaP, PC-3, DU145		Docetaxel cisplatin doxorubicin	LNCaP (+) PC-3 (+) DU145 (+)	(3) Decrease drug resistant by inactivating NF-kappaB	[22]
Ginsenosides	Red *Panax* Ginseng	Leukemic progenitor cells	20 μg/mL	Homoharringtonine cytarabine, adriamycin, and etoposide	Leukemic progenitor cells (+)	Stimulate progenitor cell proliferation by driving noncycling progenitors to enter cell cycle	[23]
Ginsenosides, Rh4 and Rk3 (unique ginsenosides of SG/RG)	Korean ginseng: WG: Korea local SG and RG: Ginseng Science Inc. Seoul, Korea	LLC-PK$_1$	10~160 μg/mL, Rh4/Rk3: 5~20 μg/mL	Cisplatin	N/A	Reduce drug-induced renal injury: (i) increase cell viability: RG/Rh4/Rk3 (+), WG (−) (ii) decrease LDH leakage: Rh4/Rk3 (+)	[24]
Ginsenoside Rd	Self-prepared from Korean ginseng	LLC-PK$_1$	25~125 μM	cisplatin	N/A	Reduce drug-induced renal toxicity: (i) Decrease LDH leakage (ii) Suppress apoptosis	[25]
Ginsenosides, protopanaxatriol ginsenosides (major Rg1, Re), protopanaxadiol ginsenosides (major Rb1, Rb2, and Rc)	Korean red ginseng: Korea Ginseng and Tobacco Research Institute, Taejeon, Korea	AML-2/D100 (overexpress Pgp), AML-2/DX100 (overexpress MRP)	50~300 ug/mL	Daunorubicin	AML-2/D100: PTG (+), others (−)	Decrease drug resistant: inhibit Pgp activity (protopanaxatriol group)	[26]

Table 2: Continued.

Ginseng products	Source	Cells	Conc.	Anticancer drugs	Direct action (cytotoxicity)	Indirect action	Reference
Acidic polysaccharide	Korean red ginseng: Korea Ginseng Cooperation, Daejeon, Korea	BALB/C mouse splenocytes and macrophages	10–1000 μg/mL	paclitaxel	N/A	Reduce drug-induced toxicity (immunosuppression): (i) restore splenocyte proliferation; (ii) increase macrophage cytotoxicity	[27]
Shengmai (Chinese herbal preparation consisting red ginseng, lilyturf root, and magnolia vine fruit)	China, no detailed description	A549, SGC-7901, MCF-7, HepG-2	30 μg/mL	gemcitabine, cisplatin, paclitaxel, and epirubicin	A549 (+) SGC-7901 (+) MCF-7 (+) HepG-2 (+)	Decrease drug resistant: downregulating mRNA expression level of MDR-1.	[28]

Human bladder cancer cell line (BIU-87); human lung cancer cell line (A549); human colon cancer cell line (SW480); human gastric cancer cell line (BGC823, MK-1, and SGC-7901); human colorectal cancer cells (HCT-116, SW-480, and SW620); human prostate cancer cell lines (LNCaP, PC-3, DU145); human breast carcinoma (MCF-7), human hepatocellular carcinoma (HepG-2), pig renal tubular epithelial cells (LLC-PK_1), two resistant acute myelogenous leukemia (AML) sublines: daunorubicin- and doxorubicin-resistant AML-2 subline (AML-2/D100 and AML-2/DX100 overexpress Pgp and MRP, respectively); red ginseng (RG), white ginseng (WG); Sun ginseng (SG); p-glycoprotein (Pgp); multidrug resistance-associated protein (MRP); "+": positive; "−": negative.

Table 3: *In vivo* studies of ginseng in combination with other anticancer drugs.

Ginseng products	Source	Animals	Dose	Anticancer drug	Direct action (inhibit tumor growth)	Indirect action	Reference
Panax ginseng extracts	Tongrentang Pharmacy, Beijing, China, major Rg1, Rb1, Rd	Rat	3.0 mg/kg po., bid, 10 days	5-FU	N/A	(1) Increase drug elimination half-life: $t_{1/2}(k_e)$ of control and ginseng treated group 79.17 and 125.72 min, respectively ($P < 0.05$) (2) Reduce drug-induced weight loss;	[16]
	Korean ginseng, Ginseng Nonghyup, Keum-san, Korea	Rat	12.5~100 mg/kg, po.,	cisplatin	N/A	Reduce drug-induced nausea and vomiting	[29]
	Korean red ginseng, Korea Ginseng Cooperation, Daejeon, Korea	Ferret	3 g/kg, po.,				[30]
acidic polysaccharides	Korean red ginseng, Korea Ginseng Cooperation, Daejeon, Korea	ICR mice bearing sarcoma 180; C57BL/6 mice bearing B16 melanoma	25, and 100 mg/kg, ip., 7 days	paclitaxel	Yes	(1) Improve survival rate (ICR mice bearing sarcoma 180): 28.6 and 42.8% increase in 30-day life-span, while no obvious effect seen on drug-treatment alone. (2) Reduce drug-induced immunosuppression: increase NK cell cytotoxicity (C57BL/6 mice)	[27]
	Korean red ginseng, Korean Tobacco, and Ginseng company	BALB/c mice	33~300 mg/kg po., 3 wks	Cyclophosphamide (CP)	N/A	Reduce drug-induced: (1) weight loss; (2) immunosuppression: (i) increase spleen weight (ii) restore splenocyte proliferation (iii) increase macrophage activity (NO production) (iv) increase NK cell cytotoxicity (v) increase serum IL-12, IFN-γ and CRP (C-reactive protein) level	[31]
	Korean ginseng	BALB/c mice: C57BL/6 mice bearing mouse lung carcinoma LLC cells	100 mg/kg, i.p.	Cyclophosphamide (CP)	Yes	(1) Improve survival rate (BALB/c mice): 53% of post-treated group increased in the 30-day life-span compared with only 10% in the drug alone treated group. (2) Reduce drug-induced immunosuppression. (i) Accelerate recovery of bone marrow cells and blood neutrophils (ii) Stimulate splenocyte proliferation and maintain its cytotoxicity. (iii) Increase cytokine mRNA expression (TNF-α, IL-1β, IL-6, SCF and GM-CSF).	[32]

Table 3: Continued.

Ginseng products	Source	Animals	Dose	Anticancer drug	Direct action (inhibit tumor growth)	Indirect action	Reference
neutral polysaccharides	*Panax* ginseng, Changbai Mountain, Jilin, China	ICR mice bearing Sarcoma 180	25~150 mg/kg, po., 10 days	5-FU	Yes	Reduce drug-induced immunosuppression: (i) increase spleen weight, (ii) stimulate lymphocyte proliferation, (iii) increase NK cell cytotoxicity, (iv) enhance macrophage activity (phagocytosis and NO production), (v) increase serum TNF-a level	[33]
Ginsenoside Rg3	American ginseng; ≥99.5%, provided by Department of Medicinal Chemistry of Preclinical Medicine of Jilin University, China	Athymic mice bearing human ovarian cancer SKOV-3	3.0 mg/kg, ip., 10 days	Cyclophosphamide (CP)	Yes: (1) Decrease tumor weight (2) Inhibit tumor cell proliferation [19] (3) Increase tumor necrosis rate [20]	(1) Improve survival: (i) Rg3, CP, and combination group had longer survival (23.72, 25.90, and 27.12 days, respectively) compared with control group (13.61 days, $P < 0.05$) ($n = 7$) [19]; (ii) 18 days after treatments, combination group resulted in increased survival rate (100%) compared with control or gemcitabine group (60% or 70%, $P < 0.05$) ($n = 10$) [20] (iii) Control group, low-dose CP or Rg3 alone group, and combination group had 29, 70 or 77, and 95 days of 50% survival rate from implantation, respectively. ($n = 20$) [35] (2) Improve life quality (3) Reduce drug-induced: weight loss, leucopenia, limited motility, and skin discoloration. (4) Inhibit tumor angiogenesis: (i) decrease microvascular density (ii) decrease vascular endothelial growth factor expression	[34]
			20 mg/kg, po. 18 days	gemcitabine			[11]
	Panax red ginseng, from Northeast China, ≥99.5%, YaTai Pharmaceutical Company, China	C57/BL6 mice with Lewis lung carcinoma					
			10 mg/kg, po. 21 days	Cyclophosphamide (CP)			[36]
Ginsenoside Rg1	*Panax* ginseng, provided by Takeda Chemical Industries, Osaka.	BALB/c mice	10 mg/kg, i.p. 3 days	Cyclophosphamide	N/A	Reduce drug-induced immunosuppression	[37]

Table 3: Continued.

Ginseng products	Source	Animals	Dose	Anticancer drug	Direct action (inhibit tumor growth)	Indirect action	Reference
Ginsenoside Rd	*Panax* ginseng	Rat	1 and 5 mg/kg, po. 30 days	cisplatin		Reduce drug-induced renal toxicity	[23]
Shengmai (Chinese herbal preparation consisting red ginseng, lilyturf root and magnolia vine fruit)	Jilin Province Ji'an Yisheng Pharmaceutical Co. Ltd., China	Rat	3 mL/kg, i.p., pre and during treatment, 4 wks	doxorubicin (DOX)	N/A	Reduce drug-induced diaphragm muscle toxicity	[38]
	Shanghai Hutchison Pharmaceuticals	Mice bearing hepatoma	3.5~14 mL/kg/d, 14 days	5-FU	Yes	(1) Improve immunological function (2) Reduce drug induced adverse reaction: (i) protective effect on liver and renal function (ii) increase WBC and PLT counts	[39]

Table 4: Clinical studies of ginseng in combination with other anticancer drugs.

Ginseng products	Source	Study design	Anticancer drug	Cancer type	Endpoints and results	Reference
Shengmai (Chinese herbal preparation consisting of red ginseng, lilyturf root, and magnolia vine fruits)	Ya, an Sanjiu Pharmaceutical Co., Ltd, China	Randomized controlled open design, two groups: (1) control group ($n = 28$): anticancer drugs alone, (2) treatment group ($n = 33$): anticancer drugs + Shengmai 100 mL/day, 14 days + Gujin Granule (a Chinese herbal remedy) 10 g, t.i.d., po. 6 months	Navelbine + cisplatin	Non-small-cell lung cancer	(i) Response rate: 48.5% (16/33) in treatment versus 32.2% (9/28) in control groups, $P < 0.05$; (ii) median survival time: 13-month treatment group versus 9-month control group, $P < 0.05$; (iii) 1-year survival rate: NS (iv) median time to progression: NS (v) hematological toxicity: NS (vi) cycles of chemotherapy: NS	[40]
	China, no detailed description	Two groups: (1) routine group ($n = 56$): infuse 80 mL Shengmai alone after chemotherapy; (2) improved group ($n = 75$): infuse Shengmai 40 mL before and after chemotherapy, respectively	No detailed description	No detailed description	Chemotherapy induced phlebitis: $P < 0.01$ (incidence 20% in improved group, 50% in routine group)	[41]
		Two groups: (1) control group ($n = 26$): anticancer drugs alone, 21-day/cycle, 2 cycles; (2) test group ($n = 33$): anticancer drugs + infuse Shengmai 50 mL 14-day/cycle, 2 cycles	Etoposide + folinic acid + 5-FU	Advanced gastric cancer	(i) Life quality improvement: $P < 0.05$ (ii) Reduce drug induced: $P < 0.05$ (iii) Gastrointestinal effect (iv) Myelosuppression	[42]
	Sen-Ten Pharmaceutical Company, Taiwan	Randomized, double-blind, two groups: (1) test group, (2) placebo-control group, 4 weeks	No detailed description	No detailed description	Antifatigue activity (ongoing)	[43]
Ginsenoside Rg3	YaTai Pharmaceutical Company, China	Two groups: (1) control group ($n = 30$): anticancer drugs + Rg3 capsule; (2) test group ($n = 61$): anticancer drugs + Rg3 table	No detailed description	Breast cancer	Immunoimprovement: (i) increase level of T cell subtype function (CD4/CD8), $P < 0.001$ (ii) improve symptoms of Q-deficiency according TCM, $P < 0.01$ or $P < 0.05$	[44]
		Randomized, prospective, multicenter, two groups: (1) placebo group ($n = 61$) (2) test group ($n = 54$): Rg3 capsule, po, b.i.d.,	vinorelbine + cisplatin	Advanced non-small-cell lung cancer	(i) Improve response rate: $P < 0.05$ (ii) Improve survival time: 9.7 months (mean) and 8.0 months (median) in placebo group; 15.3 months (mean) and 10.0 month (median) in test group; $P < 0.01$ (iii) Weight, general conditions, and adverb reactions: $P > 0.05$	[45]
		Randomized controlled trial, two groups: (1) control group ($n = 30$) (2) treatment group ($n = 30$): Rg3 capsule, po, 20 mg, b.i.d., 30 days	Gemcitabine + cisplatin	Advanced esophageal cancer	(i) Improve response rate: NS (ii) Improve quality of life: $P < 0.05$ (iii) Increase 1-year survival rate: $P < 0.05$ (iv) Reduce drug induced adverse reaction: nausea and vomiting, WBC and PLT counts; $P < 0.05$	[46]

expression of iNOS and lipid peroxidation [39]. Furthermore, Shengmai has been found to also protect liver and renal function and increase white blood cell, platelet counts, and serum alanine aminotransferase [38]. Also, ginseng is known to modulate the immune system and thus may improve chemotherapy by an "indirect effect." Shim et al. have shown Korean ginseng can increase the expression level of the cytokines, such as TNF-α, IL-1β, IL-6, SCF and, GM-CSF [32]. Polysaccharides may also improve drug-induced immunosuppression. They can significantly increase relative spleen weight (spleen weight/100 g of bodyweight, e.g., frm 2.89% cyclophosphamide alone to 3.42% combined use), stimulate lymphocyte proliferation, NK cell cytotoxicity, and macrophage activity, increase serum TNF-α, IL-12, IFN-γ, and CRP (serum C-reactive protein) levels, and so forth [27, 31, 33]. However whether the degree of elevation of these cytokines by ginseng can improve chemotherapy outcome needs further investigated.

4.3. Clinical Studies. Despite popular use of ginseng, only a limited number of clinical studies have been reported on ginseng—chemotherapeutic agent combination (see Table 4). On the basis of the traditional Chinese medicine (TCM) consideration, the Chinese ginseng preparation Shengmai is selected as a tonic in combination with chemotherapy, for example, supplementing qi (means vital energy) and nourishing yin (means passive force) [40]. Chen et al. have evaluated the efficacy and side effects of Shengmai combined with chemotherapeutic agents in treating advanced non-small-cell lung cancer (NSCLC) [40]. This study was conducted on 63 patients with stages III B and IV NSCLC receiving navelbine (trade name of Vinorelbine, a plant alkaloid) and cisplatin chemotherapy. The patients were assigned to two groups: 33 patients in the treatment group receiving Shengmai by intravenous drip and Gujin Granule (a Chinese herbal remedy in water soluble granule) orally and 30 patients in the control group. Among the 61 patients (33 from the treatment group and 28 from the control group) who completed the observation, the response rate was 48.5% (16/33) in the treatment and 32.2% (9/28) in the control groups, with a median survival time of 13 months and 9 months, respectively. This study indicated that the combined use of ginseng and anticancer drug might enhance the short-term therapeutic efficacy of NSCLC. This study however was not blinded. A randomized, double-blind, placebo-controlled trial evaluating the therapeutic efficacy of Shenmai (same as Shengmai here) in cancer patients undergoing chemotherapy or radiotherapy is ongoing [43].

In another randomized controlled trial, Huang et al. evaluated the efficacy of Shenyi (95% ginsenoside Rg3) in combination with gemcitabine plus cisplatin in 60 patients with advanced esophageal cancer. Compared to patients in the control group with chemotherapy alone, the results showed no significant difference in total response rate between the two groups during the treatment phase. After treatment, the vascular endothelial growth factor in the treatment group was found to be lower than that in the control group ($P < 0.05$), suggesting an effect of inhibiting angiogenesis. In addition, one-year survival rate in the treatment group was higher compared with the control group ($P < 0.05$). Shenyi also improved the patients' quality of life according to the Karnofsky performance status scale [46]. Rg3 had been shown to have some anticancer activities like antiproliferative, apoptotic, antiangiogenic, antimetastatic, and anti-invasive effects as well as cell cycle regulation [8, 11, 12].

5. Summary and Conclusion

Ginseng has been used primarily as a tonic to benefit cancer patients, especially in Asia. Based on our review of published *in vitro*, *in vivo,* and human studies, ginseng has excellent potential as a chemotherapy adjuvant, because of its low toxicity and many desirable properties such as antiangiogenesis, antiproliferation, anti-inflammation, antioxidation, apoptosis, and immune modulation effects [63]. Although there are substantial evidence from *in vitro* and animal studies showing the benefit of ginseng and its active constituents in enhancing antitumor activity when used in combination with other anticancer drugs, there is insufficient clinical evidence of such benefit at present.

Ginseng has already been accepted as a natural product for health promotion. For this reason, continued use of ginseng together with encouraging results from the *in vitro* and *in vivo* animal studies (see Tables 2 and 3) may provide important clues to demonstrate future clinic benefit of ginseng. Further studies of ginseng products should include quality control such as the use of activity markers and active components, as well as determination of their pharmacokinetics and pharmacodynamics. It is hoped that government support as well as development of new process patents for ginseng will provide sufficient incentive and funding to conduct well designed clinical trials leading to regulatory approval of a ginseng product for chemotherapy enhancement in the future.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Research Article

The Overlap of Dietary Supplement and Pharmaceutical Use in the MIDUS National Study

David S. Kiefer,[1] Joe C. Chase,[1] Gayle D. Love,[2] and Bruce P. Barrett[1]

[1] *Department of Family Medicine, University of Wisconsin, 1100 Delaplaine Court, Madison, WI 53715, USA*
[2] *Institute on Aging, University of Wisconsin, 1100 Delaplaine Court, Madison, WI 53715, USA*

Correspondence should be addressed to David S. Kiefer; david.kiefer@fammed.wisc.edu

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Introduction. In the United States, dietary supplement (DS) use is common, often takes place outside of the purview of health care providers, and may involve DS in combination with pharmaceuticals. This situation has led to concerns about interactions between DS and pharmaceuticals, as well as the risks from polypharmacy and polysupplement use. *Methods*. We used data from the Midlife in the US study (MIDUS 2 Survey) to examine DS and prescription pharmaceutical use in 3876 study participants in order to determine the demographics of high-users (5 or more) of DS and pharmaceuticals and the presence of DS-pharmaceutical co-use. *Results*. Over 69% of study participants regularly used DS, 49.6% regularly used both DS and pharmaceuticals, and 6.3% and 8.7% were high-users of pharmaceuticals and DS, respectively. High-users of DS, pharmaceuticals, and either were more likely than the whole cohort to be female and of lower income. *Conclusions*. These findings corroborate those of other national studies with respect to the demographics of DS users but add new information about people at risk of DS-pharmaceutical interactions, not an insignificant proportion of the population examined by this dataset.

1. Introduction

In the United States (USA), the use of complementary and alternative medicine (CAM) is common [1]. One component of CAM is a category referred to as dietary supplements (DS), which includes herbal medicines, vitamins, minerals, and other substances such as amino acids and enzymes [2].

Nationwide surveys, including Midlife in the United States (MIDUS), have begun the process of delineating the demographics of DS users, the prevalence of DS use, and other related factors such as disclosure to health care providers (HCP) and sources of DS information (Table 1). The results of such surveys show that the use of DS is not insignificant, with estimates of 20% of the US population regularly using DS [1, 3]. These rates may be even higher in some groups such as immigrant populations [4]. In recent years, the medical literature has also begun the process of assessing DS efficacy and safety, including issues surrounding the ingestion of numerous DS [5, 6], adverse dietary supplement-pharmaceutical interactions [3, 7–9], and specific DS-pharmaceutical combinations that warrant extra caution on the part of HCP [10].

With respect to the use of multiple DS, there is no generally accepted threshold at which extra risk is thought to occur, though there is some guidance about this topic in the polypharmacy literature. Polypharmacy is a situation of high risk for adverse interactions or drug effects, often defined as the simultaneous ingestion of five or more pharmaceuticals, though the most accurate determination of risk for a given individual would also take into effect other factors such as their medical history and the appropriateness of a pharmaceutical or pharmaceutical dose [11, 12]. The assessment of DS safety involves a careful examination of interactions between DS and pharmaceuticals to identify any adverse health outcomes associated with co-use [13, 14]. All of these concerns are heightened when health care providers are unaware of DS use, a common phenomenon in the USA, with data showing that nondisclosure rates approach 70% in some populations [7, 15–18].

From its inception, MIDUS, a longitudinal study of health and aging, has included a wide array of demographic and

Table 1: Demographics and DS use in four national surveys.

	National Health and Nutrition Examination Survey (NHANES)	National Health Interview Survey (NHIS)	American Association of Retired Persons (AARP)	Midlife in the United States (MIDUS 2)
Years	2007-2008	2002, 2007	2006	2004–2007
Number of participants	3364	30,427 (2002), 22,657 (2007)	1559	5895
Ages	20–69	18+	50+	35–86
DS included*	HM, M, V, O	HM, O	HM, O	HM, M, V, O
% using DS	47.7	17–19	42	69.7%
Reference	Kennedy et al., 2013 [22]	Wu et al., 2011 [23]; Barnes et al., 2009 [1]; Hanyu et al., 2002 [24]	AARP and the National Center for Complementary and Alternative Medicine, 2007 [25]	Dienberg Love et al., 2010 [21]; Radler and Ryff, 2010 [20]

*DS: dietary supplement; HM: herbal medicine, M: minerals, V: vitamins, O: other dietary supplements.

psychosocial measures as well as comprehensive assessments of health (physical and mental) and health behaviors including use of prescription pharmaceuticals (Rx) and over-the-counter medications (OTC) [19, 20]. In the first longitudinal follow-up (MIDUS 2, 2004–2006), assessments of medication use were expanded to include DS, thus creating a dataset containing detailed information about Rx, DS, and OTC use.

The aim of this analysis was to add to the DS literature by analyzing data from the MIDUS 2 survey in order to (1) improve knowledge surrounding the characteristics of users of DS, comparing these results to other large national surveys, and (2) explore the presence DS-Rx co-use and the demographics of people involved.

2. Participants and Methods

At baseline (MIDUS 1, 1995-1996) study participants (n = 7108) were noninstitutionalized, English-speaking adults in the continental USA, aged 25–74 years. As described elsewhere [20], the MIDUS 1 sample was comprised of 3 subsamples: the Main sample recruited using random-digit dialing methods (n = 4244), siblings of Main sample participants (n = 950), and a national sample of twins (n = 1914), all of whom were invited to complete telephone interviews and self-administered questionnaires. Nine to ten years after MIDUS 1 (in 2004-2005), these individuals were invited to participate in the MIDUS 2 survey, which included a phone interview and another self-administered questionnaire [20]. Medication use is assessed in the self-administered questionnaire, while the demographic data is obtained via the telephone interview. Only a subset of individuals (n = 4,006) who completed both the telephone survey and the self-administered questionnaire at MIDUS 2 were included in the current analysis; this subsample is not significantly different from the larger sample from which it is drawn [21]. Of the 4,006 study participants, only 3,876 completed study questions pertaining to DS and Rx use.

The demographic variables of interest are age in years, gender, educational level (less than high school or high school graduate (HS) or equivalent (GED), some college, and college graduate or more), and personal income, reported by the respondent as wages over the last year, an estimate of a person's financial resources and status.

The MIDUS 2 self-administered questionnaire included two sets of items assessing Rx and DS use: (1) "During the past 30 days have you taken prescription medicine for any of the following conditions?" Individuals were classified as pharmaceutical users if they said "yes" to at least one of these items; (2) "Please check below any of the following vitamin, mineral, or herbal supplements you take regularly—that is, at least a couple of times a week." The DS checklist included common herbal medicines, vitamins, and minerals and provided study participants the option to add DS not found on the list. Individuals were classified as DS users if they checked at least one item on this list. In addition, the total numbers of DS and Rx being used were tabulated.

Four categories of DS and Rx users were created: (1) neither DS nor Rx used; (2) Rx only in the past 30 days; (3) DS only used regularly; and (4) both DS and Rx used. Furthermore, three categories of "high-users" were created: (1) using five or more Rx in the past 30 days (regardless of DS use); (2) using five or more DS regularly (regardless of Rx use); and (3) using five or more of *either* DS *or* pharmaceuticals. This distinction is designed to capture study participants who were in a polypharmacy and/or polysupplement situation.

3. Data Analysis

Descriptive statistics of individual and paired variables were examined in tabular and graphic format. A chi-squared analysis for proportion was utilized to compare variables as detailed in Tables 2 and 3. Any z-test P value <0.05 was considered statistically significant; values meeting these criteria were labeled as such in the relevant tables. Logistic regression analysis was then used to evaluate the group differences between high- and low-users of DS and Rx. All analyses used SPSS (IBM SPSS Version 21, 2012). Missing data was deleted from the cases of interest for the DS and pharmaceutical variable analyses.

Table 2: Demographic profiles for study cohort as a whole and with respect to use or nonuse of dietary supplements (DS) and pharmaceuticals (Rx).

Demographic characteristic	Total sample (n = 3876)	Neither DS nor Rx (n = 474)	Any Rx (n = 2622)	Any DS (n = 2703)	Both DS and Rx (n = 1923)
Gender					
Female (%)	55.7	43.3[a]	59.4[ab]	59.4[ab]	62.3[abc]
Mean age (SD)	56.2 (12.4)	48.7 (10.2)[ab]	58.6 (12.3)[ab]	58.0 (12.2)[ab]	60.2 (11.9)[abcd]
Median income	$27,500–$29,999	$22,000–$22,499	$22,000–$22,499	$32,500–$34,999	$16,000–$17,999
Education (%)					
HS-GED or less	33.2	31.0	36.2[a]	31.1	34.0[d]
Some college	28.8	27.6	29.1	28.2	29.9
College or more	38.0	41.4	34.7[ab]	39.1	36.1[bd]

[a] $P < 0.05$ when compared to the total sample (column 2).
[b] $P < 0.05$ when compared to "neither using DS (regularly) nor pharmaceuticals (in the last 30 days)" (column 3).
[c] $P < 0.05$ when compared to "any Rx" (column 4).
[d] $P < 0.05$ when compared to "any DS" (column 5).

Table 3: Demographic profiles (in %) for "high-users" (≥5) of prescription pharmaceuticals (Rx), "high-users" (≥5) of dietary supplements (DS), and study participants using ≥5 either Rx or DS.

Demographic characteristics	Total sample (n = 3876)	"High-users" of Rx in the past 30 days (n = 241)	"High-users" of DS "regularly" (n = 333)	"High-users" of Rx or DS (n = 546)	"High-users" of Rx and DS (n = 28)
Gender					
Female	55.7	68.5[a]	65.2[a]	66.7[a]	35.7%[a,b]
Mean age (SD)	56.2 (12.4)	61.7 (11.3)[a]	59.2 (11.3)[ab]	60.0 (11.4)[a]	65.0 (10.2)[a]
Median income	$27,500–$29,000	$2,000–$3,999	$18,000–$19,999	$10,000–$11,999	$1,000–$1,999
Education					
HS-GED or less	59.7	52.7[a]	25.8[ab]	37.0	39.3[a]
Some college	22.0	31.1	29.7	29.9	39.3[a]
Bachelors+	18.2	16.2[a]	44.4[ab]	33.2[a]	21.4

[a] $P < 0.05$ when compared to the total sample (column 2).
[b] $P < 0.05$ when "high-users" of DS are compared to "high-users" of prescription pharmaceuticals.

4. Results

Demographic characteristics for the full sample, as well as categories of DS and pharmaceutical use, are summarized in Table 2. The sample is predominantly female (55.7%), aged 56.2 years on average, with a mean income of approximately $28,000 and having a high school education or less. In addition, among the 3,876 study participants, 2,703 (69.7%) were taking at least one DS recently, 2,622 (67.6%) were taking at least one pharmaceutical in the last 30 days, 1,923 (49.6%) were taking both, and 474 (12.2%) were taking neither (Figure 1).

When compared to the entire cohort (n = 3876), there were statistically significant differences in the demographics of study participants using neither, any Rx, any DS, or both DS and Rx (Table 2, columns 3–6). Notably, when compared to the whole cohort, people using neither were less likely to be female (55.7% versus 43.3%, resp.) and were younger (mean age 56.2 versus 48.7), with a higher income (about $28,000 annually versus about $38,000). In contrast, users of both DS and Rx were more likely to be women (62.3%) and were older (mean age 60.2), with a lower income ($16,000–$17,999). Those study participants who used any DS (column 5) were slightly older than the whole cohort (mean age 58.0 versus 56.2, resp.), more likely to be female (59.4% versus 55.7%, resp.), and had a slightly higher income ($32,500–$34,999 versus $27,500–$29,999), though their education was similar.

When neither category was used as the comparison, both DS users and Rx users were older (average age 48.7 versus 58.0 and 58.6, resp.), while the Rx users had more people in the high school or less education category (31.0% versus 36.2%, resp.).

The number of DS and Rx taken by study participants is shown in Figure 1. Study participants ingested between 0 and 15 DS and 0 and 12 Rx. As mentioned above, nearly half the sample (49.6%) were taking both DS and Rx; a cluster exists in the lower numbers of DS and Rx, though individuals populate even the higher number combinations (Figure 2). Examples of these higher number combinations are one person taking

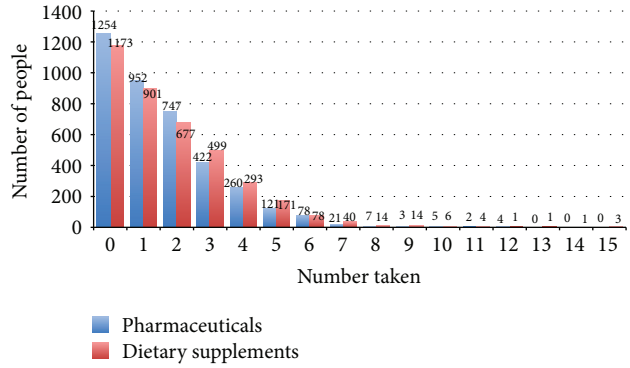

Figure 1: Number of people in MIDUS 2, Project 1, ingesting a given number of prescription pharmaceuticals (in the past 30 days) or dietary supplements ("regularly").

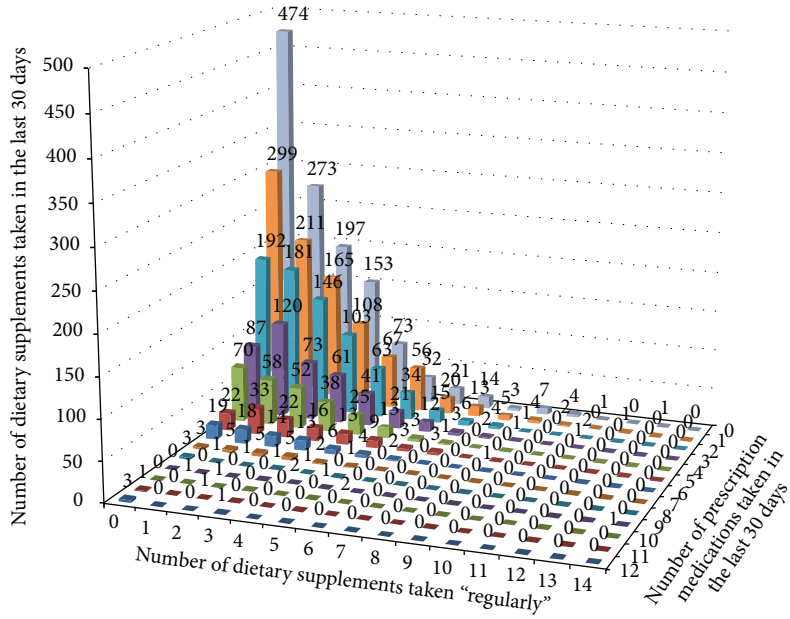

Figure 2: The MIDUS 2 Project 1 cohort (n = 3876): the number of people taking a given number of dietary supplements (0–15) and prescription medications (0–12).

nine Rx and 14 DS, one person taking five Rx and nine DS, one person taking three Rx and 11 DS, and one person taking two Rx and 11 DS (Figure 2).

The demographics of high-users (≥5) of DS (n = 333), Rx (n = 241), or either DS or Rx (n = 546) are presented in Table 3. When compared with the total sample (n = 3876), people in all three categories were more likely to be female, older (mean age 65 or greater), and with a lower median income. The low annual income result for the high-user Rx group was affected by respondents answering "zero" to wages over the last year. With respect to education, DS users tend to have more education and Rx users less education. In the high-user Rx group, more people had high school or less education, and less people had a bachelor's degree or more education, when compared to the high-user DS group. Of note, 28 individuals who were high-users of *both* DS and Rx were identified, so ≥5 DS and ≥5 Rx (Table 3, column 6).

Exploring the demographics of the high-users of either DS or Rx (Table 3, column 5), a logistic regression illustrated that female gender, lower income, and higher age make it more likely that a study participant is in the "high-use" category, whereas amount of education was less of a determinant.

5. Discussion

Large national datasets provide information that can answer questions of importance to health care delivery and decision-making. This is no exception with MIDUS 2 survey, which shows that people in this dataset use DS and pharmaceuticals simultaneously and in multiple quantities. The results presented here both corroborate past research and provide an expansion of the topic by exploring details behind DS and pharmaceutical co-use. For example, this analysis illustrates

that recent DS users (any quantity) are more likely to be older and women, in line with other prior national surveys such as the 2007-2008 update to the National Health and Nutrition Examination Survey (NHANES) and the National Health Interview Survey (NHIS) [1, 3, 22, 23]. In contrast, this analysis showed a similar amount of education between the DS cohort and the whole cohort; in other trials, DS users are often more likely to be more highly educated. Of note, the MIDUS study participants are considered highly educated at baseline [20, 21], perhaps affecting any additional education effect that might appear in subanalyses.

As presented in Table 3, the data on users of five or more DS and/or Rx ("high-users"), considered to be an important high-risk group, shows that high-users tend to be older and female. Of the high-user groups, high-users of DS and both DS and Rx had more education, whereas high-users of pharmaceuticals had less education overall. Picking apart the meaning and etiology of these trends, and finding clinical relevance, is a challenge. Clearly, women, in particular, women at a higher average age, are at risk of adverse dietary supplement-pharmaceutical interactions because they are users of both DS and pharmaceuticals in high numbers; this is a demographic worthy of a clinician's attention in this respect.

With respect to the income variable, there are several reasons why it is more difficult to draw clinically relevant conclusions. For example, more than for other variables, in MIDUS 2 there is missing income information, affecting the statistical significance of the results. Also, with increasing age, income is replaced by retirement funds, not necessarily captured by the survey questions which focus on reportable wages; this would artificially convey that someone has a lower income when they may, in fact, have significant regular retirement income. Future analyses to examine other variables in the MIDUS 2 survey dataset relevant to income, such as retirement income, will help to further examine this variable and allow a closer comparison to the NHANES results that showed a higher income in DS users.

There are several additional study limitations that could have affected the results presented and their generalizability. For example, the MIDUS 2 survey has a small percentage of people of nonwhite races and ethnicities, restricting its generalizability to the US population. Furthermore, our analysis did not include iron nor calcium supplements, even those that have been included in some, but not all, other national surveys. These variables were separate from the DS data, though still part of the MIDUS 2 dataset, and there is debate about whether or not such minerals should be considered DS. If anything, the inclusion of calcium and iron in our analysis would have further increased the DS use data for women and older individuals, given that such products are not uncommonly used in that population. Along the same lines, the questions in this survey included multivitamins as part of DS, similar to some, but not all, prior surveys (Table 1). In MIDUS 2, it is not possible to separate out multivitamin use from other DS; multivitamin users may in fact represent a different demographic from other DS users, though it is not possible to comment on this using these results.

It was beyond the scope of this analysis to include the use of over-the-counter medications, nor the specific pairings of DS with diagnoses and health parameters. Some of this data is contained in MIDUS 2, but an expansion of this information exists in MIDUS 2 Biomarker data; future analyses intend to explore these aspects of the DS-pharmaceutical overlap. The specific DS being used and overlaps with pharmaceuticals for individual study participants is an important next step in focusing efforts in a targeted way on decreasing the most concerning adverse dietary supplement-pharmaceutical interactions. In addition, numerous other variables could be involved with whether people use DS, RX, or both. One example is insurance coverage; NHIS found that DS use was higher in people with no insurance. Given that the analysis presented here found differences between the cohort as a whole and users ("any" and "high") of both DS and Rx, future analyses are intended to determine which other factors, such as insurance, are involved.

In summary, this analysis provides some insight into the demographics of DS users, pharmaceutical users, high-users or either, and those at risk of adverse DS-Rx due to co-use for the MIDUS 2 survey dataset. This large national survey shows that a not insignificant percentage of people are taking both DS and Rx and that there are important contributions to this group from gender status, age, education, and income. Merely at the beginning of the process of identifying who might be at risk for adverse dietary supplement-pharmaceutical interactions, this study illustrates a method that could be used in other large national surveys and datasets with DS and pharmaceutical data and serves as a reminder to clinicians to be aware of such co-use in some patients more than others, but ideally in all demographics. With that being said, the ideal way to prevent polypharmacy, polysupplement use, and adverse dietary supplement-pharmaceutical interactions would be to query every patient about DS use and have an informed discussion about risks and benefits in the context of their health cosmology, past medical history, and pharmaceutical use. In that way, each of the data points in Figure 2 would receive attention in the clinical setting.

Conflict of Interests

The authors declared no potential conflict of interests with respect to the authorship or publication of this paper.

Authors' Contribution

David S. Kiefer, Joe Chase, Bruce Barrett, and Gayle D. Love made substantial contributions to the conception and design of the study, data acquisition, analysis, and interpretation, as well as to the drafting and revision for substantial intellectual content. All authors gave final approval of the version to be published.

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Review Article

Beneficial Effects of Rikkunshito, a Japanese Kampo Medicine, on Gastrointestinal Dysfunction and Anorexia in Combination with Western Drug: A Systematic Review

Sachiko Mogami and Tomohisa Hattori

Tsumura Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-Machi, Inashiki-Gun, Ibaraki 300-1192, Japan

Correspondence should be addressed to Tomohisa Hattori; hattori_tomohisa@mail.tsumura.co.jp

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Background. Kampo medicines are traditional herbal medicines which have been approved for medicinal use by the Japanese Ministry of Health and Welfare and are currently being used more and more, often in combination with Western drugs. Thus, the need for investigation of interactions between Kampo medicines and Western drugs is now widely recognized. *Aim.* To summarize the effects and drug interactions of rikkunshito, a Kampo medicine often prescribed for upper gastrointestinal disorders and anorexia. *Methods.* Animal and human studies were systematically reviewed to identify published data on rikkunshito. Results describing its effects were abstracted, with an emphasis on drug interactions. *Results and Discussion.* Rikkunshito ameliorates anorexia induced by anticancer drugs, improves quality of life scores, and can even prolong survival compared with monotherapy. Rikkunshito combined with proton pump inhibitor therapy is shown to be useful in the treatment of PPI-refractory gastroesophageal reflux disease patients and patients with gastrointestinal symptoms after endoscopic submucosal dissection. Rikkunshito reduces antidepressant-induced adverse events and improves quality of life without influencing antidepressant effects. *Conclusions.* Rikkunshito shows ameliorative effects on adverse reactions induced by various Western drugs and can achieve better results (e.g., anticancer drugs and proton pump inhibitor) without influencing the efficacy and bioavailability of Western drugs.

1. Introduction

Herbal therapy has been used in Asia and other parts of the world for thousands of years and is currently manufactured in Japan as "Japanese Traditional (herbal) or Kampo medicines." These medicines are standardized with regard to quality and quantity of its ingredients and have been approved by the Japanese Ministry of Health and Welfare. At present, almost 90% of physicians in Japan use Kampo medicines in daily practice, sometimes as the first choice. Because Kampo medicines are now being used more and more, often in combination with Western drugs, the need for investigation of interactions between Kampo medicines and Western drugs is now widely recognized. An emerging therapeutic target for Kampo medicines in clinical practice is gastrointestinal functional disorders, in which conventional pharmacotherapy is either only partly effective or associated with adverse events. Rikkunshito, a Kampo medicine, is often prescribed for upper gastrointestinal disorders such as functional dyspepsia (FD), gastroesophageal reflux, and anorexia [1–3] and is prepared by compounding eight of the following crude drugs: *Atractylodis lanceae rhizoma*, *Ginseng radix*, *Pinellia tuber*, *Hoelen*, *Zizyphi fructus*, *Aurantii nobilis pericarpium*, *Glycyrrhizae radix*, and *Zingiberis rhizome*. The present paper reviews the physiology and clinical benefits of rikkunshito, with special focus on rikkunshito-Western drug combination therapies.

2. Methods

A literature search was performed on PubMed using the keywords "rikkunshito," "rikkunshi-to," "TJ-43" (product ID), and "Liu-Jun-Zi-Tang" (Chinese pronunciation). After

Table 1: Administration of rikkunshito in combination with Western drugs in human studies.

Reference	Western drugs	Patients	Outcome
[4]	Docetaxel/5-FU/cisplatin	Esophageal cancer	Improved QOL
[5]	S-1/cisplatin	Unresectable or relapsed gastric cancer	Improved anorexia
[6]	Gemcitabine	Stage III/IV pancreatic cancer with ascites	Prolonged median survival
[7]	Rabeprazole	PPI-refractory GERD	Improved GERD score
[8]	Lansoprazole	PPI-refractory LPR	Improved global sensation VAS scores
[9]	Rabeprazole	Gastrointestinal symptoms after ESD	Improved GSRS score
[10]	Fluvoxamine	Depressive disorder	Improved GSRS scores
[11]	Antidepressants or antipsychotics	Elderly patients with dementia	Increase in food intake
[12]	Anti-Parkinson drugs	Parkinson's disease	Ameliorated gastroparesis
[13]	Ofloxacin	Healthy volunteers	No effects on bioavailability
[14]	Levofloxacin	Healthy volunteers	No effects on bioavailability

ESD: endoscopic submucosal dissection; GERD: gastroesophageal reflux disease; LPR: laryngopharyngeal reflux; PPI: proton-pump inhibitor; QOL: quality of life; GSRS: Gastrointestinal Symptom Rating Scale; VAS: visual analog scale.

accumulating a combined list of studies, publications not examining the use of rikkunshito and those not written in English were excluded. Data abstraction was performed to characterize physiological and clinical effects and drug interactions of rikkunshito. Because a formal meta-analysis was not possible based on variations in study populations, disorders, and study protocols retrieved, data are presented descriptively with regard to the physiological and clinical effects of rikkunshito.

3. Results and Discussion

The initial search terms yielded 2 books, 14 reviews, and 66 original manuscripts related to rikkunshito, of which 30 were human studies and 36 were animal and *in vitro* studies. Of these 30 human studies, 10 concerned the use of Western drugs in combination with rikkunshito (Table 1); of the 36 animal and *in vitro* studies, 6 concerned the use of Western drugs in combination with rikkunshito (1 of these included a human study) and 1 investigated the effects of rikkunshito on human drug metabolic enzymes.

3.1. Clinical Applications of Rikkunshito. A double-blinded, randomized, placebo-controlled trial on rikkunshito was conducted by Tatsuta and Iishi in 42 patients with chronic idiopathic dyspepsia [15]. Gastric emptying was significantly accelerated and gastrointestinal symptoms were significantly reduced in patients treated with rikkunshito for 7 days, indicating that rikkunshito has a prokinetic action on gastric emptying and may be useful in treating FD. Arai et al. conducted a parallel, randomized controlled trial to investigate the effects of rikkunshito on upper gastrointestinal symptoms and ghrelin levels in 27 patients with FD treated with either rikkunshito or domperidone for 4 weeks [16]. Gastrointestinal Symptom Rating Scale (GSRS) questionnaire scores significantly improved in both groups but plasma ghrelin levels significantly increased only in the rikkunshito group and good correlation was found for improvements in reflux and indigestion symptoms. A study on the effects of rikkunshito on gastric accommodation reflex and duodenogastric motility was conducted in 16 patients with FD using extracorporeal ultrasonography before and after 14 days of treatment [17]. GSRS scores for abdominal pains, heartburn, and abdominal distension significantly decreased and the expansion rate of the proximal stomach was significantly greater after treatment. A study on the effects of 4-week treatment with rikkunshito in 19 patients with gastric cancer who had undergone proximal gastrectomy more than 6 months previously was conducted by Gunji et al. [18]. Patients' body weight significantly increased after the treatment course, and, in a subgroup analysis of patients showing a GSRS score of ≥2 before treatment, the mean total GSRS score significantly improved after treatment because of significant improvements in the subscale scores for abdominal pain, acid reflux, diarrhea, and constipation. Significant attenuation of post-gastrectomy gastrointestinal symptoms and increase in the ratio of acyl-/total ghrelin concentration by rikkunshito treatment for 4 weeks were also reported by Takiguchi et al. in 25 patients who had undergone distal gastrectomy or total gastrectomy [19]. Takahashi et al., in a crossover study, examined the clinical effects of rikkunshito in 11 patients who were to undergo pylorus-preserving gastrectomy for early gastric cancer [20]. Rikkunshito significantly improved emptying of solid meals from the remnant stomach according to (99 m)Tc-labeled solid scintigraphy, and stasis-related symptoms were significantly reduced during the treatment. Yagi et al. evaluated the effect of rikkunshito on symptoms and gastric myoelectric activity in 8 dyspeptic pediatric patients whose symptoms had persisted for over 1 year after gastrointestinal surgery. Following rikkunshito therapy, all patients exhibited symptomatic relief and a significant decrease in the mean symptom scores, along with increased gastric contractile activity according to electrogastrography [21]. Symptomatic relief and improved gastric emptying by rikkunshito administration were reported by Kawahara et al. in 9 handicapped patients using the ^{13}C-acetate breath test and the BreathID system [22]. Kawahara et al. also investigated the effects of rikkunshito on clinical symptoms and esophageal acid exposure in

8 children with symptomatic gastroesophageal reflux disease (GERD) before and after 7 days of therapy [23]. Treatment relieved symptoms and reduced the distal esophageal acid exposure through improvement in esophageal acid clearance. The largest and most comprehensive clinical study was conducted by Harasawa et al. in 1998 in 235 patients with dysmotility-like dyspepsia although it is reported in Japanese and not found in the PubMed search but outlined in English elsewhere [24–26].

3.2. Effects of Rikkunshito in Nonclinical Studies

3.2.1. Effects on Upper Gastrointestinal Function. The effects of rikkunshito on gastric adaptive relaxation have been reported in isolated guinea pig stomachs [27], conscious dogs [28], and isolated fundus smooth muscle from diabetic neuropathic rats with gastric dysmotility [29]. Rikkunshito not only increased gastric adaptive relaxation at the basal level but also ameliorated inhibited relaxation by the nitric oxide synthase inhibitor [27]. Improvement in gastric accommodation was also reported in virtual reality stress-imposed healthy humans [30]. Rikkunshito was reported to stimulate gastrointestinal contractions in the interdigestive state and gastric emptying in conscious dogs through cholinergic neurons and serotonin type-3 receptors [31]. The enhancement effect of rikkunshito was also reported in delayed gastric emptying induced by either nitric oxide synthase inhibitor (active components hesperidin (derived from *Aurantii nobilis pericarpium*) and L-arginine (derivation not determined)) [32] or serotonin [33]. Rikkunshito was reported to ameliorate the reduced voluntary movement in reflux esophagitis model rats and improve barrier function of the esophageal mucosa by restoring tight junction protein expression [34] and also reported to show great capacity to absorb bile salts [35]. Preventive effects of rikkunshito were also reported on gastric mucosal injury induced by either repeated electrical stimulation of the gastric artery or ethanol treatment via modulating platelet-activating factor production and oxidative granulocyte activation [36], in a nitric oxide-dependent manner [37] and by increasing surface mucin content [38].

3.2.2. Effects on Ghrelin. Ghrelin, a 28-residue octanoylated peptide, is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [39]. Ghrelin is known to play a role in both growth hormone release and stimulation of gastric motility and food intake [40–42]. Various studies are reported with regard to investigating the effects of rikkunshito on ghrelin secretion and signaling. Rikkunshito was reported to increase plasma ghrelin levels in humans and mice [43] and dogs [31] and was also reported to restore the decreased plasma ghrelin levels induced by serotonin release in rats. This rikkunshito effect is mediated by the serotonin 2B/2C receptor (5-HT_{2B}R/5-HT_{2C}R) antagonistic effect, the active components of rikkunshito including 3,3′,4′,5,6,7,8-heptamethoxyflavone and hesperidin (*Aurantii nobilis pericarpium*), and isoliquiritigenin (*Glycyrrhizae radix*) [44, 45]. Rikkunshito, hesperidin, and isoliquiritigenin ameliorated reduced hypothalamic ghrelin secretion and reduction in GHS-R signal transduction in the hypothalamus via 5-HT_{2C}R antagonism [46, 47]. The potentiating effects on ghrelin signaling *in vitro* were also demonstrated in GHS-R-expressing cells, showing significantly sustained increase in intracellular Ca^{2+} levels induced by ghrelin, mediated by the increased binding ability of ghrelin to its receptor following pretreatment by rikkunshito or its one of the active components, atractylodin (*Atractylodis lanceae rhizoma*). The inhibitory effects on activities of ghrelin metabolizing enzymes, which inactivate ghrelin to deacylated form, were investigated by Sadakane et al. [48]. Several components of rikkunshito, such as glycycoumarin (*Glycyrrhizae radix*) and pachymic acid (*Hoelen*), were reported to show inhibitory activity against human ghrelin-deacylating enzyme, butyrylcholinesterase. In surgically induced GERD model rats, the ameliorative effects of exogenous ghrelin on reduced antral motility were observed only after pretreatment with rikkunshito, indicating that this medicine had restored gastrointestinal motility by reversing impaired ghrelin signaling [49].

3.2.3. Effects on Other Hormones. Rikkunshito is reported to induce significant increases in plasma somatostatin and gastrin levels compared with a placebo group in healthy subjects but not in motilin, vasoactive intestinal peptide, calcitonin gene-related peptide, and substance P levels [50, 51]. It is also reported that rikkunshito suppresses increases in plasma adrenocorticotropic hormone levels, cortisol levels, and neuropeptide Y levels compared with the response to a placebo under stress conditions by repetitive blood sampling in health volunteers [52, 53].

3.2.4. Effects in Anorexia Models. Rikkunshito and its components nobiletin (*Aurantii nobilis pericarpium*), heptamethoxyflavone, and isoliquiritigenin reportedly ameliorated compromised ghrelin reactivity in the hypothalamus and regulation of its secretion in mice with aging-associated anorexia via inhibition of phosphodiesterase type 3 [54]. These effects are considered to be mediated by suppression of leptin signaling in the hypothalamus by increased levels of cyclic adenosine monophosphate. Rikkunshito ameliorated reduced feeding behavior in an urocortin-1-induced anorexia model, and its effect was abolished by GHS-R antagonist coadministration [55]. Rikkunshito also attenuated anorexia induced by novel environmental change, by increasing plasma ghrelin levels via antagonism to 5-$HT_{1B}/_{2C}$R [56] and 5-HT_{2B}R [57]. Effects of rikkunshito in a cancer anorexia-cachexia model were reported by Fujitsuka et al. [6], where amelioration in anorexia, gastrointestinal dysmotility, muscle wasting, and anxiety-related behavior as well as prolonged survival were recorded. These effects were mediated by rikkunshito's active components, hesperidin and atractylodin, which led to potentiated ghrelin secretion and receptor signaling, respectively. Ameliorative effects of rikkunshito on anorexia and body composition change is also reported in novel stomach cancer cachexia model by Terawaki et al. [58]. On the other hand, Tsubouchi et al. recently reported the protective effects of rikkunshito against acute lung injury by

protecting the alveolar epithelial cells and regulating lung inflammation independently of the ghrelin system [59].

3.3. Rikkunshito and Western Drug Interactions

3.3.1. Rikkunshito and Anticancer Drugs. Adverse reactions such as chemotherapy-induced nausea and vomiting often interfere with continuation of chemotherapy. The effects of rikkunshito on such adverse reactions were investigated in docetaxel/5-FU/cisplatin therapy, which is useful in the treatment of advanced esophageal cancer, as a prospective randomized study [4]. Nineteen patients who were to undergo docetaxel/5-FU/cisplatin therapy were randomly assigned to rikkunshito-treated and nontreated groups. Incidence of symptoms, nausea score, mood score, and activity of daily living score in quality of life (QOL) scoring were significantly lower in the rikkunshito-treated group than in the control group. The effects of rikkunshito on S-1/cisplatin chemotherapy-induced anorexia and ghrelin secretion were also investigated in a crossover design [5]. Ten unresectable or relapsed patients with gastric cancer were randomly divided between two groups. In the rikkunshito-on period, no cisplatin-induced decrease was observed for plasma ghrelin levels, average oral intake was significantly higher, and the grade of anorexia was significantly lower compared to those in the rikkunshito-off period. The effects of rikkunshito were also investigated as a retrospective study in 39 patients treated with gemcitabine who had pathologically proven stage III/IV pancreatic cancer with ascites. Median survival in patients treated with rikkunshito was significantly prolonged compared with that in patients treated with gemcitabine alone [6].

Combined administration of rikkunshito (1000 mg/kg) and cisplatin (2 or 4 mg/kg) was also reported in animal studies. In a cisplatin-induced anorexia model, rikkunshito was reported to ameliorate reduced food intake by reversing reduced plasma ghrelin levels, reduced hypothalamic ghrelin secretion, and decreased GHS-R signal transduction [44, 46–48]. It was also reported that combined administration of rikkunshito (500 mg/kg) and cisplatin (1 mg/kg) significantly prolonged survival in tumor-bearing rats compared with cisplatin monotherapy [6].

3.3.2. Rikkunshito and Proton-Pump Inhibitors (PPI). The relative efficacy of rikkunshito in combination with PPI, rabeprazole, and a double dose of rabeprazole was compared by Tominaga et al. in a prospective, multicenter, randomized, parallel comparative study in 104 PPI-refractory GERD patients [7]. After 4-week treatment with rabeprazole, patients were randomly assigned to either combination therapy (rikkunshito with a standard dose of rabeprazole) or a double dose of rabeprazole. Both treatment regimens significantly decreased the frequency scale for the symptoms of GERD in both groups. With regard to the therapeutic improvement rate, there were also significant effects in both groups. However, in the subgroup analysis based on reflux esophagitis/nonerosive GERD (NERD), the improvement rate for male patients with NERD in the rikkunshito group was significantly higher than that for male patients in the other group. These studies indicate that rikkunshito in combination with PPI therapy may be a useful new strategy for treatment of PPI-refractory patients. The effects of rikkunshito on laryngopharyngeal reflux (LPR) symptoms and gastric emptying in 22 patients with proton-pump inhibitor PPI-refractory LPR were investigated by Tokashiki et al. as a prospective, randomized, parallel comparative study [8]. Following 2 weeks of treatment with PPI monotherapy, patients were randomly divided between two treatment groups, rikkunshito monotherapy and rikkunshito plus lansoprazole. Following 4 weeks of treatment in both groups, the authors observed significantly decreased global sensation visual analog scale (VAS) scores, which showed significant positive correlation with improvement in gastric emptying. The VAS score for sore throat significantly decreased following treatment with rikkunshito plus PPI but not by rikkunshito alone. Effects of rikkunshito on gastrointestinal symptoms following endoscopic submucosal dissection (ESD) were evaluated in combination with rabeprazole by Uehara et al. in a prospective, randomized, parallel, comparative study [9]. Patients who were scored ≥3 more than the average GSRS score for abdominal pain or indigestion 6–8 days after ESD were randomized to either of the two groups (PPI monotreatment group, $n = 5$, or a PPI + rikkunshito group, $n = 8$). Overall GSRS score and abdominal pain score were significantly improved only in PPI plus rikkunshito group.

3.3.3. Rikkunshito and Antidepressant Agents. Upper gastrointestinal symptoms such as nausea and vomiting are common adverse events associated with the administration of selective serotonin reuptake inhibitors (SSRIs) and may result in discontinuation of drug therapy in patients with a depressive disorder. A study on the effects of rikkunshito on gastrointestinal symptoms and antidepressant effects was performed by Oka et al. [10] in a randomized, controlled study of 50 patients with depressive disorder treated by fluvoxamine. Patients were divided into two groups, fluvoxamine and fluvoxamine plus rikkunshito, with administration over 8 weeks. The numbers of patients complaining of adverse events or nausea in the combination group were lower than those in the fluvoxamine group. GSRS scores improved in the combination group but not in the fluvoxamine group. Self-Rating Depression Scale scores were not different between the two groups at all assessment points.

Functional gastrointestinal symptoms are frequently found in elderly patients with dementia and treated by the administration of antidepressants or second-generation antipsychotics, but with the risk of side effects. Although only in a preliminary study, the effects of rikkunshito on appetite loss in elderly patients with dementia were investigated by Utumi et al. [11]. Rikkunshito was administered for 4 weeks in six elderly patients with dementia suffering from appetite loss in combination with olanzapine + sulpiride, donepezil + paroxetine, trazodone, quetiapine, or donepezil + quetiapine or as monotherapy. In one patient, investigation was stopped because of the development of cholecystitis (administration

of rikkunshito was ruled out as being of relevance because of the presence of gallstones and history of cholecystitis). Significant increases in food intake were observed following administration of rikkunshito, with the side effect of mild lower limb edema occurring in two patients.

The ameliorative effects of rikkunshito (1000 mg/kg) in rats administered SSRI (fenfluramine 2 or 5 mg/kg) that had induced gastrointestinal dysmotility were also reported by Fujitsuka et al. [45]. Rikkunshito reversed the disruption of Phase III-like contractions and decreased food intake by restoring the reduced ghrelin secretion via 5-HT_{2C}R receptor antagonism. These studies suggest that rikkunshito reduces SSRI-induced adverse events and improves QOL related to gastrointestinal symptoms, without affecting the antidepressant effect of SSRI.

3.3.4. Rikkunshito and Anti-Parkinson Drugs. Effects of rikkunshito on gastroparesis in Parkinson's disease patients were reported by Doi et al. [12]. Twenty patients with mild gastrointestinal symptoms were enrolled; 14 of the 20 patients had constipation. Sixteen patients were taking levodopa/carbidopa, 2 were taking dopamine agonists, and the others were not treated yet. Twelve weeks after rikkunshito administration, 67% of patients reported improvement of their gastrointestinal symptoms, particularly appetite loss and bloating. Rikkunshito significantly shortened the gastric emptying time in these patients measured by the ^{13}C-sodium acetate expiration breath test, without any adverse effects, except for its bitter taste.

3.3.5. Rikkunshito and Bioavailability of Antimicrobial Agents. The effects of rikkunshito on the bioavailability of ofloxacin in seven healthy volunteers [13] and that of levofloxacin in eight healthy volunteers [14] were investigated in an open, random crossover study by Hasegawa et al. Subjects were administered a single oral dose of either ofloxacin or levofloxacin alone or by coadministration of rikkunshito at 1-week intervals. No significant differences in any estimated bioavailability parameters of ofloxacin or levofloxacin were observed between the two groups. Urinary recovery of ofloxacin and levofloxacin was not significantly different compared with that after coadministration of rikkunshito.

3.3.6. Rikkunshito and Human Metabolic Enzymes. The effects of rikkunshito on the activity of cytochrome P450 (CYP), a superfamily of drug-metabolizing enzymes, and P-glycoprotein (P-gp), a major drug transporter, were investigated by Ito et al. [60]. The inhibition rate of rikkunshito on human CYP3A4, 2C9, 2C19, 2D6, and 2E1 was less than 50% at concentrations below 0.1 mg/mL. Furthermore, rikkunshito did not affect ATPase activity using human P-gp membranes at concentrations lower than 0.1 mg/mL, in either the presence or absence of P-gp substrate. These findings indicate that rikkunshito is unlikely to cause clinically relevant drug interactions involving the inhibition of major CYP isozymes or P-gp.

4. Conclusion

Rikkunshito administration has shown its effects with regard to improvement in the symptoms of GERD and in functional and drug-associated dyspepsia through its effects on upper gastrointestinal functions and ghrelin secretion and signaling. Rikkunshito in combination with anticancer drugs also appeared to ameliorate anorexia, improve QOL, and even prolong survival compared with Western drug monotherapy. Rikkunshito in combination with PPI therapy also showed beneficial effects in PPI-refractory GERD patients and patients with gastrointestinal symptoms after ESD compared with monotherapy. Rikkunshito reduced antidepressant-induced adverse events and improved QOL without affecting antidepressant effects. Rikkunshito showed no significant effect on the bioavailability and renal excretion of antimicrobial agents.

However, because the studies described above were all performed in Japan on account of the nonavailability of standardized rikkunshito outside Japan, the basic and clinical effects of rikkunshito in other countries may not be consistent with these data. In addition, to confirm the safety and efficacy of rikkunshito, multiple, randomized placebo-controlled trials (preferably international) using common endpoints are required. On account of the increasing use of Kampo medicines, accurate data on interactions between these and Western drugs are required, not only for patients but also by healthcare providers.

In conclusion, at present, rikkunshito is considered to have no influence on the efficacy and bioavailability of Western drugs. More importantly, it has shown ameliorative effects on adverse reactions induced by various Western drugs and sometimes yields better results in combination with, for example, anticancer drugs and PPIs than Western drug monotherapy.

Abbreviations

CYP: Cytochrome P450
ESD: Endoscopic submucosal dissection
FD: Functional dyspepsia
GERD: Gastroesophageal reflux disease
GHS-R: Growth hormone secretagogue receptor
GSRS: Gastrointestinal Symptom Rating Scale
LPR: Laryngopharyngeal reflux
P-gp: P-glycoprotein
PPI: Proton-pump inhibitor
QOL: Quality of life
5-HTR: Serotonin receptor
SSRIs: Selective serotonin reuptake inhibitors
VAS: Visual analog scale.

Conflict of Interests

Sachiko Mogami and Tomohisa Hattori are employed by Tsumura & Co.

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Review Article

Updates on the Clinical Evidenced Herb-Warfarin Interactions

Beikang Ge, Zhen Zhang, and Zhong Zuo

School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Correspondence should be addressed to Zhong Zuo; joanzuo@cuhk.edu.hk

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Increasing and inadvertent use of herbs makes herb-drug interactions a focus of research. Concomitant use of warfarin, a highly efficacious oral anticoagulant, and herbs causes major safety concerns due to the narrow therapeutic window of warfarin. This paper presents an update overview of clinical findings regarding herb-warfarin interaction, highlighting clinical outcomes, severity of documented interactions, and quality of clinical evidence. Among thirty-eight herbs, Cannabis, Chamomile, Cranberry, Garlic, Ginkgo, Grapefruit, Lycium, Red clover, and St. John's wort were evaluated to have major severity interaction with warfarin. Herbs were also classified on account of the likelihood of their supporting evidences for interaction. Four herbs were considered as *highly probable* to interact with warfarin (level I), three were estimated as *probable* (level II), and ten and twenty-one were *possible* (level III) and *doubtful* (level IV), respectively. The general mechanism of herb-warfarin interaction almost remains unknown, yet several pharmacokinetic and pharmacodynamic factors were estimated to influence the effectiveness of warfarin. Based on limited literature and information reported, we identified corresponding mechanisms of interactions for a small amount of "interacting herbs." In summary, herb-warfarin interaction, especially the clinical effects of herbs on warfarin therapy should be further investigated through multicenter studies with larger sample sizes.

1. Introduction

Warfarin has been the most commonly used oral anticoagulants ever since its approval in 1954 [1]. Clinically, warfarin is administered as a racemic mixture of the *S*- and *R*-enantiomers. *S*-warfarin is 3–5 times more potent than *R*-enantiomer in anticoagulation effects. Warfarin is highly effective in preventing and treating deep venous thrombosis and can meliorate symptoms in patients suffering from arterial fibrillation, prosthetic heart valves, indwelling central venous catheters, and myocardial infarction [2]. The potency shown in clinical use boosts the studies on the mechanisms of warfarin anticoagulation. The anticoagulation effects are currently believed to be due to warfarin interrupting the vitamin K cycle in liver: in coagulation cascade, activated clotting factors are indispensable for the formation of blood clot. Most of these clotting factors are vitamin K dependent proteins, which suggest that reduced vitamin K is essential for activating clotting factors. Since vitamin K epoxide reductase (VKOR) is responsible for the synthesis of reduced vitamin K, warfarin, by targeting at and inhibiting VKOR, can block the activation of clotting factors and decrease the blood clot [3].

Use of warfarin is still limited despite the strong evidence for its clinical value. This may be accounted by the narrow therapeutic index, warfarin's predisposition to drug and food interaction, and its propensity to cause hemorrhage. Despite the fact that concomitant drug therapy may further increase the risk, complementary and alternative medicines including herbal medicines were widely used in the past decade [4]. Nearly 40% of patients with cardiovascular disease or stroke used concomitant herbs along with their prescribed medications [5]. Herbal medicines and food interaction are now cited as the main cause of adverse events with warfarin. A literature survey over the herb-drug interactions in clinical cases showed that warfarin accounted for 34 of the total 133 cases of interactions, making itself the most frequently involved drug in herb-drug interactions [6]. The incidence of interaction between herbs and warfarin is not yet fully known, and there is no body of reliable information currently available to draw upon when assessing the scale of any

possible problem or predicting clinical outcomes. The lack of evidence may be due to under-reporting or unrecognized interactions. In absence of good evidence, speculation has taken its place, and poor quality of available information in turn restricts future study of herb-warfarin interaction.

Herb-warfarin interaction has considerable clinical significance, so it is especially necessary to identify the herbs that interact with warfarin. Unlike what the public usually expect, herbal medicines are not always safe even if they are natural. Adverse events of herbs happen often and are reviewed recently, especially the adverse effects caused by herb-drug interactions [7–9]. Botanical extracts differ greatly from conventional medicines in that the former contain a mixture of many bioactive compounds. The diverse ingredients may result in the prevalence of herb-drug interaction. Knowing how herbs can be used safely and how to reduce the possible risk is the prerequisite for maximizing the benefits derived from herb medicine.

Reports about herb-drug interactions are far from enough, and if exist, they often miss some important items. Several literature surveys showed that interactions of clinical importance were indeed certified by case reports [6, 9–11], but mechanistic study in this field is still limited. A research group assessed the quality of data generated for the study of herb-drug interactions, suggesting that 67% cases were classified as possible interactions, 27% cases were invalid to be evaluated, and only 6% of the cases were well documented [6]. These case reports are insufficient to establish a causal relationship between herb-drug interaction and adverse effects. Patel's group reviewed the published clinical literature from the year 1971 to year 2007, including reported adverse events, descriptions of the clinical case, and case series, to assess the interactions between various herbs and warfarin. Out of 72 documented case reports of herb-warfarin interactions, 84.7% cases were evaluated as possible interactions (61/72) and 15.3% cases (11/72) as probable interactions. Cranberry juice was involved in 34.7% of (25/72) the case reports, as the most commonly involved herb [12]. In addition, western herbs interactions with warfarin are better known, while traditional Chinese medicines (TCM) are rarely studied. According to a literature survey, among 133 cases of herb-drug interactions, St. John's wort was the most common herb studied (37 cases), followed by *ginkgo* and *ginseng* [6]. There were only limited studies on Danshen *(Salvia miltiorrhiza)*, Gegen *(Pueraria lobata)* and several other TCMs. On basis of what have been done on herb-warfarin interactions, the current review aims at complementing the missing points from previous studies as summarized below:

(i) classification of clinical significance: life threaten, bleeding, INR change, and so forth;

(ii) assessment of evidence reliabilities: highly probable, probable, possible, and doubtful;

(iii) classification of evidence level: *in vitro*, animal, or human;

(iv) summary and classifications of mechanisms for herb-warfarin interactions: pharmacokinetics or pharmacodynamics;

(v) information related to interactions between TCM and warfarin.

2. Methods

In the current review, primary articles released from 1993 and 2013 were searched in both English and Chinese databases including EMBASE, MEDLINE, AMED, Cochrane Systematic Review Database, SciFinder, and CNKI. Search terms were "herb," "herbal," "traditional Chinese medicine," "complementary and alternative medicines," "warfarin," "interaction," "clinical," and "human study," Based on the data collected from the search, this paper summarized both traditional Chinese herbs and western herbs involved in clinical herb-warfarin interaction.

2.1. Effects of Interaction. A clinical study refers to research using human volunteers (also called participants) who agree to be involved to add to medical knowledge [13]. Outcomes of clinical studies are mainly reported in the form of case reports, case series, clinical trials, and clinical assessments. An overview of literature was conducted in the current study to consolidate clinical evidences on herb-warfarin interactions. Based on these documented clinical evidences, clinical effects of herb-warfarin interactions were classified into either potentiation or inhibition.

2.2. Severity of Interaction. Interactions that potentiated or inhibited the effect of warfarin were further rated as major, moderate, minor, or nonclinical (Table 1) according to the ranking scheme developed by Holbrook's group in 2005 [14]. Major potentiation was defined by death, major bleeding, or necessity to stop warfarin therapy entirely. Major bleeding episodes included those that were life-threatening as well as those that led to the loss of at least 2 units of blood in 7 days or less [15]. Moderate potentiation meant that there was an INR change requiring an adjustment in warfarin dosage. In a moderate potentiation, the INR increased to greater than 5.0 or there was an increase in INR by greater than 1.5. Minor potentiation interactions were defined as an INR increase in which no change in warfarin dosage was required. INR increased to less than 5 and the increase of INR was less than 1.5 units in a minor potentiation. Potentiation interactions were classified as nonclinical if the only evidence of warfarin augmentation was a statistically significant increase in warfarin levels without change in INR or clinical status.

Major inhibition interactions were defined by the occurrence of thrombosis. Moderate inhibition (clinically relevant but less than major) indicated a change in INR requiring an adjustment in warfarin dosage. In a moderate inhibition, INR decreased to less than 1.5 or the decrease in INR was greater than 1.5 units. Minor inhibition interactions were defined by an INR decrease requiring no change in warfarin dosage INR decreased to more than 1.5 in a minor inhibition interaction, and the decrease in INR was less than 1.5 units. Inhibition interactions were classified as nonclinical if the only evidence of warfarin inhibition was a statistically significant decrease in warfarin levels. An interaction was defined as having no

Table 1: Scoring of clinical severity of herb-warfarin interaction [14].

Clinical severity	Potentiation	Inhibition
Major	Death, major bleeding, entailing entire cease of warfarin therapy	Occurrence of thrombosis
Moderate	INR increase, entailing adjustment in warfarin dosage INR increase to greater than 5.0 INR increase by greater than 1.5 units	INR decrease, entailing adjustment in warfarin dosage; INR decrease to less than 1.5 INR decrease by greater than 1.5 units
Minor	INR increase but requiring no change in warfarin dosage INR increase to no more than 5.0 INR increase by smaller than 1.5 units	INR decrease but requiring no change in warfarin dosage INR decrease to no more than 1.5 INR decrease by smaller than 1.5 units
Nonclinical	No change in INR	No change in INR

Table 2: Criteria for defining evidence reliabilities of an interaction [14, 16].

Reliabilities of evidence	Criteria required
I: highly probable	A, B, and C plus any one or more of D to G
II: probable	A, B plus one or more of C to G
III: possible	A plus one or more of B to G
IV: doubtful	Any combination of B to G or A alone
(A) Was the timing correct for an interaction to be pharmacologically plausible?	
(B) Did laboratory test (INR, prothrombin time, and thrombotest) support the contention of an interaction?	
(C) Were other potential factors affecting warfarin pharmacokinetic or pharmacodynamics ruled out?	
(D) Was there other objective evidence?	
(E) Was a dose-response relationship shown for the interacting herb?	
(F) Was the subject rechallenged and, if so, did a similar response occur?	
(G) Did the same thing happen on previous exposure to the herb?	

Notes:
A: in patient-based studies, warfarin must been taken at a stabilized dose before initiation of the interacting herbs. In addition, the potentially interacting herbs should be consumed long enough in usual doses to attain a substantial plasma level. For volunteer-based studies, subjects had to receive warfarin, both alone and with the interacting herbs, for similar periods.
B: in patient-based articles, the coagulation variable should be out of therapeutic range, whereas for volunteer studies, a change of at least 20% was required in coagulation parameters. For conclusion of "no interaction," the absence of a statistically significant change in coagulation variables was required.
C: factors such as diet, other medications, or certain medical conditions, especially liver diseases, should be declared to be ruled out as possible causes of the outcome.
D: other objective evidences refer to changes in plasma level of warfarin or level of vitamin K dependent clotting factors (II, VII, IX, or X).
E: the alterations in the dose of the coadministrated interacting herbs correlated with subsequent changes in warfarin coagulation variables, inferring a dose-response relationship.
F: the interacting herb should be administered simultaneously with warfarin in two or more separate courses, with similar results for each course.
G: similar outcome should be found for the patient cousing the interacting herb with warfarin at a time prior to that reported.

effect if the interacting drug neither potentiated nor inhibited warfarin's effect in any way described here.

2.3. Possibility of Interaction: Reliability of Evidences. Possibility of an interaction was assessed by previously validated criteria and study design that were developed in the first systematic overview on this topic in 1994 [14, 16]. The reliability of evidences was ranked, according to 7 causation criteria, into four levels, with level 1 being highly probable and level 4 being doubtful (Table 2). The lower levels imply that no adequate evidences were available to confirm that interactions will certainly happen.

2.4. Mechanism of Herb-Warfarin Interactions. Mechanisms of herb-warfarin interaction are divided into two categories: those involving pharmacokinetics (PK) of warfarin and those involving pharmacodynamics (PD). In addition, we attempted to identify the possible corresponding mechanisms of interactions for relevant herbs.

Pharmacokinetic interactions are those that can affect the process by which warfarin is absorbed, metabolized, and distributed as summarized below. Among these, cytochrome P450s for the metabolism of warfarin is main focus. Other enzymes like UDP-glucuronyltransferase (UGT) may also be involved in herb-warfarin interaction, but little information or literature is available to confirm their effects.

2.4.1. Interference with Warfarin Absorption. Passive diffusion is known as the primary means for warfarin to cross biological membranes. Until now, no transporters have been identified for warfarin absorption in gastrointestinal (GI) environment [17]. Some herbs including Aloe, Jalap, Cascara,

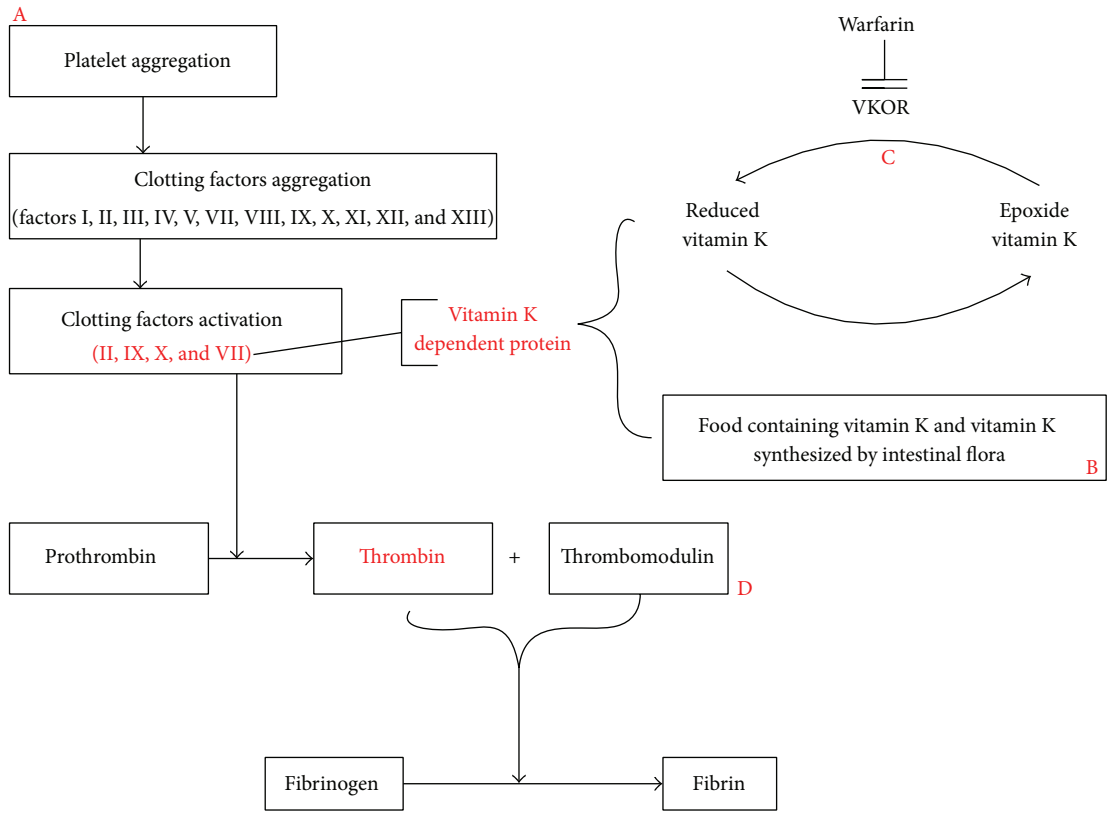

Figure 1: Schematic illustration of the potential pharmacodynamics mechanism for the interaction between warfarin and herbs.

and Rhubarb are found to be able to bind with warfarin to affect its absorption [18]. Some herbs may directly cause gastrointestinal membrane erosions leading to risk of hemorrhage after their coadministration with warfarin [19].

2.4.2. Interference with Metabolizing Enzymes of Warfarin. Warfarin is metabolized mainly by cytochrome P450s (CYPs), a very large family of related isoenzymes [20]. Of all those isoenzymes, CYP2C9 accounts for the greatest proportion for metabolism of *S*-warfarin, which is much more potent than *R*-enantiomer clinically. Herbs showing effects on cytochrome P450s, especially on CYP2C9, CYP1A2, CYP3A4, or CYP2C19, will affect the plasma concentration of warfarin, which may be one of the reasons for herb-warfarin interaction [21].

2.4.3. Interference with Protein Binding of Warfarin. Up to 99% of absorbed warfarin is bound to plasma protein, primarily albumin. Herbs competitively bound to albumin will affect the plasma concentration of warfarin, which may also be one of the reasons for herb-warfarin interaction [22]. This, however, is not regarded as the main cause of herb-warfarin interaction [23].

Most of above PK mechanisms for warfarin related interaction were direct interaction such as alteration on CYP enzyme activities and inhibition on protein binding of warfarin. However, there was indeed indirect interaction, for example, when gastrointestinal membrane was damaged.

Pharmacodynamic interactions are those where the effects of one drug are changed by the presence of another drug at its site of action. Sometimes the drugs directly compete for particular receptors, specifically VKOR, but often the reaction is more indirect and involves interference with physiological mechanisms. Therefore, pharmacodynamic interactions are more complicated to be classified neatly than pharmacokinetic interactions. Figure 1 illustrates the process of blood coagulation, pharmacodynamics effects of warfarin, and how herbal components would affect it. It has been shown that pharmacodynamics related mechanisms comprised 79.9% of all the identifiable herb-warfarin interaction mechanisms [5], which are summarized in following four aspects.

2.4.4. Interference with Platelet Function. Platelet aggregation is the first step of coagulation, and a cascade of further platelet activation initiates the formation of blood clot. Reduced platelet aggregation may inhibit thromboxane synthesis [24], thus interfering with clotting mechanisms, decreasing blood coagulation, and prolonging bleeding time [25]. Some antiplatelet herbs, for instance, Ginkgo (particularly ginkgolide B), have been shown to inhibit the binding of platelet activating factor to their receptors on platelet membranes, resulting in reduced platelet aggregation [26, 27]. It can be inferred that the concurrent administration of warfarin with ginkgo may present an additional risk of bleeding.

2.4.5. Altering Gut Vitamin K Synthesis or Containing Vitamin K. In vivo, vitamin K can be obtained from two main pathways: being taken from food and being synthesized by vitamin K cycle. Reduced vitamin K is essential for activating several key clotting factors, including II, IX, X, and VII, which are actually vitamin K dependent proteins [28]. Some herbs may stop intestinal flora from synthesizing vitamin K, such as *Thymus vulgaris* and *Allium sativum* [29], thereby enhancing the effect of warfarin. Several herbs such as green tea may contain large amount of vitamin K, which may also result in interaction with warfarin [30].

2.4.6. Interference with Vitamin K Cycle. Besides, synthetized by intestinal flora and taken from food, the primary way for reduced vitamin K synthesis is vitamin K cycle [31]. Some herbs, such as Lapachol [32], affect key enzymes in this circle such as vitamin K epoxide reductase (VKOR), regulating the amount of vitamin K *in vivo*, thereby interacting with warfarin. Warfarin is a synthetic derivative of dicoumarol. Dicoumarol, in turn, is derived from coumarin [33]. Although coumarin itself has no anticoagulant properties, it is transformed into the natural anticoagulant dicoumarol by a number of species of fungi [33]. Therefore, herbs containing coumarin or its derivatives may display similar anticoagulative effects as warfarin. Concurrent administration of warfarin with these herbs may present an additional risk of bleeding.

2.4.7. Interference with Coagulation Cascade. The process of clotting is complex and involves numerous different proteins termed clotting factors (factors I, II, III, IV, V, VII, VIII, IX, X, XI, XII, XIII, protein C, and thrombomodulin) [28, 31]. Some herbs, for instance, Danshen (*Salvia miltiorrhiza*), may affect the expression of thrombomodulin [34], changing blood coagulation *in vivo*, thereby interacting with warfarin.

3. Results

Thirty-eight herbs were listed in the current review, including herbs clinically evidenced to interact with warfarin; herbs preclinical evidenced to affect PK or PD of warfarin; herbs containing vitamin K or coumarin; and herbs with similar or opposite pharmacological actions to those of warfarin.

On basis of this overview, effects and severity of each proposed herb-warfarin interaction, as well as the possibility and potential mechanism for those interactions, were abstracted and compiled in Table 3.

3.1. Western Herbs

3.1.1. Boldo (Peumus boldus). Boldo was traditionally used for dyspepsia, digestive disturbances, constipation, and rheumatism. Recent research has shown boldine, one of the major active components from Boldo, to be a potent antioxidant [35]. Boldo also contained amount of natural coumarins. But it is unclear whether they have any anticoagulant activity [36]. No relevant pharmacokinetic data of Boldo have been found yet. One case report suggested that it might interact with warfarin. A woman taking warfarin together with 10 drops of Boldo and one capsule of Fenugreek showed a modest rise in her INR from 2 to 3.4. A week after stopping Boldo, her INR had fallen to 2.6 [36]. The mechanism of this interaction remains unknown. Interaction between Boldo and warfarin was defined as *doubtful*.

3.1.2. Cannabis (Cannabis sativa L). Cannabinoids are the major active compounds in Cannabis. Medicinal Cannabis is used to treat chronic conditions, including adjunct and neuropathic pain. There is no experimental evidence for interaction between warfarin and Cannabis [37]. However, a clinical case report described a raised INR and bleeding in a patient who smoked Cannabis (2.5 packs/day for 35 years) while taking warfarin [38]. *In vitro* study showed that a major constituent of Cannabis induced CYP2C9 [39]. This would be expected to increase the metabolism of warfarin effects, which is in contrast to the case report. Because of the existence of other factors, it is not reasonable to ascribe the INR change specifically to herb-drug interaction by a single case report. Interaction between Cannabis and warfarin was defined as *possible*.

3.1.3. Chamomile (Matricaria recutita). Chamomile is used for dyspepsia, flatulence, and nasal catarrh [40]. *In vitro* study found that the extract of Chamomileinhibited the cytochrome P450 isoenzyme CYP3A4 [41, 42]. However, the effects were weak when compared with the known potent CYP3A4 inhibitor ketoconazole [42]. A study using liver microsomes from rats pretreated with Chamomile for 4 weeks found that CYP1A2 activity was reduced to 39%, when compared with control group [43]. An isolated case of bleeding in a patient taking warfarin with Chamomile products (drinking 4 to 5 cups of Chamomile tea) daily for chest congestion, and using a chamomile-based skin lotion 4 to 5 times daily for foot oedema had been reported [44]. Because of many other factors influencing anticoagulant control, it is unreasonable to identify a drug interaction in a single case report without other supporting evidence. Interaction between Chamomile and warfarin was defined as *possible*.

3.1.4. Chitosan (Swertia chirayita). Chitosan is used as a dietary supplement for obesity and hypercholesterolemia [40]. Chitosan is an absorption enhancer and increased the permeability of hydrophilic drugs across intestinal and mucosal epithelia [45]. One case report suggested that Chitosan might increase the effects of warfarin. In this case, an 83-year-old man stabilized of warfarin treating showed an increased INR from 3.7 to 9, when taking Chitosan 1.2 g twice daily. He was advised to stop this supplement and was subsequently reestablished on warfarin. One month later, the patient restarted the chitosan, which again resulted in a raised INR [46]. Chitosan might impair the absorption of fat soluble vitamins, including vitamin K [46]. Warfarin was a vitamin K antagonist and a reduction in vitamin K would be expected to enhance its effects. Evidence was limited to this case and the mechanism was largely speculative; however, an interaction seemed *possible*.

Table 3: Summary of herb-warfarin interactions supported by clinical evidence.

Herbs (common and Latin name)	Clinical effects	Severity	Reliabilities of evidence	Mechanisms	
				PK	PD
Cranberry *(Vaccinium macrocarpon)*	Potentiation	Major [55]	I	F [52, 53, 160]	D [40]
Soya *(Glycine max* Merr.*)*	Inhibition	Moderate [115, 116]	I	F [113, 114]	B [115]
St John's wort *(Hypericum perforatum)*	Inhibition	Major [121, 122]	I	F [41, 118, 119]	NA
Danshen *(Salvia miltiorrhiza)*	Inhibition	Moderate [133, 134]	I	F, G [131, 132, 135, 136]	A,C [40]
Coenzyme Q10 *(Theobroma cacao)*	Inhibition	Minor [47, 48]	II	NA	B [50]
Chinese angelica *(Angelica sinensis)*	Potentiation	Moderate [126]	II	F [125]	C [40]
Ginger *(Zingiber officinale* Roscoe*)*	Potentiation	Moderate [161]	II	NA	A [162]
Chamomile *(Matricaria recutita)*	Potentiation	Major [44]	III	F [41, 163, 164]	NA
Chitosan *(Swertia chirayita)*	Potentiation	Moderate [46]	III	NA	B [45]
Cannabis *(Cannabis sativa* L*)*	Potentiation	Major [165]	III	F [166]	NA
Devil's claw *(Harpagophytum procumbens)*	Potentiation	Moderate [61]	III	F [60]	NA
Ginkgo *(Ginkgo biloba)*	Potentiation	Major [83]	III	F [75–77]	NA
Garlic *(Allium sativum)*	Potentiation	Major [40]	III	F [167, 168]	A [73, 169]
Ginseng *(Panax quinquefolius/Panax ginseng)*	Inhibition	Moderate [170]	III	F [40]	A [87]
Grapefruit *(Citrus paradise)*	Potentiation	Major [94]	III	F [95]	NA
Green tea *(Camellia sinensis)*	Inhibition	Moderate [97]	III	NA	B [99]
Lycium *(Lycium barbarum)*	Potentiation	Major [139]	III	F [137]	NA
Boldo *(Peumus boldus)*	Potentiation	Minor [36]	IV	NA	C [40]
Echinacea *(Echinacea purpurea)*	Inhibition	Minor [66]	IV	F [119, 171]	NA
Fenugreek *(Trigonella foenum-graecum)*	Potentiation	Minor [36]	IV	NA	B, C [40]
Melilot *(Melilotus officinalis)*	Potentiation	Moderate [102]	IV	NA	C [40]
Parsley *(Petroselinum crispum)*	Potentiation	Moderate [104]	IV	F [103]	B [40]
Pumpkin *(Cucurbita pepo)*	Potentiation	Minor [106]	IV	NA	B [40]
Red clover *(Trifolium pretense)*	Potentiation	Major [108]	IV	F [60, 107]	NA
Saw palmetto *(Serenoa repens)*	Potentiation	Minor [106, 111]	IV	F [109]	NA

Notes. (1) As to mechanisms of herb-warfarin interaction, PD factors including the following: A: interference with platelet function; B: altering gut vitamin K synthesis or containing vitamin K; C: interference with vitamin K cycle; D: interference with coagulation cascade. PK factors including the following: E: interference with warfarin absorption; F: interference with metabolizing enzymes of warfarin; G: interference with protein binding of warfarin. (2) Other nonclinical evidenced herbs defined as doubtful in Section 3 were excluded in this table.

3.1.5. Coenzyme Q10 (Theobroma cacao). Coenzyme Q10 is usually obtained from parsley, broccoli, peanuts, and grape. Coenzyme Q10 is often taken orally as a supplement to aid treatment of cardiovascular disorders including congestive heart failure, angina, and hypertension [40]. In one controlled study, coenzyme 100 mg daily Q10 for four weeks did not alter the INR or the required dose of warfarin. But another report described decreased warfarin effects in patients taking coenzyme Q10 (30 mg per day for two weeks), with an INR reduction from 2.5 to 1.4 [47]. Similar result was found in another case [48]. A study in rats showed that coenzyme Q10 reduced the anticoagulant effect of warfarin and increased the clearance of both enantiomers of warfarin [49]. The mechanism of interaction between coenzyme Q10 and warfarin was unclear. Coenzyme Q10 may have some vitamin K-like activity [50], which would explain the decrease in INR. Interaction between Coenzyme Q10 and warfarin was defined as *probable*. Until more is known, it is reasonable to increase the frequency of INR monitoring in patients taking warfarin and coenzyme Q10 together.

3.1.6. Cranberry (Vaccinium macrocarpon). Cranberry is commonly used for blood and digestive disorders. Some *in vitro* and animal studies suggested cranberry might affect CYP2C9 and CYP3A4 [51, 52]. However, clinical study found no evidence of significant effects in human [51, 53]. There were some case reports of raised INR and significant bleeding when coadministration of warfarin with Cranberry. One patient died after taking two cups of cranberry juice (approximately 300–400 mL) per day for about six weeks [54]. In the US, a case of major bleeding and a high INR had been reported in man taking warfarin, which occurred shortly after Cranberry juice 710 mL daily was started [55]. In a controlled study, twelve healthy subjects were given Cranberry juice (two capsules three times daily, which is equivalent to 57 g of fruit per day) after warfarin (Coumadin 5 × 5 mg tablets) for fifteen days. INR was increased by 28%, whereas the warfarin pharmacokinetics had no significant difference. The Cranberry juice had no effect on platelet aggregation and pharmacokinetics of either *R*- or *S*-warfarin [56]. The interaction might be therefore via a pharmacodynamics mechanism. For example, the salicylate constituent of commercial Cranberry juice might cause hypoprothrombinaemia [57]. In 2004, on the basis of these case reports, the CSM/MHRA in UK advised patients taking warfarin to avoid drinking juice [58]. They recommended frequently INR monitoring for any patient taking warfarin and having a regular intake of Cranberry juice. Interaction between Cranberry and warfarin was defined as *highly probable*, and no reports with dose-response relationship could be found.

3.1.7. Devil's Claw (Harpagophytum procumbens). Devil's claw is used as bitter tonic and for inflammatory disorders [59]. *In vitro*, extract of Devil's claw moderately inhibited the activity of CYP2C8, CYP2C9, CYP2C19, and CYP3A4. *In vitro* study showed that Devil's claw had the greatest effect on CYP2C9 and may increase the effects of warfarin and possible other coumarins [60]. A case report from a 5-year toxicological study described the development of purpura in a patient following the concurrent use of Devil's claw and warfarin (without dosage information) [61]. CYP2C9 was a key enzyme for warfarin metabolism. Limited *in vitro* study suggested that Devil's claw inhibit the metabolism of warfarin, raising its level and potentiating its effect [60]. Clinical evidence of interaction between Devil's claw and warfarin was limited to one case study reporting minor side effects. An interaction seems *possible*, but the evidence is too sparse to make any firm conclusion.

3.1.8. Echinacea (Echinacea purpurea). Echinacea is mainly used in treatment and prevention of common cold, influenza, and other infections. *In vitro* study showed that Echinacea had no significant effect on CYP2C9, CYP1A2, and CYP2D6 [62, 63]. Clinical study showed corresponding result, while a weak inhibition on CYP3A4 was found [64, 65]. In a random study, 12 healthy subjects were given a single dose of warfarin before and after taking Echinacea for 14 days. The AUC of *S*-warfarin decreased by 9%; however, the pharmacokinetic and pharmacodynamics of warfarin had no significant difference [66]. Therefore, Echinacea seemed not to affect warfarin metabolism. Interaction between Echinacea and warfarin was defined as *doubtful*.

3.1.9. Fenugreek (Trigonella foenum-graecum). The seeds of Fenugreek are used mainly for wounds and leg ulcer. It was reported to have hypocholesterol emic and hypoglycemic activity [67]. No relevant data on its pharmacokinetics had been found yet. A case report described that coadministration of one Fenugreek capsule and 10 drops of Boldo increased INR from 2 to 3.4 in patients taking warfarin [36]. However, evidence for this interaction appeared to limit to this one study and it was difficult to identify which of the two herbs is responsible for the increased INR. Therefore, interaction between Fenugreek and warfarin was defined as *doubtful*.

3.1.10. Garlic (Allium sativum). Garlic has been used for respiratory infection and cardiovascular disease. It is believed to have antithrombotic activity [26, 68]. *In vitro* studies suggested that Garlic inhibit CYP2C9, CYP3A, and CYP2D6 [69, 70]. Studies in rats suggested Garlic that inhibited CYP2E1 and induced CYP2C9 [40]. However, clinical studies found no significant effects of Garlic on cytochrome P450 isoenzymes [40]. Clinical evidences for Garlic-warfarin interaction were inconsistent with each other. Isolated case reports showed that ingestion of Garlic might cause INR increased apparently and cause bleeding in patients taking warfarin. One patient stabilized on warfarin had a more than doubled INR and showed hematuria 8 weeks after taking Garlic daily. This situation resolved when the Garlic was stopped. Another patient treated with warfarin also showed a more than doubled in INR by taking six Kwai Garlic tablets daily [40]. In contrast, in a placebo-controlled study in 48 patients stabilized on warfarin, there was no change in INR in those receiving 5 mL of aged Garlic extract (Kyolic) twice daily for twelve weeks [71]. Similarly, in a preliminary report of patients taking warfarin, there was no apparent increased

risk for bleeding or raised INRs in patients taking Garlic concomitantly [72]. Garlic decreased platelet aggregation, which might therefore increase the risk of bleeding. However, this would not cause an increase in INR, and the mechanism for this effect in the cases reported was unknown [73, 74]. Clinical evidence for Garlic-warfarin information is limited to these reports. Interaction between warfarin and Garlic was defined as *possible*. Serious interactions seem unlikely to happen between warfarin and Garlic. However, it may be prudent to consider a complication of bleeding when Garlic was given with warfarin.

3.1.11. Ginkgo (Ginkgo biloba). The ginkgolides possess antiplatelet and anti-inflammatory properties. It can be used for cerebrovascular and peripheral vascular disorders. The effects of Ginkgo on cytochrome P450 isoenzymes were relatively well studied. It appears that the flavonoid fraction of Ginkgo has more effects on cytochrome P450 isoenzymes than the terpene lactones. And these effects disappear quickly when Ginkgo is stopped [75–77]. *In vitro* and rat studies found Ginkgo have effects on CYP2C9, CYP2D6 and CYP2E1. But the effect of Ginkgo on CYP3A4 was unclear and some *in vitro* studies did not appear to be clinically relevant [78–80]. Evidence from pharmacological studies in patients and healthy subjects showed no interaction between Ginkgo and warfarin [81, 82]. However, an intracerebral hemorrhage was reported in an elderly woman when concomitant use of Ginkgo and warfarin in an isolated case. The author of that report speculated that Ginkgo may have contribution to the hemorrhage [83]. There were also a few reports of bleeding [84]. The mechanism of interaction was still uncertain. The interaction between warfarin and ginkgo was *possible*. Evidences are insufficient to justify advising patients taking warfarin to avoid ginkgo, but patients are suggested to monitor their INR when co-administrated ginkgo with warfarin.

3.1.12. Ginger (Zingiber officinale Roscoe). Ginger has anti-inflammatory, antispasmodic, and antiplatelet activities [85]. Pharmacological studies suggested that Ginger does not increase the anticoagulation effects of warfarin [86]. However, a case report described a markedly raised INR in a woman taking warfarin and pieces of Ginger root together (without dosage information) [82]. Moreover, in a prospective, longitudinal study of patients taking warfarin and herbal product, there was a statistically significant increased risk of bleeding events in patients taking warfarin and Ginger [72]. In a randomized, crossover study in twelve healthy subjects, three Ginger capsules (*Blackmores Travel Calm* Ginger capsule containing an extract equivalent to 400 mg of Ginger rhizome powder) taken three times daily for two weeks did not affect either the pharmacodynamics or pharmacokinetics of a single 25 mg dose of warfarin taken on day seven [82]. Ginger was believed to be an herb that interacts with warfarin on the basis of its inhibition on platelet aggregation *in vitro*. However, results of *in vitro* studies cannot be simply extended to clinical [84]. Despite Ginger being cited as an antiplatelet aggregation herb, there was limited evidence suggest that it can increase warfarin anticoagulation effect. There was only one case report showed markedly increased INR for patient coused warfarin and Ginger tea. Without ruling out effects of other factors, it is unreasonable to ascribe this change to Ginger-warfarin interaction. Based on above, interaction between warfarin and Ginger wasdefined as *probable*.

3.1.13. Ginseng. Ginseng is used to enhance the body's resistance to stress and to improve mental and physical performance [40]. The active constituents of Ginseng are mainly ginsenosides that are believed to inhibit the platelet aggregation and thromboxane formation [87]. One study showed that Ginseng (Ginseng capsules three times daily for two weeks) modestly decreased the anticoagulant effects of warfarin (INR decreased from 3.1 to 1.5) [88], and another patient taking warfarin was found to have thrombosis with a subtherapeutic INR of 1.4 (without dosage information) [89]. A clinical study also showed that American Ginseng (1.0 g, twice daily for three weeks) modestly reduced the AUC warfarin in healthy volunteer with INR slightly decreased [90]. In contrast, in a randomized, crossover study in 12 healthy subjects, Ginseng capsules 1 g three times daily for two weeks did not affect either the pharmacokinetics or pharmacodynamics (INR) of a single 25 mg dose of warfarin [91]. The ginsenosides have been reported to inhibit CYP1A2 to some extent, and other ginsenosides metabolites had been found to exert an inhibitory effect on CYP3A4, CYP2D6, or CYP2E1 [40]. Study in rats failed to find any evidence of an interaction between warfarin and Ginseng. Based on the above, interaction between Ginseng and warfarin was defined as *possible*. It was unclear why Ginseng might reduce the efficacy of warfarin. *In vitro* study found that *Panax ginseng* contained antiplatelet components that inhibit platelet aggregation and thromboxane formation [92]. It was reasonable to recommend the caution while combining Ginseng and warfarin.

3.1.14. Grapefruit (Citrus paradisi). Grapefruit juice can inhibit CYP3A4 irreversibly and cause drug interactions in a relatively low dose [93]. A couple, both well stabilized on warfarin, took some drops of Grapefruit seed extract products (*Estratto di Semillas di pompelmo*, Lakshmi, Italy) for 3 days. The women developed a minor hematoma and the man had a raised INR of 5.1 [94]. Mechanism of the interaction was inferred to be that Grapefruit inhibited CYP2C9 and CYP3A4 and therefore affected the metabolism of warfarin [95]. Data presented in these cases, backed by *in vitro* data, supported that Grapefruit had the potential to interact with warfarin, and this interaction wasdefined as *possible*. On this basis, it would probably be prudent to avoid coadministration of warfarin with Grapefruit or for concurrent use to be monitored closely.

3.1.15. Green Tea (Camellia sinensis). *Camellia sinensis* has been reported to contain high amounts of vitamin K, conflicting evidences indicating various amount of vitamin K in green tea [30]. It is true that the dried leaf of *Camellia sinensis* is rich in vitamin K, containing as much as 1428 μg/100 g of leaves. However, brewed tea only contains about 0.03 μg

of vitamin K per 100 g of brewed tea [96]. A 44-year-old man taking warfarin 7.5 mg once daily for stroke prevention had a significant decreased INR from 3.79 to 1.37, which was attributed to the ingestion of green tea (0.5–1 gallon). On discontinuation of the green tea, the patient's INR increased to 2.55. This interaction might be attributed to the vitamin K contained in the tea [97]. In another case, a 67-year-old white female was prescribed warfarin at a dosage of 32 mg/week atrial flutter. After receiving warfarin for 3 months, the patient stopped drinking black tea (dose not known). Within one week after discontinuing the black tea, the patient's INR of increased from 1.7 to 5.0. The weekly dose of warfarin was subsequently decreased to 26 mg/week [98]. The antagonism of warfarin by green tea has been reported to be mainly attributed to the vitamin K contained in the tea. However, there is evidence which shows the antiplatelet effect of green tea. The compounds, including catechin and caffeine, in green tea may stop arachidonic acid release from platelet and thereby inhibit blood clot formation [99]. The reason for this interaction is still unclear, but patients are suggested to take this *possible* interaction into attention when receiving warfarin treatment.

3.1.16. Melilot (Melilotus officinalis). Melilot is used mainly to treat inflammation, oedema, and capillary fragility [100]. There was no relevant pharmacokinetic data for Melilot. In one case, a 66-year-old women's INR rises from 2 to 5.8 after 7-day intake of Melilot with acenocoumarol [101]. Another report showed a woman developed a prolonged prothrombin time when taking large quantities of a herbal tea containing Melilot [102]. However, experimental evidence for this interaction was blank. The mechanism of interaction was unclear. Some studies inferred that the natural coumarins contained in Melilot might be a reason for the interaction between warfarin and Melilot. On the basis of limited case reports and lacking of mechanistic study, this interaction may only be considered as *doubtful*.

3.1.17. Parsley (Petroselinum crispum). Parsley is used as a diuretic and may significantly ameliorate symptoms of arthritis, rheumatism, and inflammatory disorders. A study showed that Parsley reduced the content of cytochrome P450 in rat liver [103]. There were no experimental data of the interaction between Parsley and warfarin. Only one case report showed that a 72-year-old man taking warfarin had a raised INR of 4.43, and after that he stopped 7-year intake of herbal products containing Parsley [104]. It was therefore likely that the Parsley contained sufficient vitamin K to antagonize the effect of warfarin. Interaction between warfarin and Parsley can be considered as *doubtful*. Nevertheless, some consider that increased INR monitoring is required in any patient wanting to start any herbal medicine or nutritional plant.

3.1.18. Pumpkin (Cucurbita pepo). Pumpkin is traditionally used to treat tapeworm infection and has been recently used to treat benign prostatic hyperplasia [105]. There was no experimental data of interaction between Pumpkin and warfarin. One clinical case report showed that an elderly man stable taking warfarin who had a raised INR to 3.4 after starting herbal products containing Pumpkin for 6 days [106]. The mechanism was unclear but may be due to the amount of vitamin K contained in Pumpkin. Because of the limitation of information, the interaction between warfarin and Pumpkin might be considered as *doubtful*.

3.1.19. Red Clover (Trifolium pratense). Red clover is used to reduce the symptoms of the menopause. It could also be used for mastalgia, premenstrual syndrome, and cancer prevention. An *in vitro* study showed that Red clover reduced the activity of CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19, and CYP3A4 and especially inhibited CYP2C8 and CYP2C9 [60]. Some reviews listed Red clover as having the potential to increase the risk of bleeding or potentiate the effects of warfarin, based on the fact that Red clover inhibited CYP2C9 and contained natural coumarins [107]. But there was only one case report support this statement A 53-year-old woman developed spontaneous subarachnoid hemorrhage when she was taking warfarin with herbal supplements containing Red clover, Chinese Angelica, Ginseng, and others for 4 months [108]. With this case reports, it is not possible to identify which, if any, of these constituents in the herbal supplements might have contribution to the hemorrhage. Therefore, the interaction between Red clover and warfarin was defined as *doubtful*.

3.1.20. Saw Palmetto (Serenoa repens). The primary use of Saw palmetto fruit is to treat benign prostatic hyperplasia. It can also be used as an endocrine agent. *In vitro* studies suggested that Saw palmetto inhibits some cytochrome P450 isoenzymes, including CYP2D6, CYP2C9, and CYP3A4 [109]. However, clinical studies found that Saw palmetto did not appear to have a clinically relevant effect on the majority of cytochrome P450 isoenzymes [110]. A case report showed that the INR of a 61-year-old man modestly increased from 2.4 to 3.4, and after that he took Saw palmetto containing product (Curbicin, five tablets daily for six days) together with warfarin [106]. In addition, Saw palmetto (without dosage information) has been reported to cause excessive bleeding in a 53-year-old man undergoing a surgical procedure to remove a brain tumor [111]. Experimental evidence found that Saw palmetto inhibited the CYP2C9, which may be one of the reasons for interaction between warfarin and Saw palmetto [109]. However, evidences were limited to case reports and an experimental study of unknown clinical relevance, which reduced the possibility of interaction. Therefore, the interaction between Saw palmetto and warfarin was defined as *doubtful*.

3.1.21. Soya (Glycine max Merr.). Soya is widely used in Japanese and Chinese cuisine. There are numerous purported benefits of Soya protein, including hyperlipidemia, menopausal symptoms, and osteoporosis [112]. An *in vitro* study showed that Soya bean products inhibited CYP3A4 and CYP2C9 [113, 114]. However, the findings of *in vitro* studies cannot be directly extrapolated to clinical situations.

Fermented Soya bean products contain high level of vitamin K and may therefore decrease the activity of warfarin and related anticoagulants [115]. Experiments in rabbits found that Natto, a Japanese food made from fermented Soya bean containing high levels of vitamin K, strongly antagonized the effects of warfarin [116]. Clinical case reports also showed marked reduction in effects of acenocoumarol, a warfarin derivative anticoagulant, when coadministrated with Natto (100 g/day) for two weeks [117]. In this study no dose-response relationship could be concluded. In another clinical study, Soybean protein also modestly reduced the effects of warfarin [116]. A similar interaction was reported for a 70-year-old man whose INR decreased from 2.5 to 1.6 when taking 480 mL Soy milk daily after warfarin treatment for four weeks [115]. Mechanistic study suggested that the antagonistic interaction between warfarin and Soya was due to the high level of vitamin K in Soya and the inhibition effect of Soya extracts on CYP2C9 and CYP3A4. The interaction between warfarin and fermented Soya bean production was established and marked and was *highly probable* to be clinically relevant in all patients.

3.1.22. St. John's Wort (Hypericum perforatum). St John's wort is an herbal medicine mainly used for treatment of depression. An amount of interactions related to St John's wort have been reported in clinical case reports. *In vitro* studies demonstrated that St John's wort inhibited CYP2C9, CYP2D6, and CYP3A4 [41, 118]. Paradoxically, *in vivo* studies found that St John's wort induced CYP2D6, CYP2E1, and CYP3A4 [119, 120]. There are several case reports suggesting that coadministration of St John's wort decreased the effects of warfarin. From 1998 to 1999 period, the Swedish Medical Products Agency (MPA, Uppsala, Sweden) has received seven case reports of a reduced anticoagulant effect and decreased INR of warfarin associated with coadministration of St John's wort [121]. In a randomized, crossover study in 12 healthy subjects, one tablet of St John's wort (each tablet containing standardized dry extract equivalent to 1 g *Hypericum perforatum* flowering herb top, 0.825 mg hypericin and 12.5 mg hyperforin) three times daily for two weeks modestly decreased the AUC of both *R*- and *S*-warfarin by about 25% after a single 25 mg dose of warfarin [91]. However, in another case, an 85-year-old patient taking warfarin 5 mg daily was reported to develop upper gastrointestinal bleeding one month after starting St John's wort (without dosage information) [122]. Until now, the potential interactions between warfarin and St John's wort have not been systemically investigated. Concomitant intake of St John's wort was associated with a loss of anticoagulant activity in patients stabilized on warfarin. Although no thromboembolic episodes occurred, the decrease in anticoagulant activity was considered clinically significant. Anticoagulant activity was restored when St John's wort was terminated or the warfarin dose was increased. These observations suggest an increased clearance of warfarin, possibly due to the induction of CYPs, particular CY2C9, and 3A4. Based on the above findings, a modest pharmacokinetic interaction between St John's wort and coumarins would be established, which might be clinically important in some patients. The possibility of interactions between St. John's wort and warfarin has been considered as *highly probable*. And it is recommended to closely monitor INR in patients taking warfarin after ingestion of St. John's wort.

3.2. TCM Herbs. Among the reported herbs, *Ginseng, Andrographis Paniculata,* and *Melilotus Officinalis* are also commonly used as TCM. In addition, we summarized a few more TCM herbs commonly used in Chinese population that may have interactions with warfarin as follows.

3.2.1. Chinese Angelica (Angelica sinensis). Chinese Angelica is mostly used for the treatment of menopausal symptoms, menstrual disorders, hypertension, and allergic conditions [123]. A study in rabbits showed an increase in prothrombin time but no changes in the pharmacokinetic parameters of warfarin [124]. In contrast, most experimental evidences showed that Chinese Angelica inhibited CYP2C9 and CYP3A4, which indicated the potential risk of interaction between Chinese Angelica and a wide range of conventional drugs [125]. In one clinical case, the INR and prothrombin time of a 46-year-old woman doubled after Chinese Angelica (one 565 mg tablet 1-2 times/day for four weeks) and warfarin treatment. And these indexes went back to normal when stopped Chinese Angelica [126]. Another case report also described a very marked increase of INR to 10 when coadministration of warfarin with Chinese Angelica for a month (without dosage information) [127]. The reasons for this interaction are not fully understood. A high level of coumarin derivatives may be included. Other studies suggest that the herb may inhibit CYP2C9, which is the main route of warfarin metabolism. On basis of limited clinical evidence, the interaction between Chinese Angelica and warfarin is not fully established and may be defined as *probable*. More studies are needed to certify this interaction. However, for safety, the use of Chinese angelica should be avoided unless the effects on anticoagulation can be monitored.

3.2.2. Danshen (Salvia miltiorrhiza). Although not commonly used in the western cultures, Danshen (the dried root of *Salvia miltiorrhiza Bunge*) is a very popular TCM recommended in China and many other Asian countries for promoting circulation and improving blood flow. Primary clinical application of Danshen is treatment of various cardiovascular and cerebrovascular diseases, including angina pectoris, hyperlipidemia, and acute ischemic stroke [128]. Previous studies indicated that Danshen extracts could increase the absorption rate, area under the plasma concentration-versus-time curve (AUC), as well as the maximum concentration (C_{max}) of warfarin, and reduce the elimination half-life ($t_{1/2}$) in rats. Danshen injection was reported to significantly increase the plasma concentration of warfarin in rats [129, 130]. Plenty of rat/mouse *in vivo* and cell-based *in vitro* studies showed inconsistent effects of Danshen products on cytochrome P450 isoenzymes. In a study on mice, a commercial pharmaceutical extract of Danshen induced the activity of CYP1A2 by about 60%. A purified extract of tanshinone IIA had a similar effect in this study [131]. In converse, another

study using mice and human liver microsomes showed that tanshinone IIA inhibited CYP1A2 [132]. In clinical study, INR of patients taking warfarin significantly increased after ingestion of Danshen. There are several case reports about the warfarin-Danshen interaction. A 62-year-old man stabilized with warfarin had a raised INR to more than 8.4 after consuming Danshen extract for two weeks (without dosage information) [133]. In another case, after consuming Danshen for three days (without dosage information), a 66-year-old man who had been receiving warfarin 2–2.5 mg/day for nearly a year was hospitalized for bleeding accompanied with INR increasing from 2 to 5.5 [134]. Most mechanistic studies focus on the expression and metabolic activities of various cytochrome P450 enzymes. After rapidly and completely absorbed in GI tract, warfarin is metabolized mainly in the liver by CYP2C9, CYP1A2, and CYP3A4 in human. Clinical studies have been conducted to investigate the effects of Danshen or its single component on the metabolic activity of several CYP isoenzymes. A sequential, open-label, two-period clinical investigation indicated that Danshen may have induction effect on CYP3A and CYP1A2 [135]. Danshen or its components could also alter the distribution of warfarin. After entering into the blood, around 99% warfarin would bind to the plasma protein, mainly albumin, to form a warfarin-albumin complex that has no therapeutic effect. Danshen and its major component danshinone IIA could competitively bind to albumin and therefore inhibit the protein binding of warfarin [136]. Reduced protein binding may result in over-anticoagulant because of the increased blood concentration of warfarin. Though less investigated, the pharmacodynamics effects of Danshen on the warfarin cannot be ignored. Danshen is widely used for removing blood stasis to improve the blood flow. With the similar clinically therapeutic effect to warfarin, concomitant use of warfarin and Danshen may cause a synergistic effect and result in over-anticoagulation. On basis of previous studies, interaction between Danshen and warfarin can be considered as *highly probable*. Therefore, it may be prudent to advice against concurrent use of Danshen with warfarin.

3.2.3. Lycium (Lycium barbarum). Lycium have anti-inflammatory, antioxidation, and anticancer properties. It could be used for diabetes, hypertension and erectile dysfunction [137]. *In vitro* study found that Lycium was a weak CYP2C9 inhibitor, but it was insufficient to cause a drug interaction [137]. A Chinese women stabilized on warfarin had a significantly rise in her INR from 2 to 4.1 when concurrently taking Lycium (3-4 glasses daily) for 4 days. And the INR returned to normal when herbal treatment stopped [138]. In another case, after 4-day coadministration of Lycium juice (30 mL each morning and evening) and warfarin, a 71-year-old woman had nosebleeds, bruising, and rectal bleeding [139]. These cases reports showed that Lycium might potentiate effects of warfarin. Warfarin is mostly metabolized by CYP2C9. Inhibition of CYP2C9 may therefore lead to increased warfarin levels and effects. However, other mechanisms cannot be ruled out. On basis of the INR changes in several cases, the interaction between warfarin and Lycium may be considered as *possible*.

In addition, herbs preclinically evidenced to affect pharmacokinetics of warfarin (Andrographis [140–143], *Horse chestnut* [63, 82], *Schisandra* [144–146], *Gegen* [147–149], and *Liquorice* [150, 151]) and pharmacodynamics of warfarin (*Clove* [40, 152, 153], *Lapacho* [32, 154]), or both (*Evening primrose* [70, 155], *Feverfew* [60, 107, 156]); herbs containing vitamin K (Alfalfa [40, 157, 158], *Asparagus* [40, 159]) or coumarin (*Bogbean* [18, 40], *Celery* [160, 161], and *Horse chestnut* [40]) and herbs with similar or opposite pharmacological actions to those of warfarin may also interact with warfarin. As no clinical evidence is now available to support an interaction, these herbs were defined as *doubtful* in the current review.

4. Discussions

The basic issues involved in assessing the importance of interactions between herbs and drugs are similar to those in evaluating interactions between conventional drugs, but for herbal medicines the picture is complicated by their very nature: the herbs are complex mixtures and there is also lack of reliable information about the occurrence and relevance of interactions. This review attempts to answer following questions.

(i) Is the herb-warfarin interaction clinically evidenced or only theoretical and speculative?

(ii) If they do interact, how serious is it?

(iii) Has this interaction been identified many times or only once?

(iv) What is the possible mechanism for the interaction?

The current review scales the clinical severity and evidence reliabilities of herb-warfarin interaction according to previously validated criteria. The uniqueness of this study is demonstrated in Table 3, which summarizes clinical effects of proposed herb-warfarin interaction, severity, and possibility of these interactions as well as their possible mechanisms. The outcome can be harmful if the interaction causes an increase in the effect of warfarin. A potential example of this is bleeding related to coadministration of cranberry with warfarin. However, a reduction in warfarin efficacy due to an interaction can sometimes be just as harmful as an increase. For example, the reduction in warfarin effect caused by St. John's wort may lead to thrombosis. In regard to this, it seems extremely dangerous for patients to take warfarin and herbs together. But this could be an overestimation of the outcome. First, human beings do not respond uniformly to drugs or herbal medicines due to many elements including genetic makeup, sex, age, diseases, renal and hepatic functions, ethnic background, nutritional state, and other factors. Second, a good deal of evidence on herb-warfarin interactions discussed in this paper is based on case reports, which are sometimes incomplete and do not allow one to infer a causal relationship. According to our current review, out of 38 herbs with clinical evidence, only 4 interactions are regarded as *highly probable*. It is worth noting that even documented case reports could never establish a causal relationship between

herb administration and an adverse event, as sometimes only one case report has been used, and in many cases, the quality of documented case report is poor. The often underregulated quality of herbal medicines is another safety issue. Contamination or adulteration of herbal medicines, including adulteration with synthetic drugs, may be relatively frequent and can cause drug interactions. In other words, the possibility that a contaminant adulterant instead of herbal ingredient causing drug interactions cannot be ruled out. Therefore, it is difficult to make conclusion on whether or not coadministration of certain herb with warfarin contributes to the adverse event. Although it is impossible to identify all clinically important herb-warfarin interaction, some general principles can be reached as follow.

(i) Herbs containing large amount of vitamin K have high possibility to interact with warfarin.

(ii) Blood vitalizing herbs and herbs with antiplatelet effects are liable to have interaction with warfarin.

(iii) The elderly are at greater risk because of reduced liver and renal function on which drug clearance depends.

Since herb usage could be quite variable, the current review only focuses on single herb without herb remedies included. In addition, variability in the dosage of both warfarin and proposed herbs is critical for managing an interaction. As a narrow therapeutic drug, the dosage of warfarin is adjusted according to the INR of patient. For therapeutic purpose, the value of INR should be maintained at a range of 2 to 3. While for healthy subjects, the normal value of INR is 0.9 to 1.2. For most of the clinical studies mentioned in current review, warfarin doses and intensity of anticoagulation were stable before initiation of herbs. Therefore the intensity of interactions was mainly correlated with the dosage of coadministrated herbs. Unfortunately, most of the existing case reports failed to mention the relevant dosage of interacting herbs. For example, a report describes an intracerebral hemorrhage, which occurred in an elderly woman within 2 months of her starting to take ginkgo. She had been taking warfarin uneventfully for 5 years. The author of the report speculated that ginkgo may have contributed towards the hemorrhage [83]. However, the dosage of ginkgo was not reported. Conversely, in a randomized, crossover study in 21 patients stabilized on warfarin, a tablet of *Ginkgo biloba* 100 mg daily for 4 weeks, did not alter the INR or the required dose of warfarin, when compared with placebo [81]. Therefore, despite the prevalence report about herb-warfarin interaction, the intensity of these interactions may be overestimated. All the herbal medicines have side effects more or less, but when used at a therapeutic dosage in clinical treatment, it can be accepted as a safe medicine. On this basis, the authors suggest further studies with corresponding information on herb dosage. In addition, herbs of different producing regions, medical parts, and processing techniques may contain various amounts of active compounds. Therefore, it is reasonable to develop qualified and standardized herbal products such as EGb 761, a standardized and commercially available extract of *Ginkgo biloba* leaves.

5. Conclusions

An overview of the clinical data regarding herb-warfarin interactions was conducted in this paper, highlighting clinical effects, severity of documented interaction, and quality of clinical evidence. Among thirty-eight of selected herbs, four were evaluated as *highly probable* to interact with warfarin (Level I evidence), three were *probable* interaction (Level II evidence), and ten and twenty-one were *possible* (Level III evidence) and *doubtful* (Level IV evidence), respectively. Herbs defined as *highly probable* (Cranberry, Soya, St John's wort, and Danshen) and *probable* (coenzyme Q10, Chinese Angelica, Ginger) are strongly suggested to be avoided from concomitant use with warfarin. For herbs defined as *possible and doubtful*, although insufficient evidences supporting the interaction yet, for safety reason, it is recommended to closely monitor INR in patients taking warfarin. Patients and physicians are advised to use herbal medicines within a safety dosage. Qualified and standardized herbal products such as EGb 761 are recommended for scientific researches, especially for clinical studies. Although several corresponding pharmacokinetic or pharmacodynamic mechanisms of interactions were able to be identified for a small amount of "interacting herbs," there are still a great number of unexplored aspects of herb-warfarin interactions. The clinical effects of herbs on warfarin therapy should be further investigated through multicenter studies with large sample sizes.

Conflict of Interests

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

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Research Article

Bench to Bed Evidences for Pharmacokinetic and Pharmacodynamic Interactions Involving Oseltamivir and Chinese Medicine

Qi Chang,[1,2] Siukwan Wo,[2] Karry L. K. Ngai,[3] Xiaoan Wang,[2] Benny Fok,[4] Teresa M. Ngan,[5] Vivian T. Wong,[5] Thomas Y. K. Chan,[4] Vincent H. L. Lee,[1] Brian Tomlinson,[4] Paul K. S. Chan,[3] Moses S. S. Chow,[6] and Zhong Zuo[2]

[1] *Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100193, China*
[2] *School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong*
[3] *Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong*
[4] *Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong*
[5] *Hospital Authority, Hong Kong*
[6] *Center for Advancement of Drug Research and Evaluation, College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766-1854, USA*

Correspondence should be addressed to Zhong Zuo; joanzuo@cuhk.edu.hk

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Oseltamivir (OA), an ethyl ester prodrug of oseltamivir carboxylate (OC), is clinically used as a potent and selective inhibitor of neuraminidase. Chinese medicines have been advocated to combine with conventional drug for avian influenza. The current study aims to investigate the potential pharmacokinetic and pharmacodynamic interactions of a Chinese medicine formula, namely, Yin Qiao San and Sang Ju Yin (CMF1), commonly used for anti-influenza in combination with OA in both rat and human, and to reveal the underlined mechanisms. It was found that although C_{max}, AUC and urinary recovery of OC, as well as metabolic ratio (AUC_{OC}/AUC_{OA}), were significantly decreased in a dose-dependent manner following combination use of CMF1 and OA in rat studies ($P < 0.01$), such coadministration in 14 healthy volunteers only resulted in a trend of minor decrease in the related parameters. Further mechanistic studies found that although CMF1 could reduce absorption and metabolism of OA, it appears to enhance viral inhibition of OA ($P < 0.01$). In summary, although there was potential interaction between OA and CMF1 found in rat studies, its clinical impact was expected to be minimal. The coadministration of OA and CMF1 at the clinical recommended dosages is, therefore, considered to be safe.

1. Introduction

Oseltamivir (OA) is clinically used as a potent and selective inhibitor of neuraminidase essential for replication of influenza A and B viruses. The normal adult dose of OA for the treatment of avian influenza is 75 mg orally twice a day for 5 days. Following 50 mg doses, the maximum plasma oseltamivir carboxylate concentration is about 230 μg/L, which is above of 50% inhibitory concentrations (IC_{50}) of many influenza A viruses [1]. The pharmacokinetics of both OA and its active metabolite oseltamivir carboxylate (OC) have been studied in young healthy adults and children, as well as elderly subjects [1–4]. Following oral administration, OA is rapidly absorbed and extensively converted to OC, primarily by hepatic carboxylesterase enzymes, resulting in a much higher concentration *in vivo* than OA. The absolute

oral bioavailability of OC from orally administered OA is 80% with a half-life of 6–10 hours and food has no significant effect on its bioavailability [1, 5].

The potential advantage of OA in combination with Chinese medicine (CM) is of interest since avian influenza can be deadly and it is an important health care goal in many Asian countries. In Hong Kong, the Hospital Authority Central Committee on Infectious Disease and Infection Control Branch Centre for Health Protection has jointly recommended the drug OA (Tamiflu) for prophylaxis and treatment of avian influenza. In addition, a panel of the CM experts from HA together with the Task Force on Herb-Drug Interaction Research has recommended four specific CM formulae for the prophylaxis and treatment of influenza with OA. It is expected that many Hong Kong citizens will be prescribed with such "western" medicine (WM) and CM for avian influenza if there is an outbreak. Whether coadministration of the CM formulae as recommended by HA expert will cause any change in plasma oseltamivir carboxylate concentration or whether there is an additive antiviral effect with the combination is unknown. Although there is a report on the effect of a few CM (*Flos Lonicerae, Folium Perillae, Radix isatidis*) on OA [6], the findings are mainly from *in vitro* cell studies. The present study aims to determine, in animal and human studies, the potential pharmacokinetic and pharmacodynamic interactions of OA in combination with the most recognized and thirteen herb containing CM formulae (CMF1, Table 1), which is a combined formula of two traditional Chinese herb preparations, Yin Qiao San and Sang Ju Yin, for avian influenza as recommended by a CM expert panel from the HA in Hong Kong.

Table 1: The herbal composition of the CMF1.

Latin name	Chinese (pinyin) name
Flos Chrysanthemi	Ju Hua
Flos Lonicerae Japonicae	Jin Yin Hua
Folium Mori	Sang Ye
Fructus Arctii	Niu Bang Zi
Fructus Forsythiae	Lian Qiao
Herba Lophatheri	Dan Zhu Ye
Herba Menthae	Bo He
Radix Et Rhizoma Glycyrrhizae	Gan Cao
Radix Platycodonis	Jie Geng
Rhizoma Phragmitis	Lu Gen
Semen Armeniacae Amarum	Ku Xing Ren
Semen Sojae Praeparatum	Dan Dou Chi
Spica Schizonepetae	Jing Jie Sui

2. Materials and Methods

2.1. Chemicals and Reagents. OA (RO-64-0796) and D-tartrate salt of OC (RO-64-0802) were kindly provided by F. Hoffmann-La Roche Ltd. (Switzerland). Cephalexin hydrate was purchased from Sigma (USP Science, Rockville, MD, USA). Dichlorvos (as carboxylase inhibitor) with purity of 99.4% was purchased from Riedel-de Haën (Germany). Methanol (HPLC grade) was purchased from Merck KGaA (Germany). Unless specified elsewhere, all reagents were used without further purification. Distilled and deionized water (ddH2O) was prepared from Millipore water purification system (Millipore, Milford, USA). Tamiflu 75 mg capsule (batch number B1280B01) (Hong Kong registration number HK-46484) was manufactured by F. Hoffmann-La Roche Ltd.

For cell culture, Dulbecco's modified Eagle's medium, fetal bovine serum, 0.05% Trypsin-EDTA, penicillin-streptomycin, and nonessential amino acids were obtained from Gibco BRL (Carlsbad, CA, USA) and Life Technologies (Grand Island, NY, USA). Phosphate buffered saline tablets were purchased from Sigma.

CMF1 was manufactured by Purapharm (Nanning) Pharmaceuticals Co. Ltd. in accordance with GMP standard. CMF1 (batch number A090943-01) was formulated as granules and received in package of 10 g granules per sachet for human study. The safety measures including heavy metals (arsenic, lead, mercury, and cadmium), microbial examination, and pesticides residue of the CM products were conducted by an independent Hong Kong accredited laboratory and were found to comply with the 2005 Hong Kong Traditional Chinese Medicine requirements. HPLC/DAD was used to obtain a chemical profile of potential active components of CMF1. Briefly, 30 mg of CMF1 powder was accurately weighted into a glass container with tight cap, and 5 mL of methanol water (50 : 50 v/v) was added and sonicated for 15 min for extraction. After centrifugation at 13,000 rpm for 10 min, the supernatant (10 μL) was then injected into HPLC-DAD (Waters, Milford, MA, USA) for assay of active components. The sample was separated by a Thermo ODS Hypersil column (4.6 × 250 mm, 5 μm) connected to a ODS guard column (Thermo). The mobile phase consisted of 0.2% formic acid in water (solvent A) and in acetonitrile (solvent B) with linear gradient elution at a flow rate of 1 mL/min. Solvent B was set at 5% from 0 to 5 min and increased to 40% from 5 to 45 min and then back to 5% in 10 min with 5 min. The PDA detector was set for collection of spectral data from 210 nm to 400 nm. The contents of arctiin and forsythoside A, the identified marker components for CMF1, are 3.54 mg/g and 0.364 mg/g, respectively, which fulfilled the requirement of related formula in the Chinese Pharmacopoeia [7].

2.2. Drug Administration and Samplings in Rats. The study was approved by the Animal Ethics Committee of The Chinese University of Hong Kong. Male Sprague-Dawley rats (230–250 g) were utilized and supplied by the Laboratory Animal Service Center at The Chinese University of Hong Kong. The rats were housed under standard conditions of temperature, humidity, and light and randomly divided into six groups with 10–12 rats in each group. In order to achieve full pharmacokinetics profiles of both OA and OC, our preliminary experiments suggested a dose of 30 mg/kg for OA oral administrations. The dose of 1.95 g/kg CMF1 is calculated based on the human dose recommended by the Chinese physicians and a doubled dose of CMF1 at 3.90 mg/kg is also used in the current study due to the potential large dosing range for Chinese medicine adopted in the practice. The

rats in Group 1 received OA alone (30 mg/kg), and those in Groups 2 and 3 received OA (30 mg/kg) in combination with CMF1 at low (1.95 g/kg) and high (3.90 g/kg) doses, respectively. Rats in Groups 4 and 5 were only treated with CMF1 at low (1.95 g/kg) and high (3.90 g/kg) doses, respectively, whereas Group 6 rats only received regular diet without OA or CMF1.

For dosing, OA (4 mg/mL) was freshly prepared by dissolving it in water and orally given to rats by gavage, bid (twice daily) for 4 days (9:00am and 6:00pm for each day), and CMF1 was also freshly suspended in water and then orally given to rats 2 h later of OA dosing (11:00 am and 8:00 pm for each day).

For Groups of 1 to 3, a surgery for jugular vein cannulation was performed 1 h after CMF1 second dosing on day 4. A polyethylene catheter (0.50 mm ID, 1.00 mm OD, Portex Limited, Hythe, Kent, England) was cannulated into the right jugular vein under anesthesia. After surgery, the rat was placed in separated metabolic cage and allowed to recover and fasted overnight with free access to water. In the morning of day 5, blood samples (0.2 mL each) of the rats in the Groups 1–3 were collected via the catheter at 0, 15, 30, 60, 90, 120, 180, 240, 360, and 480 min after dosing of OA. After which, 0.2 mL of normal saline containing 20 units/mL of heparin was then injected into the catheter to flush the catheter. The collected blood samples were immediately placed in heparinized tubes containing dichlorvos (5 μL of 8 mg/mL dichlorvos in normal saline) for inhibition of carboxylesterase [8], followed by centrifugation to obtain the plasma and stored at −80°C. Urine samples were collected over 8 h postdose and combined with water used for rinsing the metabolic cage, further diluted to 200 mL, and then stored at −80°C until assay.

Following the last blood sampling, the rats in Groups 1–3 received the last dose of OA or OA together with CMF1 similar to above dose, respectively. At 90 min after dosing (i.e., absorption had taken place), all rats were sacrificed and ~2 mL plasma was collected for determination of antiviral activity. Rats in Groups 4 to 5 were sacrificed at 90 min after last dosing and ~2 mL of plasma was collected for determination of antiviral activity. The plasma samples collected from the rats in Group 6 (without any treatment) were served as negative control.

2.3. Human Study

2.3.1. Ethics. Prior to the clinical study, the ethics approval was obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. The clinical study was conducted according to Good Clinical Practice (GCP) and ICH guidelines and the Declaration of Helsinki. All subjects were fully informed about the study and a written informed consent was obtained from each subject prior to the study.

2.3.2. Subjects. Normal healthy Chinese male subjects aged 20–45 years were recruited in this study. Subjects were excluded if they had a history of clinically significant hepatic, renal, biliary, cardiovascular, gastrointestinal, haematologic and other chronic and acute diseases within 3 months prior to the study; had clinically relevant abnormality in physical examination, ECG evaluation, urine test, blood chemistry or haematological test during screening test; received any prescription or hypersensitivity to Tamiflu or related CM formulae/herbal components; a history of smoking, drug or abuse of alcohol; blood donation within 4 weeks prior to the start of study.

The screening process included physical examination, ECG evaluation, urinalysis, blood chemistry, and haematological tests. During the study, subjects were abstained from any prescription or nonprescription medications 2 weeks before and throughout the study; alcohol, grapefruit juice, caffeine, or xanthine-containing foods or beverages for 72 h prior to and during sampling; smoking for 72 h prior to and during sampling.

2.3.3. Clinical Study Design. A sample size of 14 was calculated by assuming that a 15% difference (based on our preliminary study) in the mean pharmacokinetic parameter is significant between 2 groups (WM *versus* WM + CMF) and a 30% standard deviation to achieve 80% power at $\alpha = 0.05$. The study was conducted using a single-center, randomized, open-labeled, multiple dose (5 days), two-treatment, two-period, two-sequence crossover design. Subjects were randomized to one of two groups (Groups 1A and 1B) and received either western medicine (WM) alone or in combination with CMF1. Group 1A received WM first followed by WM in combination of CMF1, while Group 1B received WM in combination of CMF1 first followed by WM. Each subject underwent two treatment sessions (periods I and II), and each session consists of 5-day treatment (twice daily for day 1 to day 4, morning dose for day 5). The two treatment sessions were separated by a washout period of 2 weeks. 250 mL water was used for WM (Tamiflu capsule, 75 mg per dose) administration. CMF1 (10 g extracts per dose) was mixed to 250 mL water before administration (2 h after receiving WM, based on the common practice for combination use of western drugs and Chinese medicines recommended by local practitioners). Subjects were fast for 10 h before and 4 h after drug administration on day 5 of each treatment session. Drinking water was not allowed from 1 h predose to 1 h postdose except that needed for drug dosing. Meals were standardized and consumed at 4 h and 10 h postdosing.

2.3.4. Blood and Urine Collection. All blood and urine collection was taken on day 5 of each treatment session. Venous blood samples were collected at pre-dose (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h post-dose. Blood samples were collected from a catheter, which was placed in the forearm vein before dosing. At the specified time, 5 mL of blood was drawn (except for 0 time and at 2 h which was 10 mL each for additional antiviral activity determination) and stored in vacuette lithium heparin tubes (Greiner Bio-One). Dichlorvos (a carboxylase inhibitor) was then added (200 μg/mL) into the blood samples (except for those used for antiviral activity determination) to prevent *in vitro* hydrolysis

from OA to OC [8–10]. Plasma samples were collected after centrifugation and then stored at −80°C until assay. Urine was also collected (and the volume was recorded) at pre-dose, 0–4, 4–8, and 8–12 h intervals. The urine samples (~10 mL) were stored at −80°C until assay.

2.4. Determination of OA and OC in Plasma and Urine by LC/MS/MS. The rat/human plasma and urine samples were treated and analyzed by an LC/MS/MS system as previously described [10]. Briefly, 200 μL plasma/urine samples was mixed with cephalexin hydrate (internal standard, IS) working solution (final 2 μg/mL) and acidified with 1 mL of 10% perchloric acid in water (if necessary, dilution with blank human urine was required for urine samples). After mixing and centrifugation, the supernatant was loaded in prewashed Oasis MCX cartridge (1 cc, 30 mg, Waters) and the cartridge was rinsed subsequently with 1% formic acid, water, and methanol followed by vacuum dried for 20 min. The analytes were then eluted with 1 mL of 1% ammonia in methanol. The eluting solvent was dried by a vacuum concentrator and the residue was reconstituted with 200 μL of 0.1% formic acid : methanol (1 : 1 v/v) prior to HPLC/MS/MS analysis using an ABI 2000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems) coupled with PE-200 series micropumps and autosampler (Perkin-Elmer). The chromatographic separation was achieved by using a Nova-Pak CN HP column (75 × 3.9 mm i.d., 4 μm particle size, Waters) and the HPLC solvent system consisted of methanol (A) and 0.1% formic acid in water (B), with 50% A (for plasma samples) or 60% A (for urine samples) at 1 mL/min. The temperatures of autosampler and the analytical column were set at 4°C and ambient, respectively, and the sample injection volume was 20 μL. Prior to the mass spectrometric system, 60% of the LC eluent was split off and only 40% of the eluent was introduced into the ESI source.

The mass spectrometer was operated at positive ionization mode. Ion spray voltage was set to 5500 V; heater probe temperature was set at 400°C; nitrogen was used as nebulizer (30 psi), heater (70 psi), curtain (30 psi), and collision gas (medium). Other instrumental parameters were analyte specific and were optimized prior to analysis. Data acquisition was conducted at multiple reaction monitoring (MRM) mode, with m/z 313 → m/z 166 for O, m/z 285 → m/z 138 for OC, and m/z 348 → m/z 158 for IS. Dwell time was set at 300 ms for each channel.

Calibration standards were prepared by spiking 200 μL blank human plasma (premixed with dichlorvos to a final concentration of 200 μg/mL)/200 μL blank human urine with 20 μL each of working standard mixture and internal standard solution. The linearity of analytes (as OA phosphate or OC tartrate) was 2–1000 ng/mL (OA) and 10–10000 ng/mL (OC) in plasma and 6–1000 ng/mL (OA) and 30–10000 ng/mL (OC) in urine. The LOQ of OA and OC, defined as the signal-to-noise ratio ≥5 and being reproducible with precision of 20% RSD and accuracy between 80% and 120%, was the lowest concentration of the calibration curve.

The method validation was conducted with reference to the Guidance for Industry, Bioanalytical Method Validation from USFDA (May 2001) with satisfied accuracy and precision of OA and OC at low, medium, and high concentration levels in either plasma (6, 80, and 750 ng/mL for OA, 20, 800, and 7500 ng/mL for OC) or urine (15, 150, and 750 ng/mL for OA, 60, 1500, and 7500 ng/mL for OC) were found to comply with the criteria of accuracy (within 15% bias) and precision (within 15% RSD) as stated in the guidance. The extraction recoveries of OA (87%–109%), OC (73%–81%), and IS (70%–88%) in both plasma and urine were consistent over the concentration range studied. The analytes in both plasma and urine under three freeze (−80°C) thaw (room temperature) cycles, short term (2 h at ambient), and in autosampler (at 4°C for 12 h for plasma and 6 h for urine samples) were found to be stable (accuracy of 88.0%–106.1%) and reproducible (within 10.1% RSD) over the concentration range investigated.

2.5. Antiviral Effects Measurement

2.5.1. Cells and Viruses. Madin-Darby canine kidney (MDCK) cells were cultured in Eagle's minimum essential medium (MEM) (Invitrogen, California, USA). Media were supplemented with 10% fetal bovine serum (FBS) (Invitrogen, California, USA), except the assays of influenza virus. Infections with human influenza A H3N2 virus (A/HongKong/CUHK-22910/2004) were carried out in serum-free medium formulated with 1 μg/mL of trypsin treated with tolylsulfonyl phenylalanyl chloromethyl ketone (TPCK-treated trypsin) (Sigma-Aldrich, Munich, Germany).

2.5.2. Plaque Reduction Assay. The antiviral effects of rat plasma samples at 120 min after oral administration of OA or OA in combination with CMF1 were evaluated by the plaque reduction assay [11]. Briefly, the plasma samples obtained from Groups 1 to 6 were first ultrafiltrated at 4°C using an Amicon Ultra 3 K filter unit (Millipore) to remove protein and then diluted with serum-free medium in 250-fold dilution. An equal volume (0.25 mL) of diluted plasma was mixed with virus culture medium containing 400 PFU/mL and incubated for 1 h at 37°C. Confluent monolayer of MDCK cells in 24-well plate (Nunc, Denmark) was washed with infectious medium and inoculated with 0.5 mL plasma-containing virus mixture. After 1 h of viral absorption at 37°C, virus inoculums were removed before adding 0.5 mL agarose overlay medium containing 0.4% agarose and 500-fold diluted plasma. Duplicates of each plasma sample, virus control, and cell control were performed in each experiment. Plaques were stained with neutral red staining (0.05%) after 24 h incubation at 37°C. The plaques were counted and antiviral activity was calculated as the percentage of virus control. Blank rat plasma spiked with 2 μg/mL of OC was treated as mentioned above and served as the positive control for the assay.

Similarly, human plasma samples (~2 mL) collected on day 5 at pre-dose (0 h) and 2 h after medications in human were ultrafiltered before conducting the plaque reduction assay. Prior to analysis, the human plasma filtrate was diluted in 1 : 10 with maintenance medium and the diluted samples were mixed with equal volume of virus. Influenza virus H3N2

strain was used for the assay. The results were presented as percentage inhibition (*versus* control, i.e., drug-free plasma filtrate).

2.6. Mechanistic Studies on the Effect of CMF1 on the Metabolism and Absorption of OA

2.6.1. Effect of CMF1 on the Hydrolysis of OA in Rat Plasma. For testing the metabolic activity of OA in rat plasma, 20 male Sprague-Dawley rats (230–250 g) were sacrificed with an intramuscular injection of a mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg). Rat blood was obtained via cardiac puncture with a 10 mL syringe containing 0.1 mL heparin (5000 I.U./mL) followed by centrifugation at 13000 rpm for 5 min. The obtained plasma from all rats was pooled together and stored at −80°C for enzyme incubation experiments.

Rat plasma with a volume of 400 μL was spiked with 4 μL CMF1, which was dissolved in DMSO (control group was spiked with DMSO only). Final tested concentrations for CMF1 ranged from 25 μg/mL to 300 μg/mL. Working solutions of OA, in H_2O, were then spiked into the above reaction mixture to reach a final concentration of 5 μg/mL (for reactions at room temperature) or 10 μg/mL (for reactions at 37°C). The final mixture was incubated for 60 min (for reactions at room temperature) or 30 min (for reactions at 37°C) and terminated by adding 10 μL dichlorvos (2 mg/mL) into 100 μL reaction mixture. Samples are prepared and analyzed by a developed method with modifications [10].

2.6.2. Effect of CMF on the Absorption of OA in Rat In Situ Intestinal Perfusion Model. In view of the consistent effect of CMF1 on OA and OC, further confirmation was performed using rat *in situ* intestinal perfusion model as described previously [12]. Perfusion concentrations were set at 6.8 mg/mL for CMF1. The flow rate of perfusate applied to the intestinal lumen was set at 0.3 mL/min. Samples obtained from the mesenteric vein were collected into the preweighted 2 mL centrifuge tubes (each containing 30 μL of 10 mg/mL saline solution of dichlorvos) at every 5 min. All collected samples were weighted and centrifuged at 13,000 rpm for 4 min immediately. The plasma samples were stored at −80°C refrigerator until further treated by SPE and analyzed by LC/MS/MS assay as described above.

2.7. Data Analyses

2.7.1. Pharmacokinetics and Enzyme Kinetics Parameters. The plasma/urine OA and OC concentrations versus time profiles were analyzed using WinNonlin software standard edition version 2.1 (Pharsight Corporation). The noncompartmental model was employed to estimate the pharmacokinetic parameters including time of maximum observed concentration (T_{max}), concentration corresponding to T_{max} (C_{max}), terminal half-life ($t_{1/2}$) and area under curve from time zero to the last sampling time (AUC_{0-t}) for plasma samples, and the 12 h cumulative amount of analytes (A_e) excreted in urine. In human study, trough concentration (C_{trough}) was defined as the minimum concentration obtained and was obtained at 12 h post-dose. The renal clearance (Cl_r) was calculated as the 12 h cumulative amount of analyte in urine divided by the plasma $AUC_{0-12\,h}$. The AUC ratio of OC/OA in plasma and the 12 h cumulative amount ratio of OC/OA in urine were also evaluated.

Percentage of inhibition of CMF1 on the metabolism of OA is calculated according to the following equation: % inhibition = $[1 - (OC/OA)_{CMF\ treatment}/(OC/OA)_{control}] \times 100$, in which OC/OA refers to the ratio of OC and OA concentration in the incubated samples.

Percentage of inhibition for antivirus activity in the plaque reduction assay was calculated by the following equation: % inhibition contributed by treatment = $(N_{virus\ control} - N_{treatment})/N_{virus\ control} \times 100$, where N refers the number of plaques.

2.7.2. Statistic Analyses. For rat studies, all data obtained were expressed as mean ± standard deviation. Unpaired Students t-test was used to compare the pharmacokinetic parameters obtained between the two different treatment groups. ANOVA followed by post hoc test was used for the antiviral effect comparisons among different treatment groups. A $P < 0.05$ was considered to be significant.

In human studies, the comparison of WM + CMF1 to WM alone treatments was evaluated using a 90% confidence intervals (90% CI) approach (USFDA guideline, Drug Interactions Studies, September 2006) [13]. Analysis of variance (ANOVA) using General Linear Model (GLM) procedure was performed on logarithmically (natural logarithm) transformed C_{max}, $AUC_{0-12\,h}$ and the 12 h cumulative amount of OA and OC, as well as the $AUC_{0-12\,h}$ ratio and 12 h cumulative amount ratio of OC/OA. The statistical model included terms describing the effects attributable to sequence, subject (nested in sequence), period, and treatment (formulation). The 90% confidence intervals (CIs) for the differences in the means of logarithmically transformed C_{max}, $AUC_{0-12\,h}$ and the 12 h cumulative amount of OA and OC, and their $AUC_{0-12\,h}$ and 12 h cumulative amount ratio between the combined treatment (WM + CMF1) and western medicine (WM alone) were calculated using two one-sided t-tests. The antilogs of the CIs obtained constitute the 90% confidence interval for the geometric mean ratio, that is, (WM + CMF1)/WM, between both treatments. Difference in the median T_{max} between both treatments was evaluated using Wilcoxon signed-rank test. For comparison of the human antiviral activities, one-way analysis of variance in conjunction with post hoc Turkey's range test was performed. A P value of <0.05 was considered significant.

3. Results

3.1. Effect of CMF1 on the Pharmacokinetics of OA and OC

3.1.1. Findings from Rat Studies. Effect of CMF1 on the pharmacokinetics of OA and OC in rats was evaluated by comparing the pharmacokinetics profiles of OA and OC among Group 1 to Group 3 that have received various types of

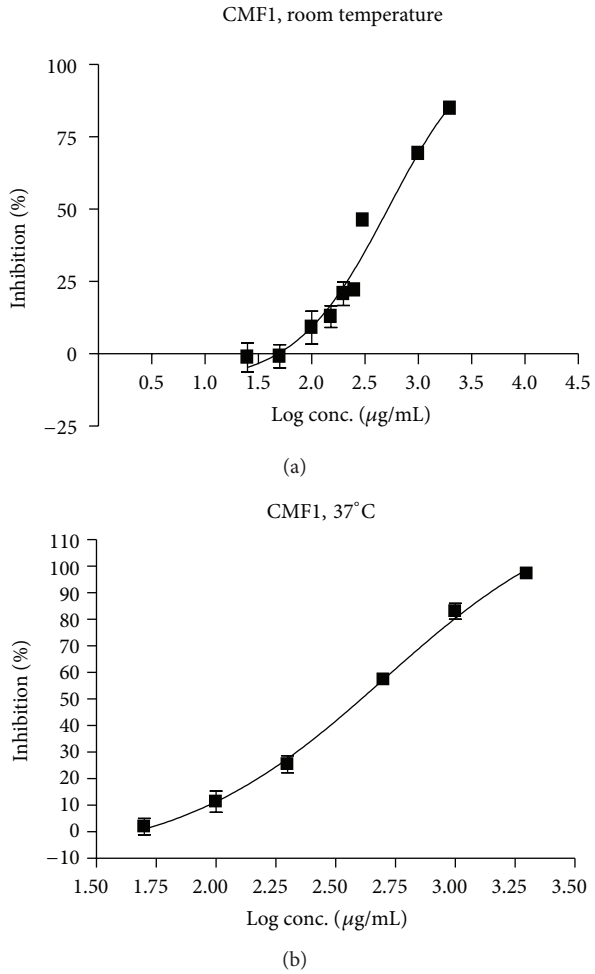

FIGURE 1: *In vitro* inhibition effect of CMF1 on the hydrolysis metabolism of OA in rat plasma at room temperature (a) and 37°C (b).

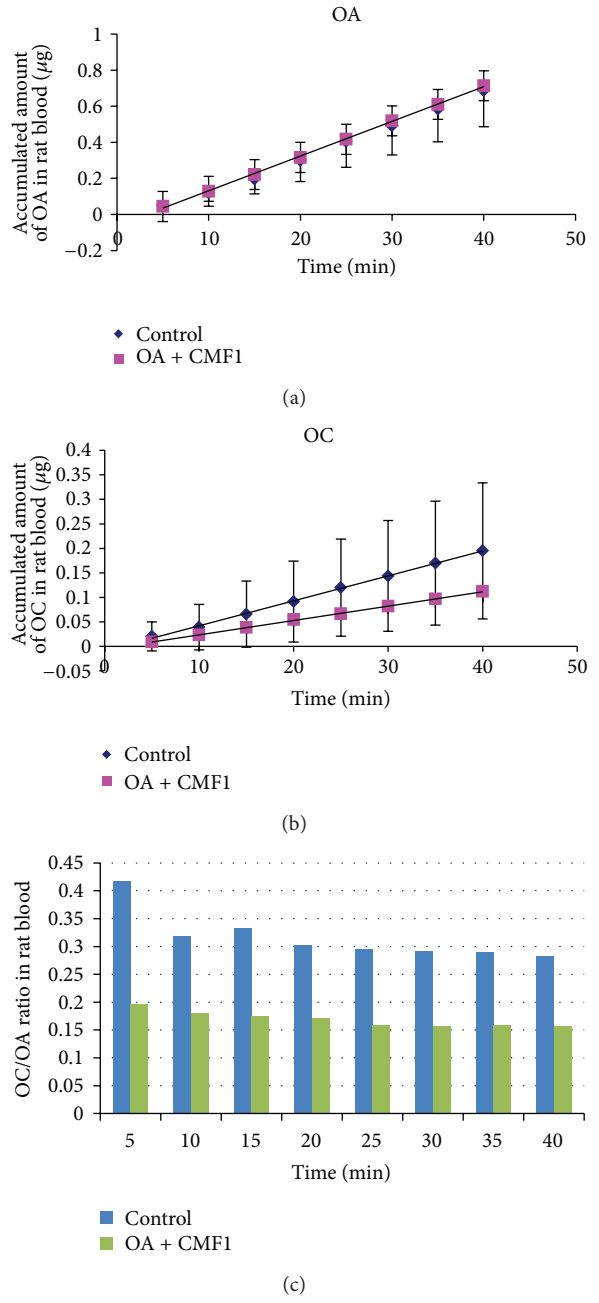

FIGURE 2: Effect of CMF1 on the accumulated amount of OA (a), OC (b), and OC/OA ratio (c) in rat mesenteric blood samples collected at different time points. Control: intestinal perfusion with OA alone.

treatment with OA. The calculated pharmacokinetic parameters of different treatment groups are shown in Table 2.

In comparing Groups 2 or 3 (two different doses of the CMF1 in combination with OA) with Group 1 (OA alone) (Table 3), the mean plasma concentrations of OA and OC were lower in Groups 2 and 3 than that in Group 1. In Group 3, the peak plasma concentration, AUC, and urinary recovery of OC as well as the AUC ratio of OC *versus* OA were significantly decreased in comparison to those in Group 1 ($P < 0.01$). These results suggested that CMF1 probably inhibited the hydrolysis of OA to OC, and such inhibition effect might be dose-dependent since there was a trend of decreased value in Group 2 compared to Group 1 even though no significant differences were found. Since the urinary excretion of OC was significantly decreased with no change in half-life of OA, a decrease of absorption of OA after administration of high dose of CMF1 cannot be ruled out.

Further mechanistic studies revealed that CMF1 exhibited dose-dependent inhibition on the metabolism of OA at both 37°C and room temperature (Figure 1), with a greater % inhibition that occurred at 37°C. Further rat *in situ* intestinal perfusion study indicated that the accumulated OA detected in rat mesenteric blood was not affected by CMF1, whereas accumulation of OC was decreased without statistical significance (Figure 2). However, OC/OA ratio was consistently decreased in presence of CMF1 with time, indicating potential decrease of hydrolysis of OA in presence of CMF1 (Figure 2).

3.1.2. Findings from Human Study. Totally 14 healthy Chinese male adults with average age of 25.2 ± 6.7 years, height of 1.73 ± 0.07 m, weight of 66.9 ± 7.2 kg, and BMI of 22.3 ± 1.8

Table 2: Pharmacokinetic parameters of oseltamivir (OA) and oseltamivir carboxylate (OC) in rats after oral administration of OA alone (30 mg/kg) (Group 1) or OA (30 mg/kg) in combination with CMF1 at 1.95 mg/kg (Group 2) or at 3.90 mg/kg (Group 3), bid for 5 days.

Analyte	PK parameters	Group 1 ($n = 10$)	Group 2 ($n = 11$)	Group 3 ($n = 10$)
OA	T_{max} (min)	33.00 ± 19.75	35.45 ± 24.44	51.00 ± 14.49*
	C_{max} (μg/mL)	1.16 ± 0.30	0.94 ± 0.13#	0.95 ± 0.23
	$t_{1/2,\lambda z}$ (min)	103.18 ± 8.77	100.91 ± 17.72	99.17 ± 11.63
	$AUC_{0-8\,h}$ (μg∗min/mL)	199.85 ± 56.88	172.16 ± 23.70	195.56 ± 35.38
	AUC_{0-inf} (μg∗min/mL)	209.14 ± 60.23	180.00 ± 26.40	213.28 ± 38.76
	$V_{d,\lambda z}$/F (L/k)g	23.39 ± 8.17	24.44 ± 3.75	20.75 ± 4.46
	CL/F (mL/min/kg)	158.34 ± 59.61	169.92 ± 24.63	144.75 ± 25.39
	CL renal (mL/min)	6.10 ± 1.32	5.47 ± 1.21	5.43 ± 0.97
	Renal recovery (% of dose)	15.84 ± 2.88	13.39 ± 3.07#	14.02 ± 1.41
OC	T_{max} (min)	105.00 ± 32.40	106.36 ± 20.63	120.00 ± 24.49
	C_{max} (μg/mL)	1.65 ± 0.40	1.36 ± 0.28#	1.19 ± 0.23**
	$t_{1/2,\lambda z}$ (min)	151.70 ± 25.10	146.45 ± 19.10	156.83 ± 16.78
	$AUC_{0-8\,h}$ (μg∗min/mL)	418.81 ± 90.86	344.13 ± 91.46#	304.84 ± 40.32**
	AUC_{0-inf} (μg∗min/mL)	485.45 ± 113.03	395.67 ± 109.41#	359.21 ± 41.30**
	CL renal (mL/min)	6.33 ± 1.27	6.27 ± 1.76	6.34 ± 0.69
	Renal recovery (% of dose)	35.26 ± 5.40	29.73 ± 7.33#	25.94 ± 3.09**
OC/OA	$AUC_{0-8\,h}$	2.28 ± 0.85	2.03 ± 0.60	1.60 ± 0.35*
	AUC_{0-inf}	2.51 ± 0.86	2.22 ± 0.61	1.73 ± 0.35*

*$P < 0.05$ and **$P < 0.01$ in comparison with those in Group 1; #$0.05 < P < 0.08$ in comparison with those in Group 1.

were recruited and completed in this study, among which 7 subjects were randomly assigned to Group 1A and the other 7 subjects were assigned to Group 1B. Two out of 14 subjects reported mild discomforts during the washout period. These adverse events are mild and unlikely to be related to the study WM and/or WM + CMF1 treatment.

The plasma concentrations of OA and OC *versus* time profiles are presented in Figure 3 and the related pharmacokinetic parameters of OA and OC in plasma and urine from different treatment groups are presented in Table 3. Upon oral administration of WM, OA (prodrug) was rapidly absorbed and converted to OC (active metabolite). In general, the concentration of OC in plasma was in order of magnitude (~10-fold) higher than the respective OA, indicating that OA was extensively metabolized after drug administration. OA (in plasma) reached peak maximum in ~0.5–1 h, which was considerably faster than that of OC (~4–5 h). The concentration of OA in plasma at pre-dose (0 h) on day 5 of the treatment session and 12 h post-dose (trough concentration) was much smaller than that of OC (Table 3), indicating a faster clearance of OA than OC, and is evidenced by the larger renal clearance of OA (~22 L/h) when compared with OC (~16 L/h). Both OA and OC are in large quantity in urine than in plasma, indicating that both compounds are readily eliminated via renal excretion.

As shown in Table 3 and Figure 3, coadministration of WM + CMF1 generally lowered the mean C_{max} of OA and OC in plasma, while only slightly reduction in $AUC_{0-12\,h}$ was observed. The cumulative amount of OA excreted in urine remained unchanged in both treatments, though a slightly lower mean value of OC in urine was found when subjects administered with WM + CMF1. There was no significant difference in T_{max} between both treatments. The C_{trough}, $T_{1/2}$, and renal clearance of OA and OC were also comparable between both treatments. The geometric mean, geometric mean ratio of (WM + CMF1)/WM, and the 90% confidence intervals of C_{max} and $AUC_{0-12\,h}$ of OA and OC, and the OC/OA $AUC_{0-12\,h}$ are summarized in Table 4. The C_{max} (OA) for WM + CMF1 treatment was 21.81% lower than that from WM alone, and the 90% CI ranged from 60.82% to 100.51%, which was lower than the 90% CI criteria from 80% to 125%. More importantly, the geometric mean ratio of $AUC_{0-12\,h}$ of OA, the C_{max} and $AUC_{0-12\,h}$ of the active metabolite (OC), and the OC/OA $AUC_{0-12\,h}$ ratio between WM + CMF1 and WM treatments were near unity, and the 90% CI was found to be within the 80%–125% criteria. The point estimates for 12 h cumulative amount (in urine) of OA, OC, and their OC/O ratio were within 85.97% to 99.32% (Table 4). The 90% CI interval for OA excreted (85.56–115.3%) was within the 80%–125% criteria [13], though a slightly lower interval of 90% CI was observed for OC and OC/OA ratio.

3.2. Effect of Coadministration of CMF1 with OA on the Antivirus Effect of Tamiflu

3.2.1. Findings from Rat Studies. Inhibitory effects of ultrafiltrated plasma collected from the rats in all six Groups on the replication of human influenza A virus (H3N2) are shown in Figure 4. The inhibition effects from all treatment groups were significantly different from those of the control group (Group 6) ($P < 0.01$). The antiviral activities of Group 1 treated with OA alone were found to be significantly enhanced when OA was used in combination with CMF1 at the dose of 1.95 mg/kg (Group 2) ($P = 0.006$) or 3.90 mg

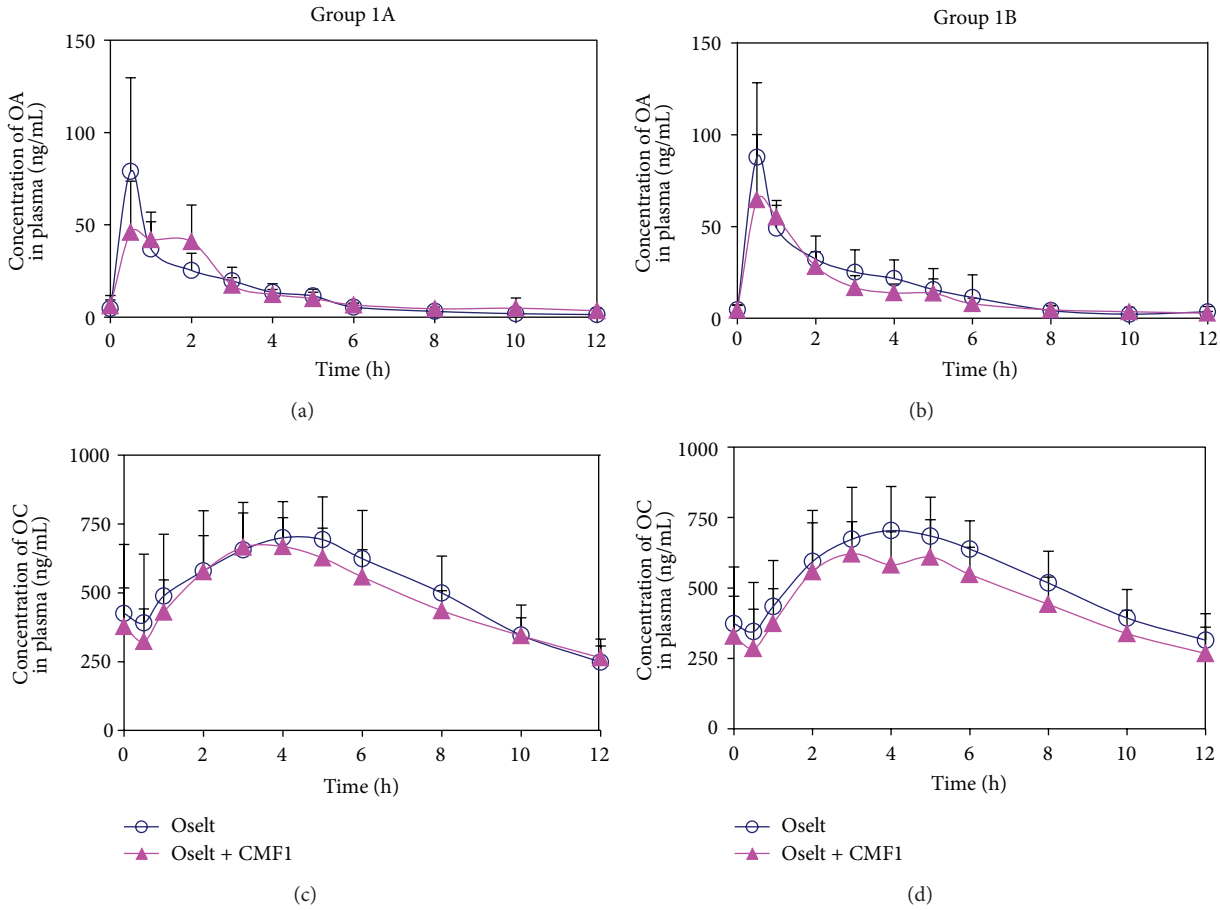

FIGURE 3: Plasma concentration versus time profiles of OA and OC in Groups 1A ($n = 7$) and 1B ($n = 7$) after oral administrations of oseltamivir (Oselt) alone and oseltamivir in combination with CMF1 (Oselt + CMF1) in 14 Chinese male healthy volunteers.

(Group 3) ($P = 0.008$). Treatment with CMF1 alone (Groups 4 and 5) seemed to be able to exhibit significant antiviral activities in a dose-dependent manner. Inhibition effects from OA in combination with CMF1 (Groups 2 and 3) were comparable to those from the positive control with plasma spiked with OC.

3.2.2. Findings from Human Studies. Figure 4 also shows the comparison of the inhibitory effects on virus replication of H3N2 strain of human plasma samples collected at 2 h after drug administration on day 5 from both groups and at different treatment sessions. By comparing the data of Group 1A, no significant difference in the inhibitory effect was observed between WM and WM + CMF1 treatments. On the other hand, subjects treated with WM in the second period, that is, Group 1B (II), had significantly lower ($P < 0.001$) inhibitory effect than those with WM + CMF1 treatment. In fact, the antiviral activity in this Group 1B (II) was also significantly lower than Group 1A with either WM ($P < 0.001$) or WM + CMF1 ($P < 0.01$) treatment. Similar trend was also observed in 0 h data.

4. Discussions

In order to mimic the clinical practice of both OA and CMF1, their human equivalent doses (OA at 30 mg/kg, CMF1 at 1.95 and 3.90 g/kg), dosing frequency (5-day dosing regimen), and dosing methods (oral) have been adopted for the current rat study. In addition, OA and CMF1 have been given in 2 h apart in order to mimic the clinical practice recommended for combination administrations of western and Chinese medicines in Hong Kong.

Our animal pharmacokinetic study indicated that CMF1 can significantly decrease OC concentration and urinary excretion, possibly resulting from a decrease of absorption or inhibition of presystemic metabolism of OA. Based on our *in vitro* and *in situ* intestinal perfusion studies, CMF1 was found to inhibit carboxylesterase activity both in the plasma and liver without a change of OA accumulation in the mesenteric vein. Thus, the observed decrease in OC concentration *in vivo* from administration of CMF1 is most likely a result of inhibition of pre-systemic OA metabolism by CMF1 at the site of mesenteric-portal vein area rather than a decrease of its absorption at the gastrointestinal site. Further studies on the specific components from CMF1 that play the major role

Table 3: Summary of pharmacokinetic parameters following the administration of (a) WM and (b) WM + CMF1 in Chinese male healthy volunteers.

Analytes	PK parameters[a,b]	Treatment	
		WM (n = 14)	WM + CMF1 (n = 14)
OA	C_{trough} (ng/mL)	1.86 ± 1.72	2.36 ± 0.83
	C_{max} (ng/mL)	67.9 ± 27.1	51.3 ± 17.4
	$AUC_{0-12\,h}$ (ng·h/mL)	134.4 ± 41.1	127.8 ± 28.8
	T_{max} (h)	0.61 ± 0.21	0.93 ± 0.51
	$T_{1/2}$ (h)	1.98 ± 0.40	2.94 ± 0.74
	12 h Cum. amt. in urine (μg)	2883 ± 985	2810 ± 743
	Renal clearance (L/h)	22.1 ± 6.8	22.4 ± 5.2
OC	C_{trough} (ng/mL)	206.9 ± 67.7	195.3 ± 51.2
	C_{max} (ng/mL)	535.6 ± 102.2	487.4 ± 82.7
	$AUC_{0-12\,h}$ (ng·h/mL)	4585 ± 1155	4142 ± 783
	T_{max} (h)	4.21 ± 0.97	3.50 ± 0.94
	$T_{1/2}$ (h)	5.16 ± 1.27	5.70 ± 1.35
	12 h Cum. amt. in urine (μg)	72851 ± 15312	62087 ± 10741
	Renal clearance (L/h)	16.3 ± 3.8	15.4 ± 3.2
OC/OA	$AUC_{0-12\,h}$ ratio	37.0 ± 14.4	34.2 ± 10.7
	12 h Cum. amt. ratio	27.2 ± 8.4	23.2 ± 5.9

[a]C_{trough}: plasma concentration of analyte at 12 h postdose; C_{max}: plasma concentration of analyte corresponding to T_{max}; T_{max}: time of maximum observed concentration; $AUC_{0-12\,h}$: area under curve from 0 to 12 h; $T_{1/2}$: Terminal half-life; Cum. amt.: cumulative amount.

[b]Data was presented as arithmetic mean ± SD.

Table 4: Summary of geometric mean, geometric mean ratio, and 90% confidence internal (90% CI) of pharmacokinetic parameters between WM and WM + CMF1.

Analyte	Parameters	Geometric mean		GM Ratio, %[b]	90% CI, %[c]
		WM (n = 14)	WM + CMF1 (n = 14)		
OA	C_{max} (ng/mL)	62.42	48.81	78.19	60.82–100.51
	$AUC_{0-12\,h}$ (ng·h/mL)	129.86	124.76	96.08	88.48–104.33
	12 h Cum. amt. (μg)[a]	2745.53	2726.98	99.32	85.56–115.30
OC	C_{max} (ng/mL)	526.27	480.59	91.32	84.81–98.33
	$AUC_{0-12\,h}$ (ng·h/mL)	4453.91	4067.40	91.32	83.80–99.52
	12 h Cum. amt. (μg)[a]	71215.61	61224.10	85.97	76.64–96.44
OC/OA	$AUC_{0-12\,h}$ ratio	34.30	32.60	95.05	85.64–105.50
	12 h Cum. amt. ratio	25.94	22.45	86.56	76.49–97.94

[a]12 h cumulative amount of analyte in urine.

[b]Geometric mean ratio of (WM + CMF1)/WM.

[c]90% CI criteria of 80%–125% [10].

in the inhibition of OA hydrolysis are warrant explaining such phenomenon. Although the *in vitro* incubation of CMF1 with OA in plasma may not entirely reflect the *in vivo* situation since not every CMF1 component could be absorbed as it appears in the extract, such study is used to preliminarily investigate the potential inhibition of hydrolysis of OA by CMF1, whereas the *in situ* intestinal perfusion study could reflect more of the absorption process in animal.

In rat studies, by comparson with those from OA alone, the C_{max}, urinary recovery and AUC of OC, and the OC/OA AUC ratio in OA + CMF1 groups were significantly decreased (26%-27%) in a dose-dependent manner. The results presented in human study are also in line with the general decreasing trend when OA is co-administered with CMF1, but the extent of reduction is relatively small when compared to the animal studies. To study the effect of CMF1 on the pharmacokinetic parameters of OA and OC in human, a drug interaction approach with 90% CI is adopted in this study [12]. This approach is generally applicable to the interacting drug with one or a few active ingredients. For Chinese medicines formulation that constitutes at least tens of active compounds, it would not be feasible to single out each of them for the study. In this study, the CMF1 is considered to be the interacting drug. The co-administration of CMF1 did not alter the C_{max} and $AUC_{0-12\,h}$ of the active metabolite (OC) to a great extent. The point estimates were found to be near unity

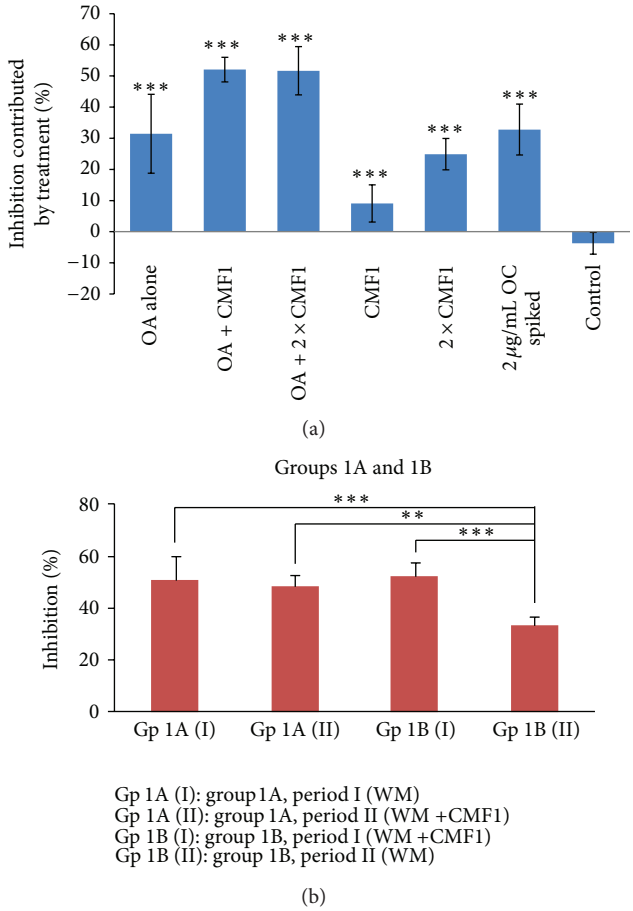

Figure 4: Comparison of inhibitory effect on virus replication of H3N2 of *ex vivo* plasma samples of (a) Rat treatment groups (n = 10 ~ 12 in each group) with all six treatment groups significantly different from control group ($^{***}P < 0.01$), and (b) Human studies groups 1A and 1B collected at 2 h after drug administration on Day 5 ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

for both C_{max} and $AUC_{0-12\,h}$ of OC, with 90% CI of which within the 80%–125% criteria. The C_{max} of OA was reduced to ~78% when OA was co-administered with CMF1. This interaction was deemed unlikely to be clinically relevant, as OA is the inactive form (i.e., the prodrug). More importantly, the $AUC_{0-12\,h}$ of OC (the active metabolite) and the OC/OA $AUC_{0-12\,h}$ ratio (metabolic ratio) are similar between WM and WM + CMF1. The co-administration of CMF1 tends to lower (~14%) 12 h cumulative amount of OC excreted in urine but did not significantly change their pharmacokinetics parameters in plasma. However, care should be considered for renal impaired patients.

To compare the antivirus effects of OA alone or in combination with CMF1 present in rat plasma by plaque reduction assay, plasma sample was required to be ultracentrifuged followed by dilution with maintenance medium in 1 : 500 to avoid cytotoxicity to MDCK cells by removing the protein or the other matrix from plasma. Numerous plasma sample preparations methods have been tried in addition to ultracentrifugation such as liquid-liquid extractions with organic solvents or solid phase extractions of plasma samples followed by evaporating with nitrogen and reconstitute with buffer. Ultracentrifugation of collected plasma samples turns out to be the most efficient method to provide the least cytotoxicity to the MDCK cells with a single step of sample treatment. The enhancement of viral inhibition found in rats treated with OA + CMF1 (when compared with OA alone) was not observed in human study, probably due to the lower dosage of CMF1 used in human study. In addition, results on plaque reduction assay showed that there is a period effect on the inhibition of virus replication in Group 1B. It is noted that the 2 h post-dose plasma concentrations of OC between both treatments were similar (410–436 ng/mL). As shown in Table 3, the mean C_{trough} of OC was around 200 ng/mL (equivalent to ~704 nM) for both WM and WM + CMF1 treatments, which is over 1000-fold higher than the inhibitory concentrations (IC_{50}) of OC against H3N2 (0.2–0.6 nM) or over 3- to 20000-fold higher than those against influenza virus strains (0.01–69.2 nM) [14]. It is expected that the concentration of OC (the active metabolite) would not be significantly reduced with the co-administration of WM + CMF1 and even a decreased inhibitory effect is observed.

Although the current study also indicated that CMF1 appeared to inhibit OA absorption and metabolism, combination of CMF1 with OA led to enhanced viral inhibition of OA as demonstrated by both rat and human studies. Mechanistic study in rat *in situ* intestinal perfusion demonstrated that CMF1 exhibited similar effects as our *in vivo* pharmacokinetic findings with inhibition on the formation of OC and no effect on the blood concentration of OA, which is also consistent with our *in vitro* rat plasma inhibition results. This further confirms the necessity to simultaneously monitor the western drug's pharmacokinetics and overall pharmacodynamics changes for such herb-drug interaction studies since their changes could be contradictory to each other. Such discrepancy is mainly due to the potential contribution of the pharmacodynamics activities from the multicomponents containing Chinese medicines, whose *in vitro* and *in vivo* levels could barely be monitored.

In addition, our present study showed for the first time an enhanced *in vivo* antiviral effect (using plaque reduction assay for *ex vivo* plasma samples) against influenza A virus (H3N2) when a CM formula, CMF1, was combined with OA. Thus enhanced effect from OA + CMF1 was observed despite a decrease in OC plasma concentration. CMF1 itself was also found to possess antiviral effect in a dose-dependent manner. The significant enhancement of antiviral effect by addition of CMF1 to OA may provide a new therapeutic approach for the treatment of resistant avian influenza in the future. Further study on the mechanism of its antiviral effect of CMF1 would warrant the translation of our current findings to the clinical practice.

5. Conclusion

The results show that co-administration with CMF1 in rat and Chinese male healthy volunteers had no clinically significant effect on the pharmacokinetics of OA and OC, although a

generally lower trend was observed in both rat and human studies. Both OA and CMF1 were found to be well tolerated. Thus, the combination therapy of WM (75 mg bid for 5 days) and CMF1 (10 g extract per dose, bid for 5 days) in human at the recommended dosages is therefore considered to be safe and without significant pharmacokinetic consequences. The co-administration of OA and CMF1 can be complementary to each other for the treatment and prophylaxis of influenza.

Conflict of Interests

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

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Research Article

Effect of the African Traditional Medicine, *Sutherlandia frutescens*, on the Bioavailability of the Antiretroviral Protease Inhibitor, Atazanavir

Adrienne C. Müller,[1] Michael F. Skinner,[2] and Isadore Kanfer[1]

[1] *Division of Pharmaceutics, Faculty of Pharmacy, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*
[2] *Biopharmaceutics Research Institute, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*

Correspondence should be addressed to Isadore Kanfer; i.kanfer@ru.ac.za

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The objective of this study was to investigate the effect of *Sutherlandia frutescens* (SF) on the bioavailability of atazanavir (ATV) in twelve healthy male subjects. During Phase I (Day 1), subjects ingested a single dose of ATV and blood samples were drawn before dose and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 9.0, 12, 18, and 24 hours after dose. From Day 3 to Day 14, a single dose of milled SF was administered twice daily to each subject. During Phase II, Day 15, subjects ingested single doses of ATV and SF. Blood samples were drawn as previously described. Plasma was harvested from blood samples and the concentration of ATV therein was determined. For each phase, the mean ATV plasma concentration-time profile was plotted and the means of AUC_{0-24} and C_{max} for ATV were computed. The geometric mean ratios and confidence intervals (CIs) for C_{max} and $AUC_{0-24\,hr}$ were 0.783 (0.609–1.00) and 0.801 (0.634–1.01), respectively. The CIs for both PK parameters fell below the limits of the "no-effect" boundary, set at 0.8–1.25, indicating that SF significantly reduced the bioavailability of ATV. This may potentially result in subtherapeutic plasma concentrations and thus reduced anti-HIV efficacy of ATV.

1. Introduction

The use of African traditional medicines (ATMs) by HIV/AIDS patients in South Africa is a common phenomenon [1–4]. Anecdotal reports detail the use of the indigenous South African plants, *Hypoxis hemerocallidea* (African potato) and *Sutherlandia frutescens* (SF) for the treatment of HIV/AIDS [5]. Despite the widespread use of ATMs, the impact of these medicines on the safety and efficacy of antiretrovirals (ARVs) when used concurrently has not yet been fully determined.

The protease inhibitor (PI), atazanavir (ATV), has a favourable adverse effect profile in comparison to lopinavir; therefore it has been included in the South African clinical guidelines for the management of HIV/AIDS in adults and adolescents (2010), as an alternative to lopinavir in patients who experience intolerable gastrointestinal problems, hyperlipidaemia or hyperglycemia [6]. Like other PIs, ATV is a substrate of the efflux transporter, P-gp [7, 8], which has a role to play in mediating absorption in the small intestine, as well as CYP3A4 and CYP3A5 [9] which facilitate metabolism in the small intestine and liver. ATV may thus be susceptible to pharmacokinetic (PK) interactions with agents able to modulate the activities of this transporter and family of CYP enzymes.

SF is a South African plant which has a long history of use in the practice of traditional medicine, particularly by the *isiZulu*, *isiSotho*, *isiXhosa,* and *Khoi-San* people [10, 11]. Anecdotal reports claim that SF may be useful for alleviating the cachexia (muscle-wasting) associated with HIV/AIDS [11]. Pharmaceutical dosage forms, such as tablets and capsules which contain milled SF leaves are now also available for purchase in pharmacies, health shops, and even online. Triterpenoid and flavonol glycosides (kaempferol and quercetin glycosides) have been isolated from SF plant material [12, 13] and are known to be present in several different

samples of the plant [14]. In addition, SF is also purported to contain the non-protein amino-acid, L-canavanine, the inhibitory neurotransmitter, L-GABA, and the sugar, D-pinitol [11]. We recently conducted *in vitro* studies [15] which showed that aqueous extracts (10 mg/mL) of SF may have the potential to reduce ATV absorption and to inhibit ATV metabolism. A methanolic extract of SF in which less polar constituents in comparison to the aqueous extract are likely present may also inhibit ATV metabolism. A triterpenoid glycoside fraction isolated from SF enhanced absorption of the PI and enhanced its metabolism. These *in vitro* studies therefore alluded to the potential for traditional aqueous decoctions, as well as commercial preparations of SF to modulate the functional activity of ATV influx and/or efflux transporters, such as P-gp and ATV metabolic enzymes, such as CYP3A4 in the human small intestine and liver, respectively. However, the varying effects of the extracts and constituents of SF on ATV absorption and metabolism *in vitro* indicate that the true potential for SF to alter the bioavailability and PK of ATV may only be revealed by undertaking an *in vivo* study. Whilst ATV alone or unboosted is not used *per se* in the management of HIV/AIDs and ARV dosage regimens usually contain other classes of ARVs, the main objective of this study was to investigate the possibility of a drug-drug interaction between an ATM such as SF and ATV and to explore the implications thereof. An ATV single dose/SF multiple dose, one sequence crossover drug-drug interaction study in healthy male subjects was therefore conducted.

2. Materials and Methods

2.1. Materials. ATV sulphate (100.9%) was donated by Aspen Pharmacare (Port Elizabeth, South Africa), and diazepam (DIAZ) was obtained from the Biopharmaceutics Research Institute (Rhodes University, Grahamstown, South Africa). HPLC grade acetonitrile was purchased from Romil Ltd. (Cambridge, United Kingdom). Water was purified by reverse osmosis and filtration through a Milli-Q purification system (Millipore, Milford, M A, USA). Sodium carbonate (99.5%) and ethyl acetate (99–101%) were provided by BDH Laboratory Reagents (Poole, England) and formic acid (99.9%) from Associated Chemical Enterprises Pty Ltd. (Johannesburg, South Africa), whilst n-Hexane (≥98%) was purchased from Merck (Darmstadt, Germany). Fresh human plasma with potassium edetate (K-EDTA) as an anticoagulant was obtained from the South African National Blood Services, Eastern Cape Headquarters (Port Elizabeth, South Africa) and was stored at 4 ± 2°C. Medication used in the clinical study was Reyataz 200 mg ATV sulphate capsules from Bristol-Myers Squibb, Bedfordview, Gauteng, South Africa, and Sutherlandia SU1 300 mg SF tablets from Phyto Nova, Cape Town, Western Cape, South Africa. Analysis of these tablets revealed that triterpenoid glycosides, namely, Sutherlandiosides A, B, C, and D were present at 0.05, 3.02, 0.93, and 0.46 mg/tablet, respectively, while 0.63, 0.67, 1.49, and 0.99 mg/tablet of flavonol glycosides, namely, Sutherlandins A, B, C, and D respectively, were quantified.

2.2. Study Population. Twelve non-smoking, HIV-negative, healthy male subjects between the ages 18 and 55 years and with a body mass index (BMI) between 19 and 30 kg/m^2 were enrolled into the study after giving informed consent and passing a medical, physical, and laboratory screening within one month prior to commencement of the study. The screening included laboratory tests for liver function, hepatitis B and C, and HIV, as well as blood biochemistry, urinalysis, and drugs-of-abuse, such as amphetamines, barbiturates, benzodiazepines, cocaine, methamphetamine, morphine, phencyclidine, tetrahydrocannabinol, tricyclic antidepressants, and alcohol.

The subjects were required to adhere to study restrictions which included no prescription or over-the-counter medicines from one week before the start of the study, no alcohol from four days before the start of the study, and no caffeine and grapefruit juice from 48 hours before the start of the study. Subjects were also prohibited from participating in strenuous exercise from 24 hours before each of the two phases of the study.

2.3. Study Design. Ethical approval was granted by the Rhodes University Ethical Standards Committee to conduct a one-sequence crossover, two phase clinical study with a single dose/multiple dose regimen combination for ATV and SF, respectively. The study was conducted according to the South African Good Clinical Trials guideline [16] and the Declaration of Helsinki and its amendments [17].

The night before the start of the study (Day 0), subjects checked into the clinic and were screened for drugs of abuse and probed to determine compliance with the study restrictions. On Day 1 of the study (start of Phase I), subjects received a light meal before a single 400 mg (2 × 200 mg capsules) dose of ATV (Reyataz, Bristol-Myers Squibb, Bedfordview, Gauteng, South Africa) was administered to each with a 240 mL glass of water. A mouth and hand check was conducted to confirm that the dose had been ingested. Subjects were not permitted to lie down or sleep until 4 hours after dose, unless this was necessary due to an adverse event. Standard meals were provided until 24 hours post-dose. The time at which each subject commenced and ended each meal was recorded as well as the approximate amount consumed.

Ten-millilitre blood samples were collected into BD Vacutainer blood collection tubes (Becton Dickinson, Woodmead, Gauteng, South Africa) containing potassium EDTA as the anticoagulant, at the following time intervals: before dosing (0) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 9.0, 12, 18, and 24 hours post-dose. The exact time at which each sample was withdrawn was also recorded.

From Day 3, each subject started a twice daily regimen (one tablet twice a day) of Sutherlandia SU1 tablets (Phyto Nova, Cape Town, Western Cape, South Africa). The label of Sutherlandia SU1 stated that each tablet contained 300 mg of SF plant material. Subjects reported to the study investigator every day between 08h00 and 09h00 and between 20h00 and 21h00 to receive these doses with a 240 mL glass of water. A hand and mouth check was carried out to confirm that these doses were taken. On Day 14, the subjects checked into the clinic for screening as described for Day 0. On Day 15

(start of Phase II), each subject received a single oral dose of ATV (2 × 200 mg capsules) and a dose (1 × 300 mg tablet) of Sutherlandia SU1, 30 minutes after a light meal. The rest of Phase II was conducted according to the same procedures described for Phase I. Dropouts at any time during the study were not replaced.

Blood samples from both phases were stored in an ice-bath immediately after withdrawal up until centrifugation at 2800 ×g for 10 minutes at 4°C, which was done within 30 minutes. Duplicate aliquots of harvested plasma were stored in polypropylene tubes at −80 ± 10°C until transfer to the analytical laboratory, where the samples were stored at −10 ± 2°C. The tubes were labelled with the study number, phase number, subject number, sample number, and sampling time.

2.4. Safety and Tolerability. A pilot clinical study [18] has been conducted which was the first to provide a scientific basis for the safety of SF in healthy human subjects. Baseline medical and laboratory data were recorded before the study and were compared to post-study results of the tests conducted within three days of the last day of the study. During the study, blood pressure, pulse, and body temperature were monitored before and four hours after dosing with ATV. Subjects were also probed about their well-being using open-ended questions, and any adverse effects experienced were documented.

2.5. Analysis of Plasma Samples. The HPLC system consisted of an Alliance 2695 Separations module and a 2996 Waters photodiode array UV detector coupled to empower data acquisition software (Waters, Milford, MA, USA). A Luna C_{18} (2) (5 μm, 150 × 2.0 mm ID) column (Phenomenex, USA) protected by a Luna C_{18} guard column (Phenomenex, USA) with the same ID was used to achieve chromatographic separation. The mobile phase was filtered under reduced pressure through a 0.45 μm (PVDF) membrane (Durapore, Millipore, Bedford, MA, USA) and degassed using an Eyela Aspirator A-25 (Tokyo Rikakikai Co. Ltd., Tokyo, Japan) prior to use. A validated HPLC-UV method developed and validated in our laboratory [19] was used to analyse the plasma samples.

2.6. Non-Compartmental Analysis. The PK parameters of ATV before and after co-administration with SF were determined by non-compartmental analyses. Exposure measures, $AUC_{0-24\,hr}$ and C_{max} were the primary PK parameters used to evaluate whether multiple dosing of SF altered the single-dose PK of ATV. Other parameters which were monitored included $t_{1/2}$, t_{max}, and k_{el}. These analyses were all conducted using the SAS software (SAS Institute Inc., Cary, NC, USA).

2.7. Statistical Analysis. Equiv Test (Statcon, Witzenhausen, Germany) was used to construct 90% CIs about the equivalence parameter and geometric mean ratio (difference of means on natural log scale) for $AUC_{0-24\,hr}$ and C_{max}. The ratio was computed as Phase II/Phase I, namely, ATV + SF/ATV alone. A clinically significant interaction was concluded if the 90% CIs for $AUC_{0-24\,hr}$ and C_{max} were found to be outside the limits of 0.8–1.25. Based on the CIs for $AUC_{0-24\,hr}$ and C_{max} and the number of subjects, an intra-subject CV% was estimated, which, with the geometric mean ratio for each systemic exposure, was utilised to compute the power of the study.

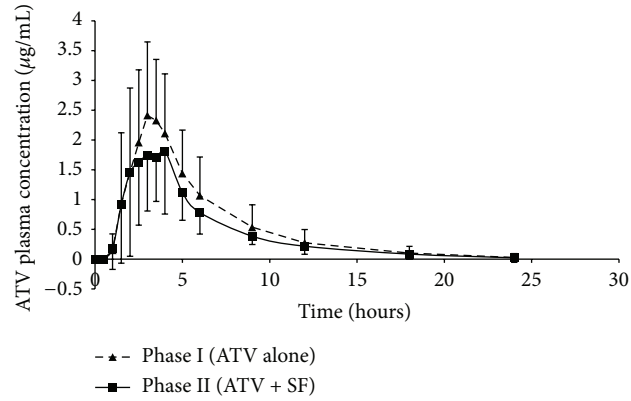

Figure 1: Comparison of ATV plasma concentration-time profiles for Phase I (ATV alone) and Phase II (ATV + SF). Each point represents the mean ± SD; $n = 12$.

3. Results and Discussion

Of the 12 healthy male subjects enrolled to participate in the study, 8 (66.7%) were black, 3 (25%) were white, and 1 (8.3%) was Indian; the mean age was 23 years (range, 19–30 years) and an average BMI of 23.5 Kg/m^2 (range, 20.2–27.6 Kg/m^2) was recorded. The study was completed without any major protocol deviations. There were no subject dropouts and no adverse events were reported.

Figure 1 shows the mean plasma concentration versus time profiles of ATV alone (Phase I) and ATV co-administered with SF (Phase II). It is evident that the two profiles were superimposable for the first 2 hours after dose. From 2 to 4 hours post-dose, the rate and extent of ATV absorption appeared to be reduced when ATV was co-administered with SF in comparison to ATV administered alone. From 4 to 24 hours post-dose, a similar rate of elimination was observed between the two phases. The statistical analysis (Table 1) revealed that for both C_{max} and $AUC_{0-24\,hours}$, the geometric mean ratio (point estimate) and the lower limit of the 90% CI fell well below the lower limit of the "no-effect" boundary of 0.8–1.25, which suggests that a two-week regimen of Sutherlandia SU1 tablets significantly reduced the bioavailability of a single dose of ATV in this cohort of 12 healthy male subjects. Moreover, the power of the study was determined to be 95% and 93% when calculated from the C_{max} and $AUC_{0-24\,hr}$ data, respectively. The sensitivity of the statistical approach applied to the data from 12 subjects was thus adequate to confirm that there was in fact a drug-drug interaction.

The significant reduction in the bioavailability of ATV, in the presence of SF may have occurred due to a decrease in absorption and/or enhanced metabolism of ATV. Potential mechanisms underlying the effect include an increase in

Table 1: Non-compartmental and statistical analysis of PK parameters of ATV.

Pharmacokinetic parameter	Phase I (ATV alone) Arithmetic mean (CV%)	Phase II (ATV + SF) Arithmetic mean (CV%)	Phase II/Phase I Geometric mean ratio (90% CI)
AUC_{0-24} (μg/mL·hour)	13.0 (50.3)	10.0 (38.6)	0.801 (0.634–1.01)
C_{max} (μg/mL)	3.17 (30.3)	2.59 (43.6)	0.783 (0.609–1.00)
T_{max} (hours)	2.71 (26.7)	2.67 (33.3)	N/A
$T_{1/2}$ (hours)	3.77 (39.5)	3.82 (35.1)	N/A
k_{el} ($hour^{-1}$)	0.205 (31.2)	0.200 (30.9)	N/A

the activities and/or the expression of influx and/or efflux transporters, such as P-gp and/or metabolic enzymes such as CYP3A4/5 in the small intestines and/or livers of some of the subjects. Protein expression via induction may only occur after chronic rather than acute administration of a xenobiotic, whilst modulation of the activity of the transporters and enzymes may manifest even after acute administration of the potential interacting agent. *In vitro* studies [15] have shown the potential for an aqueous extract of SF to reduce ATV absorption and for a triterpenoid glycoside fraction from SF to enhance ATV metabolism after acute exposure to the agents. In rats, a single dose of SF (12 mg/kg) had no significant effect on the PK parameters of a single dose of nevirapine [20], which may indicate that, *in vivo*, altered enzyme or transporter activity by SF may not have a major role to play in reducing the bioavailability of this NNRTI. However, ATV is a substrate of both P-gp and CYP3A4, whereas, nevirapine is only a substrate of CYP3A4. This indicates that modulation of the bioavailability of ATV by SF through altered transporter or enzyme activity may occur through both P-gp and CYP3A4 and may thus not necessarily exhibit the same effect as observed for nevirapine. On the other hand, chronic dosing of SF reduced the plasma levels of a single dose of nevirapine in rats, which correlated with an increase in the expression of CYP3A4 [20]. This effect may also have manifested to reduce plasma ATV levels after chronic dosing of SF in healthy humans. *In vitro* and *in vivo* studies to conclusively establish whether the PK interaction between ATV and SF is P-gp- and/or CYP 3A4-mediated should be conducted. The value of this lies in the potential to predict the role of genetic polymorphisms in the transporter and/or enzyme involved in the interaction.

The similar rate of elimination observed between the two phases from 4 to 24 hours post-dose may indicate that SF did not alter post-absorption metabolism or transport pathways of ATV which occur as part of the elimination process in the liver and that the observed decrease in bioavailability of ATV was more likely due to a reduction in the transport and/or an increase in metabolism of ATV during the absorption process in the small intestine. This may imply that the change in activity and/or expression of transporters and/or metabolic enzymes in the small intestines was greater than in the livers of susceptible subjects. To exert an effect in the liver, the "active" phytochemical constituents of SF must be absorbed across the intestinal epithelium into the systemic circulation. The absorption of at least one of the triterpenoid glycosides present in SF may be impeded, since *in vitro* studies demonstrated that it is subject to P-gp-mediated efflux in Caco-2 cells [21]. Similarly, the absorption of other SF constituents may also be hindered; therefore the concentrations of these which reach the liver may not be sufficiently high to exert the effect. This hypothesis needs to be investigated further by application of PK modelling to the data.

The clinical relevance of the potential interaction between SF and ATV is difficult to predict since only a single dose of ATV was evaluated, yet clinically, ATV is dosed chronically. It is therefore not known how SF may affect the steady-state PK of ATV, and thus whether subtherapeutic levels of ATV may result. The bioavailability of ATV is reduced in HIV patients in comparison to healthy subjects [22]; therefore the effect may be more pronounced and severe in patients. On the other hand, the effects of SF may also be diminished if ritonavir, a CYP3A4 inhibitor, is co-administered with ATV as a booster in ARV-experienced patients [22] Moreover, other covariates such as comorbidities and comedication, which also influence enzyme and/or transporter activity or expression [23] were not considered.

The PK of ATV has been found to be influenced by CYP3A5 and P-gp genetic polymorphisms [24]. CYP3A5 expressors had a faster clearance which resulted in a lower C_{min} of ATV than non-expressors. Individuals who possess the wild-type ABCB1 (P-gp) CGC haplotype had a slower clearance of ATV. The primary implication of CYP3A5 polymorphism for ATV-drug PK interactions is that CYP3A5 non-expressors may be more susceptible to CYP3A4-mediated interactions, whether through altered activity or expression, since these individuals rely entirely on CYP3A4 for metabolism of ATV. The association of wild-type ABCB1 (P-gp) CGC haplotype with a slower clearance may suggest that, in individuals who possess this P-gp genetic polymorph, P-gp-mediated transport of ATV

is less significant compared to those who exhibit other P-gp polymorphs. These individuals may therefore be at a lower risk of ATV-drug interactions mediated through P-gp. If studies to determine the mechanism by which SF alters the bioavailability of ATV reveal that the effect is mediated by P-gp and/or CYP3A4, then the potential for this ARV-ATM interaction in a population may be largely governed by the predominance of certain genotypes of the transporter and/or enzyme. In the present study, the effect of SF on the bioavailability of ATV was more significant in a few individuals in comparison to the majority. This suggests that genetic polymorphisms of the transporters and/or enzymes involved may have had a role to play in aggravating or mitigating the effect.

A significant decrease in the steady state C_{max} and AUC of other PIs, indinavir and saquinavir, has been demonstrated when these were coadministered with complementary medicines (CAMs), St. John's wort (indinavir) [25], vitamin C (indinavir) [26], and garlic (saquinavir) [27] to ≤10 healthy subjects, also in one-sequence crossover studies. The approximately 20% decrease in ATV C_{max} and $AUC_{0-24\,hr}$ by SF in this study was comparable to the effect of vitamin C on indinavir but not as significant as the effects of St. John's Wort and garlic on indinavir and saquinavir, respectively, where up to a 57% decrease in one of the PK parameters was observed. These studies in the literature, together with the findings of this study highlight the potential susceptibility of the PIs to PK interactions which result in reduced bioavailability. The need for health care providers to be aware of the CAMs and/or ATMs which patients may be using is therefore emphasised so that (i) preclinical and clinical data on the potential for PK interactions between specific PIs and CAMs or ATMs known to be used concomitantly may be generated and (ii) so that patients may be advised accordingly to prevent clinically proven interactions between CAMs or ATMs and the PIs from occurring.

4. Conclusions

A two-week regimen of Phyto Nova Sutherlandia SU1 tablets which contain SF plant material significantly reduced the C_{max} and $AUC_{0-24\,hr}$ of a single dose of ATV in healthy male subjects, implying that the bioavailability of ATV may be reduced in the presence of SF. SF and ATV co-administration may thus potentially result in subtherapeutic plasma levels of ATV which may in turn cause ATV resistance and treatment failure. Clearly, the presence of other classes of ARVs used in dosing regimens and possible effects during concurrent therapy with ATMs requires investigation. This research has highlighted the potential risk for a reduction in efficacy of an ARV regimen which may ocurr when ATMs and PIs are used concurrently and that patients and health care practitioners alike should be aware of these perils.

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Research Article

PXR-Mediated Upregulation of CYP3A Expression by Herb Compound Praeruptorin C from *Peucedanum praeruptorum* Dunn

Ling Huang,[1,2] Qian Wu,[2] Yu-Hua Li,[1] Yi-Tao Wang,[3] and Hui-Chang Bi[1]

[1] *Laboratory of Drug Metabolism and Pharmacokinetics, School of Pharmaceutical Sciences, Sun Yat-sen University, 132 Waihuan Dong Road, Guangzhou 510006, China*
[2] *School of Pharmaceutical Sciences, Hainan Medical University, Haikou 571010, China*
[3] *Institute of Chinese Medical Sciences, Macau University, Macau 999078, China*

Correspondence should be addressed to Hui-Chang Bi; bihchang@mail.sysu.edu.cn

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We recently reported that Praeruptorin C effectively transactivated the mRNA, protein expression, and catalytic activity of CYP3A4 via the CAR-mediated pathway, but whether and how PC could affect the expression and catalytic activity of CYP3A4 via PXR pathway remains unknown. Therefore, in this study, the effect of PC on the *CYP3A* gene expression was investigated in mice primary hepatocytes after knockdown of PXR by transient transfection of PXR siRNA, and the gene expression, protein expression, and catalytic activity of CYP3A4 in the LS174T cells with PXR overexpression were determined by real-time PCR, western blot analysis, and LC-MS/MS-based CYP3A4 substrate assay, respectively. We found that the level of *CYP3a11* gene expression in mouse primary hepatocytes was significantly increased by praeruptorin C, but such an induction was suppressed after knockdown of pregnane X receptor by its siRNA. In PXR-overexpressed LS174T cells, PC significantly enhanced CYP3A4 mRNA, protein expression, and functional activity through PXR-mediated pathway; conversely, no such increase was found in the untransfected cells. These findings suggest that PC can significantly upregulate CYP3A level via the PXR-mediated pathway, and this should be taken into consideration to predict any potential herb-drug interactions between PC, Qianhu, and the other coadministered drugs.

1. Introduction

The root of *Peucedanum praeruptorum* Dunn. (Qianhu) is widely used in traditional Chinese medicine as antitussive, anti-inflammatory, and antiasthma component and as a remedy for angina. In China, Qianhu is an important ingredient in many kinds of famous traditional Chinese medicine preparations. Most traditional Chinese medicine prescriptions for antitussive contain Qianhu. In recent years, pharmacological evaluations have also revealed a wide variety of activities of Qianhu, including hypotensive [1], coronary dilatory [2], and myocardial dysfunction [3]. Praeruptorin C (PC) is the major active constituent isolated from *Peucedanum praeruptorum* Dunn. PC has been proved to possess multiple pharmacological activities such as prevention and treatment of vascular hyperplastic disease [4], relaxation of the smooth muscle of tracheas and pulmonary arteries [1], relaxation of coronary artery, and decreased contractility activity in left atria [5]. For the increasing wide use of Qianhu and its active component PC in the clinical practice, potential of clinical herb-drug interactions is strikingly increased, and thus, it is important to predict these potential herb-drug interactions.

The underlying mechanisms for most reported herb-drug interactions have not been clearly elucidated, but induction or inhibition of cytochrome P450 (CYP) enzymes is one of the most important risk factors for drug-drug interactions (DDIs). CYP3A4 is responsible for metabolic conversion of more than 50% of the currently clinical drugs to more polar metabolites for easier excretion [6]. Induction or inhibition of CYP3A4 by xenobiotics contributes to the pronounced interindividual variability of its expression and often results in clinically relevant DDIs or herb-drug interactions [7–9].

Clinically and preclinically relevant interactions have been hugely reported between herbs and drugs such as St. John's wort, pomelo, and grapefruit juice, and induction or inhibition of CYP3A4 by xenobiotics often results in these herb-drug interactions [10, 11]. Therefore, CYP3A4-related DDIs have significant clinical impacts.

In recent years, important advances have been made in mechanisms involved in induction or inhibition of CYP3A4. A family of ligand-activated transcription factors, known as nuclear receptors (NRs), has been identified as mediators of drug-induced expression of CYP3A4. Among them, the pregnane X receptor (PXR, NR1i2) and the constitutive androstane receptor (CAR, NR1i3) are the mainly mediator of CYP3A4 [12–14].

To date, pregnane X receptor- (PXR-) mediated CYP3A4 induction has been well studied. PXR can be activated by a wide variety of small, hydrophobic endogenous and exogenous compounds. A number of naturally occurring compounds from herbs such as St John's wort [15], Ginkgo (*Ginkgo biloba*) [16, 17], Gugulipid (*Commiphora mukul*) [18], Wu Wei Zi (*Schisandra chinensis*), Licorice (*Glycyrrhiza uralensis*) [19], and Dan Shen (*Salvia miltiorrhiza*) [20] have been reported to activate PXR. Upon activation by a ligand, PXR unites with RXRα to bind and transactivate several specific elements, such as the everted repeat with a six-nucleotide spacer (ER6) or a direct repeat with a three-nucleotide spacer (DR3), in the 5′ upstream regulatory region of the *CYP3A4* gene [21, 22], thus play a role in regulating transcription of CYP3A4. Therefore, over 64% pharmaceutical companies in the US have adopted cell-based PXR reporter assays routinely to assess the potential for DDIs due to CYP3A4 inductions [23]. In our most recent studies, we found that the active ingredients PA and PD of Qianhu could upregulate CYP3A4 expression by PXR [24, 25], but whether and how PC could regulate CYP3A4 transcription through PXR pathway remains unclear.

The nuclear hormone receptor CAR is a sister xenobiotic receptor of PXR and plays a pivotal role in the induction of drug metabolism. CAR has been reported to synergistically regulate the transcriptional activity of the *CYP3A4* with PXR [26]. According to our previous results [27], PC induction of CYP3A4 at the transcriptional level could be activated through CAR pathways. PC effectively transactivated the mRNA, protein expression, and catalytic activity of CYP3A4 via the CAR-mediated pathway in human LS174T cells. We also found that CYP3A4 luciferase expression was enhanced through PXR pathway by PC [28]. However, the results of luciferase reporter assay could not fully prove that PC could affect the CYP3A4 expression through PXR nuclear receptor pathway. Further study is needed to investigate whether PC could upregulate CYP3A transcription by activation of PXR pathway and whether PC could affect CYP3A4 activity by the cross-talk mediated effect of PXR and CAR.

Therefore, in this study, regulation of *Cyp3a11* (CYP3A4's homolog in mouse) gene expression by PC through PXR pathway was confirmed in mice primary hepatocytes after knockdown of PXR by transient transfection of PXR siRNA. On the other hand, the gene expression, protein expression, and catalytic activity of CYP3A4 were compared in LS174T cells and PXR overexpressed LS174T cells to further prove the effect of PC on PXR and CYP3A and thus to predict any potential herb-drug interactions between PC, Qianhu, and the coadministered drugs.

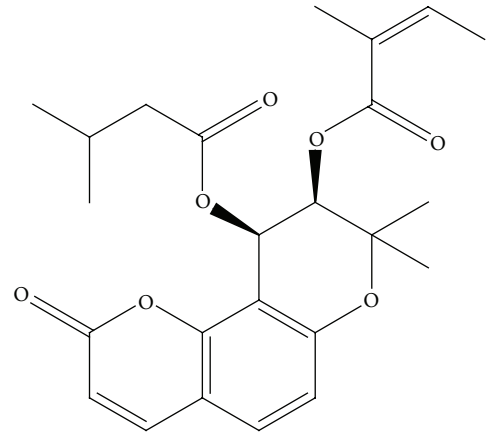

Figure 1: PC physicochemical properties investigated in this study. CAS Number: 73069-27-9. Molecular Formula: $C_{22}H_{22}O_8$.

2. Material and Methods

2.1. Ethics Statement. All procedures of animal experiments, in this study, were in accordance with the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of the People's Republic of China. The animal study protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Sun Yat-sen University (Guangzhou, China) on October 2010, and the approved number was IACUC-01101028.

2.2. Animals. Male BALB/c mice (6–8 weeks old) were purchased from Medical Experimental Animal Center of Guangdong Province and kept in a room at 22–24°C with a light/dark cycle of 12/12 h and 55–60% relative humidity in Experimental Animal Center of Sun Yat-sen University. They had free access to standard rodent chow and clean tap water.

2.3. Chemicals and Reagents. Praeruptorin C (Figure 1) (purity > 99%) was available from Kui Qing Chemical Company (Tianjin, China). Dimethyl sulfoxide (DMSO), dexamethasone (DEX), nifedipine (NIF), dehydronifedipine (DNIF), and loratadine were purchased from Sigma-Aldrich (St. Louis, MO, USA). The validated siRNA targeted to the PXR gene and nontargeting siRNA as a silencer negative control were purchased from Guangzhou RiboBio Co., Ltd (Guangzhou, China). siRNA Transfection Reagent was purchased from Roche (New Jersey, USA). RNAiso Plus and PrimeScriptTM RT Reagent were obtained from Takara (Kyoto, Japan). The *Cyp3a11*, PXR, CYP3A4, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers used in real-time PCR were synthesized by Takara. Anti-CYP3A4 polyclonal antibody was purchased from Millipore Corporation (Rosemont, IL, USA); anti-Cyp3a11 polyclonal antibody

and GADPH antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-rabbit IG-HRP antibody was purchased from R&D Systems (Minneapolis, MN, USA). SDS-PAGE Gel Preparation Kit was purchased from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). Plasmocin TM Ant-mpp and complete protease inhibitor cocktail were purchased from Invitrogen (San Diego, CA, USA) and Roche Diagnostics (Basel, Switzerland), respectively. The cytotoxicity of LS174T cells were detected by the 3-(4,5)-dimethylthiahiazo (-z-yl)-3,5-di-phenytetrazoliumromide (MTT) cytotoxicity assay, and PA did not show cytotoxicity in LS174T cells under the maximum dosage (40 μM).

2.4. Plasmid. The pSG5-hPXR expression vector was provided generously by Dr. Steven Kliewer (University of Texas Southwestern Medical Center, Dallas, TX, USA) [29]. The pGL3-CYP3A4-XREM luciferase reporter construct containing the basal promoter (−362/+53) with the proximal PXR response element (ER6) and the distal xenobiotic responsive enhancer module (XREM, −7836/−7208) of the CYP3A4 gene 5-flanking region inserted to pGL3-Basic reporter vector was provided generously by Dr. Jeff Staudinger, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS, USA [30]. The pRL-TK *R. reniformis* control vector and pSG5-empty vector were obtained from Promega (Madison, WI, USA).

2.5. Preparation of Primary Cultures of Mice Hepatocytes. Liver cells were isolated from BALB/c male mice at 6–8 weeks of age by the two-step collagenase perfusion technique previously described [31] with slight modifications [32]. A density of 2-3 $\times 10^6$ mice primary hepatocyte per g liver tissue with more than 70% cell viability was obtained. Standard culture conditions were as follows: the cells were dispersed in William's medium E containing 10% FBS, insulin (0.5 U/mL), and hepatocytes were seeded into 24-well collagen-coated plate at a density of 1×10^5 cells/well. The hepatocytes anchored to the collagen-precoated plates within 8 h and subsequently formed a monolayer, and only hepatocytes with viability greater than 90% were used for this study. The 24-well collagen-coated plate was maintained at 37°C in 5% CO_2-humidified incubator.

2.6. Transfection of siRNA and Treatment. Mice primary hepatocytes were placed on a 24-well plate at a density of 1×10^5 cells/well. For transfection, hepatocytes were transfected with siRNA targeted to the *mPXR* gene and nontargeting siRNA as a silencer negative control using siRNA transfection reagent (Roche, USA) according to the manufacturer's instructions. For each well, a mixture of siRNA transfection reagent complex was delivered to cells with final concentrations of 50 nM. For monitoring the gene silencing effect, cells were harvested after 72 h, then total RNA was extracted from mice hepatocytes and *mPXR* gene expression was investigated by Q-PCR. Then, the PXR siRNA transfection hepatocytes and negative siRNA transfection hepatocytes were further incubated with 10 μM DEX, 2.5 μM, 10 μM, and 40 μM PC for 72 h. Total RNA was extracted and *Cyp3a11* gene expression was investigated by qPCR. The si-RNA untransfected wild mice primary hepatocytes were exposed to DMSO (0.1%), dexamethasone at 10 μM, PC at 2.5, 10, and 40 μM for 72 h to observe whether PC has the induction effect on mPXR.

2.7. Real-Time PCR Analysis of Cyp3a11 mRNA and mPXR mRNA. Total RNA of mice hepatocytes was isolated using Trizol reagent (Invitrogen) according to the manufacturer's instruction. Total RNA was quantified and reversely transcribed into cDNA using PrimeScript RT reagent Kit (Takara, Kyoto, Japan). The primers for *Cyp3a11* mRNA and m*PXR* mRNA detection were designed as described in our previous report [26]. All the PCR reactions were carried out using SYBR Premix Ex TaqTM kit (Takara, Kyoto, Japan) and according to manufacturer's instructions. Amplification was performed in PCR-Capillarys on a Light Cycler 2.0 Real Time Detection System (Roche, Hercules, CA, USA). Amplification of predenatured products was conducted at 94°C for 60 sec, followed by 45 cycles at 95°C for 30 sec, 59°C for 30 sec, 72°C for 30 sec, 95°C for 10 sec, 65°C for 45 sec, and 40°C for 60 Sec. Fold induction values were calculated according to the equation $2^{-\Delta\Delta Ct}$, where ΔCt represents the differences in cycle threshold numbers between the target gene and *GADPH*, and $\Delta\Delta$Ct represents the relative change in the differences between control group and treatment group. The data presented are the mean ± S.E.M of triplicate experiments.

2.8. LS174T Cells Culture. LS174T cells (derived from Caucasian colon adenocarcinoma) were purchased from Shanghai Institute for Biological Sciences cell resource center. LS174T cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA). Cell lines were cultured at 37°C under a humidified atmosphere of 5% CO_2.

2.9. PXR Expression Plasmid Transient Transfection and Total RNA Isolation. LS174T cells (1.2×10^5 per well) were seeded into 24-well plates, cultured for 24 h, and then were transfected with PXR expression plasmids (300 ng/well). Appropriate cell samples were exposed to PC at a concentration of 2.5, 10, and 40 μM for 48 h. Incubations with 10 μM RIF and DMSO (0.1%) were also included as the controls. Total RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer's instruction. Total RNA was quantified and reverse transcribed into cDNA using PrimeScriptTM RT reagent Kit (Takara, Kyoto, Japan).

2.10. Real-Time PCR Analysis of CYP3A4 mRNA. The primers for *CYP3A4* mRNA detection were designed as described in our previous paper [24]. All the PCR reactions were carried out using SYBR Premix Ex TaqTM kit (Takara, Kyoto, Japan) and according to manufacturer's instructions. Amplification of predenatured products was conducted at 94°C for 60 sec, followed by 45 cycles at 95°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, 95°C for 10 sec, 65°C for 45 sec, and 40°C for 60 Sec. Fold induction values were calculated according to

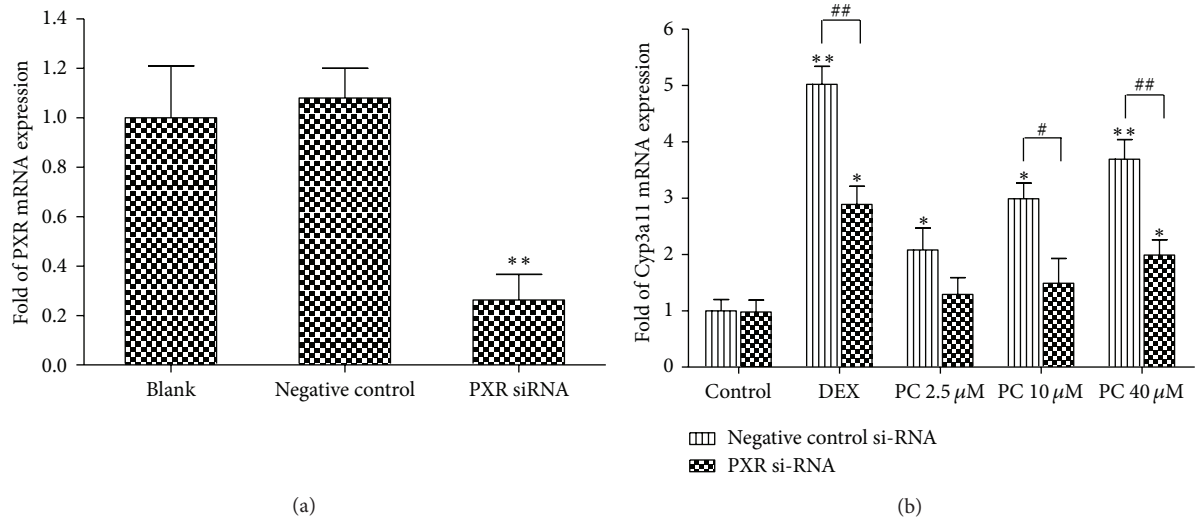

Figure 2: Effects of PC on *Cyp3a11* mRNA expression in PXR knockdown mice primary hepatocytes cells. (a) Mice primary hepatocyte were transfected with negative control siRNA (50 nM) or PXR siRNAs (50 nM). *PXR* mRNA levels were analyzed by real-time PCR. $^{**}P < 0.01$. (b) Mice primary hepatocyte were transfected with negative control (50 nM) or PXR siRNAs (50 nM) then were treated with 10 μM DEX and 2.5, 10, and 40 μM PC for 72 h, respectively. The *Cyp3a11* mRNA expression was analyzed using real-time quantitative PCR. $^{*}P < 0.05$, $^{**}P < 0.01$, for compared with the blank in control siRNA transfected groups, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for comparison between negative control siRNA and PXR siRNA. Values are expressed as mean ± S.E.M ($n = 3$).

the equation $2^{-\Delta\Delta Ct}$, where ΔCt represents the differences in cycle threshold numbers between the target gene and *β-actin*, and ΔΔCt represents the relative change in the differences between control group and treatment group. The effect of PA on *CYP3A4* mRNA levels is presented as fold mRNA expression to vehicle control.

2.11. PXR Expression Plasmid Transient Transfection and Western Blotting Analysis. LS174T cells (1.0×10^6 per well) were seeded into 6-well plates, cultivated for 24 h, and then transfected with hPXR expression plasmids (1 μg/well). Appropriate cell samples were exposed to DMSO (0.1%), RIF at 10 μM, and PC at 2.5, 10, and 40 μM for 72 h. All the proteins were extracted by high-speed centrifugation and quantified using Coomassie Protein Assay Kit (Pierce, Rockford, IL,USA). CYP3A4 protein levels were measured by Western blot analysis, and the Western blot analysis was conducted as described in our previous study [27]. The data were expressed as relative folds over vehicle controls.

2.12. PXR Expression Plasmid Transient Transfection and Functional Analysis of CYP3A4 Activity. LS174T cells (1.0×10^6 per well) were seeded into 6-well plates and cultivated for 24 h. Cells were transfected or untransfected with hPXR expression plasmids (1 μg/well) and then exposed to PC at a concentration of 2.5, 10, and 40 μM for 72 h. The cells were lysed using radio immunoprecipitation assay (RIPA) buffer plus phenylmethylsulfonyl fluoride (PMSF); total protein was extracted by high-speed centrifugation and quantified using Coomassie Protein Assay Kit (Pierce, Rockford, IL,USA). The protein pretreatment was performed as described in our previous report [27], and the concentration of the nifedipine metabolite was determined using an established LC-MS/MS method [33].

2.13. Statistical Analysis. One-way ANOVA followed by Dunnett's multiple comparison post hoc test or unpaired Student's *t*-test was used for statistical analysis of data using SPSS version 13.0 software (SPSS Inc, Chicago, IL, USA). Probability values <0.05 were considered to be statistically significant.

3. Results

The primary hepatocytes used in the study were isolated from wild-type mice and PXR knockdown mice primary hepatocytes were further obtained by transient transfection of PXR siRNA. A density of 2-3 $\times 10^6$ cells per g liver tissue was obtained. Approximately $3–5 \times 10^6$ cells per mouse were harvested with viability and attachment efficiency above 90%. The silencing effects of siRNA transfection on *PXR* gene expression were confirmed by quantitative real-time PCR. Figure 2(a) shows that the *PXR* mRNA level were significantly decreased to 26% in mice primary hepatocytes after transfection of PXR siRNA at 50 nM. Furthermore, no inhibitory effect on *PXR* gene expression was observed in mice primary hepatocytes after transfection of the negative control siRNA.

The effect of PC on the *Cyp3a11* mRNA levels in mice primary hepatocytes with or without PXR knockdown was measured. Figure 2(b) shows that significantly enhanced induction of *Cyp3a11* mRNA expressions by PC were observed in mice primary hepatocytes transfected with the negative control siRNA. After treating the negative control siRNA transfected hepatocytes with PC at 10 μM and 40 μM for 72 h, the level of *Cyp3a11* mRNA was increased to 3.01-fold and 3.69-fold, respectively ($P < 0.05$, $P < 0.01$). However, compared with the PXR unknockdown group, the induction of *Cyp3a11* mRNA expression by PC (10 μM and 40 μM) was

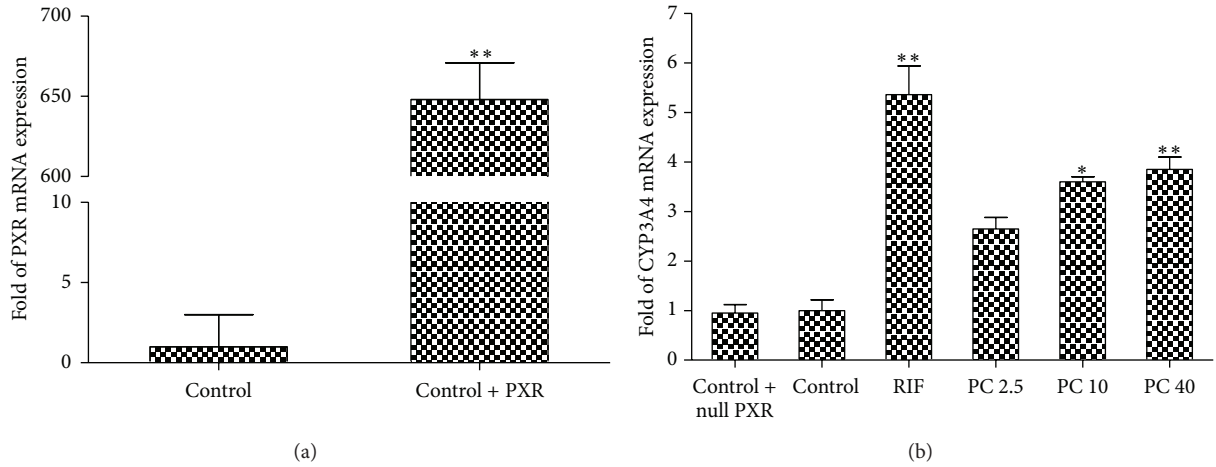

Figure 3: Effects of PC on the expression of *CYP3A4* mRNA in LS174T cells. (a) LS174T cells were transfected with hPXR expression plasmids for 6 h. Total RNA of LS174T cells was isolated, and *hPXR mRNA* levels were analyzed by real-time PCR. The effect of herbal compounds on *hPXR* mRNA levels is presented as fold mRNA expression to control vehicle treated cells. (b) LS174T cells were transfected with hPXR expression plasmids for 6 h. The cells were treated with vehicle control (0.1% DMSO); 10 μM CITCO; and 2.5, 10, and 40 μM PC for 48 h, respectively. The *CYP3A4* mRNA levels were analyzed by real-time PCR. The effect of herbal compounds on *CYP3A4* mRNA levels is presented as fold mRNA expression to control vehicle treated cells. $^{*}P < 0.05$, $^{**}P < 0.01$ for comparison with the control groups. Values are expressed as mean ± S.E.M ($n = 3$).

significantly suppressed 41.8% and 46.3% when PXR siRNA was transfected ($P < 0.05$).

Previously, we found that PC significantly transactivated CYP3A4 reporter gene construct in PXR transiently transfected LS174T cells [27]. But *CYP3A4* mRNA level was not significantly increased in the untransfected LS174T cells after administered PC. In order to determine whether or not PC induces CYP3A4 expression by PXR-mediated pathway, LS174T cells were transfected with pSG5-hPXR expression plasmids, and cells were exposed to PC at 2.5, 10, and 40 μM for 48 h, then the mRNA levels of *PXR* and *CYP3A4* were detected by real-time PCR. As shown in Figure 3(a), compared with the untransfected LS174T cells, the PXR expression had significantly increased after transfection with pSG5-hPXR expression plasmids, indicating that the PXR overexpression cells model was successfully established. Figure 3(b) shows that significant increase of *CYP3A4* mRNA by RIF (as the positive control) was found to compare with the vehicle control (5.36-fold at 10 μM, $P < 0.01$). At 10 μM and 40 μM, PC can significantly induce *CYP3A4* mRNA expression in transfected LS174T cells to 3.6- and 3.85-fold ($P < 0.05$, $P < 0.01$), respectively. However, in our previous study, we did not observe significant increase of *CYP3A4* mRNA level in the untransfected LS174T cells which was relatively low expression of PXR [28].

To confirm that PC induces CYP3A4 through PXR-mediated pathway, CYP3A4 protein expression in PXR overexpressed LS174T cells were measured by Western blot analysis. After transfection with pSG5-hPXR expression plasmids, cells were exposed to PC at 2.5, 10, and 40 μM for 72 h; CYP3A4 protein expression was subsequently measured by Western blot assay. As shown in Figure 4(a), LS174T cells transfected with PXR plasmid yielded significant increase to 2.28-fold ($P < 0.05$) in CYP3A4 protein expression after 72 h exposure to RIF at 10 μM. Compared with the vehicle control, the expression was significantly induced to 2.19- and 2.22-fold by PC at 10 μM and 40 μM, respectively ($P < 0.05$). These results were generally consistent with those observed in the real-time PCR assay. Also, Figure 4(b) shows that the CYP3A4 protein expression was not increased by PC in untransfected LS174T cells.

Furthermore, effect of PC on CYP3A4 enzyme activity was measured in PXR-overexpressed cells by LC-MS/MS assay. After transfection with PXR expression plasmids, cells were exposed to PC at 2.5, 10, and 40 μM for 72 h; CYP3A4 activity was subsequently determined by LC-MS/MS assay based on measurement of specific CYP3A4-mediated Nifedipine dehydrogenation. Figure 5 shows that LS174T cells transfected with plasmid encoding PXR plasmids yielded significant increase to 2.4-fold in CYP3A4 catalytic activity after 72 h exposure to RIF at 10 μM ($P < 0.01$). LS174T cells transfected with plasmid encoding PXR plasmids yielded significant increase to 1.81- and 2.1-fold in CYP3A4 catalytic activity after 72 h exposure to PC at 10 μM and 40 μM, respectively, compared with control group ($P < 0.05$, $P < 0.01$). At the same time, we can also observe that CYP3A4 catalytic activity was not increased by PC in untransfected LS174T cells.

4. Discussion

As for the increasing wide use of herbal medicines, the potential of clinical herb-drug interactions are dramatically increased, which is frequently caused by induction or inhibition of metabolizing enzymes. CYP3A4 is the most abundant and important CYP isoform expressed in human liver.

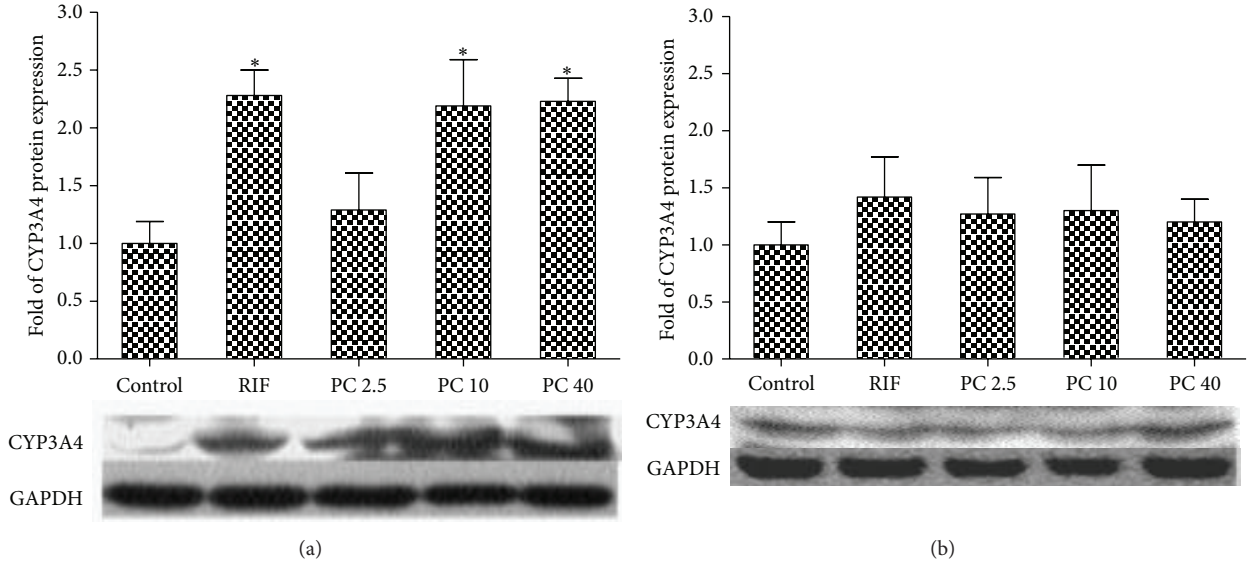

Figure 4: Effects of PC on the protein expression of CYP3A4 in LS174T cells. (a) LS174T cells were transfected with pSG5-hPXR expression plasmids for 6 h. The LS174T cells were treated with vehicle control (0.1% DMSO); 10 μM RIF; and 2.5, 10, and 40 μM PC for 72 h, respectively. The cell homogenates were subjected to western blot. (b) LS174T cells were untransfected with pSG5-hPXR expression plasmids for 6 h. The LS174T cells were treated with vehicle control (0.1% DMSO); 10 μM RIF; and 2.5, 10, and 40 μM PC for 72 h, respectively. The cell homogenates were subjected to western blot. The data were expressed as relative folds over vehicle controls. $^{*}P < 0.05$ for comparison with the control groups. Values are expressed as mean ± S.E.M ($n = 3$).

Important advances have been made in the understanding of the mechanisms involved in induction or inhibition of CYP3A4 [34]. Nuclear receptors, especially the PXR and CAR, are the most significant regulator of *CYP3A4* gene expression. Xenobiotics metabolism research has found that CYP3A4 expression and activity were affected by many ligands through both PXR and CAR pathways. PXR and CAR could generate the "cross talk" regulation effect on CYP3A4 transcription [35, 36].

Our previous results demonstrate that PC effectively transactivated mRNA expression, protein expression, and catalytic activity of CYP3A4 via the CAR-mediated pathway in human LS174T cells [27]. However, the effect of PC on the transactivation of CYP3A4 via PXR pathway is not fully understood, and it will be of clinical significance to elucidate the effect of PC on CYP3A4 through the PXR-mediated pathway, and then to find whether PC has "cross-talk" effect on PXR and CAR pathways. Therefore, in this study, we investigated the effect of PC on the *Cyp3a11* mRNA expression in PXR knockdown or PXR unknockdown mice primary hepatocytes, and regulations of CYP3A4 mRNA, protein expression, and catalytic activity by PC through PXR pathway were further investigated in human LS174T cells with overexpression of PXR.

Mice primary hepatocyte is an ideal model used for *PXR* mRNA knockdown for its higher endogenous PXR expression and easier obtain than human primary hepatocyte. PXR knockdown mice primary hepatocytes were successfully obtained by transient transfection of PXR siRNA and further validated by measuring the *PXR* mRNA level 72 h after transfection. We found that PC could significantly increase the level of *CYP3a11* gene expression in negative transfection groups, and the induction of *Cyp3a11* mRNA by PC was significantly suppressed in the *PXR* knockdown mice primary hepatocytes. These results demonstrate that knockdown of PXR suppresses the upregulation of *Cyp3a11* mRNA by PC, indicating that PXR pathway is causally involved as a contributing mediator.

On the other hand, LS174T cells has lower endogenous expression of PXR; thus, it is more ideal to transfect PXR into LS174T cells to produce the PXR high expression cell model. To confirm that PC induces CYP3A4 directly through PXR-mediated pathway, CYP3A4 mRNA, protein expression, and catalytic activity in PXR-overexpressed LS174T cells were measured by real-time PCR, western blot analysis, and LC-MS/MS assay. According to our previous results [28], *CYP3A4* mRNA expression could not be induced by PC in the LS174T cells without transfection with PXR plasmid. However, in the current study, after transfection with PXR plasmid, PC can significantly induce the level of *CYP3A4* mRNA. These results suggest that PC-mediated transactivation of *CYP3A4* gene via interaction with PXR pathway. Finally, we analyzed CYP3A4 protein expression and enzymatic activity in LS174T cells exposed to PA for 72 h after PXR transfection. Significant increase of CYP3A4 activity was observed in LS174T cells transfected with PXR plasmid. In order to further confirm the impacts of PC on the untransfected LS174T cells, the cells were treated with PC directly. The results show that increase of CYP3A4 protein expression could not be observed in untransfected LS174T cells, which was consistent with the results of mRNA expression and catalytic activity. The results indicate that PC

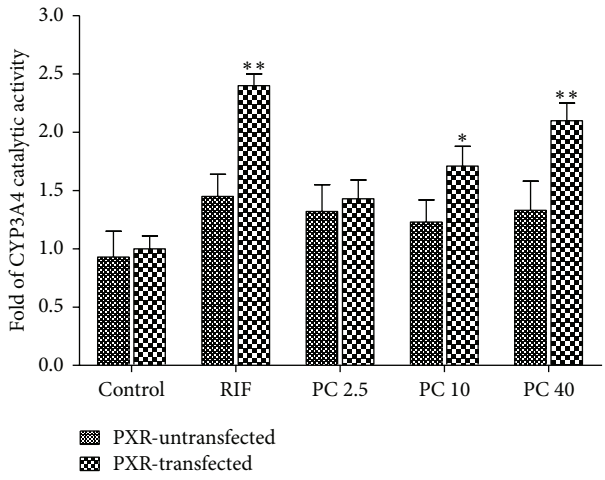

Figure 5: Determination of CYP3A4 catalytic activity. LS174T cells were transfected or untransfected with pSG5-hPXR expression plasmids for 6 h. The LS174T cells were treated with vehicle controls (0.1% DMSO); 10 μM RIF; and 2.5, 10, and 40 μM PC for 72 h, respectively. Total protein was isolated; total protein (1 mg/mL) was incubated with NIF and NADPH. The concentration of the DNIF, which is the metabolite of NIF through CYP3A4 pathway, was determined using the previously developed LC-MS/MS method. $^{*}P < 0.05$, $^{**}P < 0.01$, compared to control (DMSO) treatment group with PXR expression plasmid. Values are expressed as mean ± S.E.M ($n = 3$).

can upregulate CYP3A4 protein level and its catalytic activity through PXR pathway.

As mentioned above, PXR and CAR are critical determinants of xenobiotics-induced CYP3A4 expression and they can generate "cross-talk" regulation on CYP3A4 transcription. It is not a surprise that compounds that regulate CYP3A4 via PXR pathway might also interact with CAR to activate CYP3A4. We recently reported that PC can effectively transactivate luciferase activity and mRNA and protein expression of CYP3A4 via CAR-mediated pathway in LS174T cells; the results demonstrate that CAR also plays a role in the activation of CYP3A4 by PC [27]. In current results, it was elucidated for the first time that PC can effectively transactivate CYP3A4 mRNA expression, protein expression, and catalytic activity via the PXR-mediated pathway in human LS174T cells. Combined with current results, PC can coactivate the CAR-mediated and PXR-mediated pathway to coregulate CYP3A expression. Therefore, PC could regulate CYP3A4 gene expression, protein expression, and activity through both PXR- and CAR-mediated pathways and thus accelerate detoxification and metabolism of CYP3A4 substrates. Further mechanistic studies are needed to investigate how the herbal compounds interact with PXR, CAR, and their coactivators and corepressors.

Besides PC, Praeruptorin A (PA) and Praeruptorin D (PD) are the other two major active constituents isolated from *Peucedanum praeruptorum* Dunn (Qianhu). For the increasing wide use of Qianhu and its active components such as PA, PC, and PD in the clinical practice, it is important to predict any potential herb-drug interactions between PA, PC, PD, Qianhu, and the other coadministered drugs. Most recently, we reported that PA and PD can also significantly upregulate CYP3A level via the PXR-mediated pathway [24, 25]. From the results of this study and our published data, we found that PD has the stronger regulation on CYP3A4 mRNA, protein, and enzyme activity via PXR pathway than PA and PC. However, there is no significant difference in the regulation of CYP3A by these three compounds. Very recently, we also found that PA and PC can significantly upregulate the expression of MRP2 and UGT1A1 via CAR-mediated pathway (data not shown). Taken together, all these findings suggest that PA, PC, and PD have varieties of effects on metabolizing enzymes or transporters via PXR or CAR mediated pathways; thus, this information should be taken into consideration to predict any potential herb-drug interactions between PA, PC, PD, *Peucedanum praeruptorum* Dunn, and the other coadministered drugs.

5. Conclusion

In summary, this study found that the level of *CYP3a11* gene expression in mouse primary hepatocytes was significantly increased by PC, but such an induction was suppressed after knockdown of PXR by its siRNA. PC significantly enhanced CYP3A4 mRNA, protein expression, and functional activity through PXR-mediated pathway in PXR-overexpressed LS174T cells; conversely, no such increase was found in the untransfected cells. These findings suggest that PC can significantly upregulate CYP3A level via the PXR-mediated pathway and this should be taken into consideration to predict any potential herb-drug interactions between PC, Qianhu, and the other coadministered drugs. More attentions should be paid to ensure the safety in clinical utilization of Qianhu and PC.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Research Article

Opposite Effects of Single-Dose and Multidose Administration of the Ethanol Extract of Danshen on CYP3A in Healthy Volunteers

Furong Qiu,[1] Jian Jiang,[1] Yueming Ma,[2] Guangji Wang,[3] Chenglu Gao,[1] Xinfeng Zhang,[1] Liang Zhang,[1] Songcan Liu,[1] Min He,[1] Leilei Zhu,[1] Yujie Ye,[1] Qiuye Li,[1] and Ping Miao[1]

[1] *Lab of Clinical Pharmacokinetics, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[2] *Lab of Pharmacokinetics, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[3] *Key Lab of Drug Metabolism & Pharmacokinetics, China Pharmaceutics University, Nanjing 210038, China*

Correspondence should be addressed to Furong Qiu; furong_qiu@126.com and Jian Jiang; jiangjiansg@126.com

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The aim of this study was to investigate the effect of single- and multidose administration of the ethanol extract of danshen on in vivo CYP3A activity in healthy volunteers. A sequential, open-label, and three-period pharmacokinetic interaction study design was used based on 12 healthy male individuals. The plasma concentrations of midazolam and its metabolite 1-hydroxymidazolam were measured. Treatment with single dose of the extract caused the mean C_{max} of midazolam to increase by 87% compared with control. After 10 days of the danshen extract intake, the mean AUC_{0-12}, C_{max}, and $t_{1/2}$ of midazolam were decreased by 79.9%, 66.6%, and 43.8%, respectively. The mean clearance of midazolam was increased by 501.6% compared with control. The in vitro study showed that dihydrotanshinone I in the extract could inhibit CYP3A, while tanshinone IIA and cryptotanshinone could induce CYP3A. In conclusion, a single-dose administration of the danshen extract can inhibit intestinal CYP3A, but multidose administration can induce intestinal and hepatic CYP3A.

1. Introduction

Danshen, the dried root of *Salvia miltiorrhiza*, has been used for hundreds of years to treat coronary heart and cerebrovascular disease [1]. The herbal medicine is also available as a prescription or an over-the-counter drug in countries such as China, Singapore, Korea, Janpan, Russia, Cuba, and South Africa and as a dietary supplement in the United States [2–6]. Danshen as a medicine or dietary supplement is often administered in combination with therapeutic drugs, causing clinically important herb-drug interactions and adverse outcomes [3]. Thus, safety of administrated danshen is of great concern. At present, many danshen preparations (e.g., crude drug, lipophilic extract, and hydrophilic extract) are commercially available, and the ethanol extract of danshen rich in lipophilic constituents is commonly used in Chinese clinics. The main constituents in the extract are tanshinones including cryptotanshinone, tanshinone IIA, tanshinone I, and dihydrotanshinone I.

CYP enzymes (CYPs) play an important role in detoxification and systemic clearance of xenobiotics. Of >55 human CYP isozymes presently known, CYP3A is considered to be the most important drug-metabolizing enzyme. It participates in metabolism of >60% of all marketed drugs. As the critical role of CYP3A in drug metabolism, inhibition or induction of this enzyme often leads to drug interactions [5]. In recent years, some studies revealed the effect of danshen extract on CYP3A4, especially the danshen extract rich in lipophilic constituents. Kuo et al. reported that the ethyl acetate extract of danshen could induce CYP3A in C57BL/6J mice [7]. By using a reporter gene assay and RT-PCR, Yu et al. demonstrated that cryptotanshinone and tanshinone IIA in the ethanol extract of danshen could activate the human pregnane and xenobiotic receptor (PXR) and consequently induce the expression of the *CYP3A4* gene [8]. It was found in the in vitro study using human liver microsomes that the ethanol extract of danshen had a significant inhibition toward CYP3A4-mediated midazolam metabolism [9].

The aim of this study was to investigate the effect of single- and multi-dose administration of the ethanol extract of danshen on the in vivo CYP3A activity in healthy volunteers. The constituent(s) inhibited or induced to CYP3A was also investigated using human liver microsomes and human cryopreserved hepatocytes. It will provide valuable information for using the danshen preparation in clinical practice.

2. Meterials and Methods

2.1. Meterials. Tanshinone I, cryptotanshinone, tanshinone IIA, and dihydrotanshinone I were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). NADPH, midazolam, and 1-hydroxymidazolam were purchased from Sigma-Aldrich (St. Louis, MO). HPLC-grade acetonitrile, methanol, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore Corporation, Billerica, MA). Midazolam tablets (15 mg/tablet, Lot 20100801) were manufactured by Jiangsu Nhwa Pharma Corporation. The ethanol extract of danshen in the form of capsule (250 mg/capsule, Lot 20090904) was manufactured, and the quality control was established and enforced strictly by Hebei Xinlong XiLi Pharmaceuticals Ltd according to state drug standard (China State Food and Drug Administration, Ws3-B-3140-98-009). The lipophilic components (tanshinone I, tanshinone IIA, cryptotanshinone, and dihydroxytanshinone I) and fingerprint of the ethanol extract of danshen were determined by HPLC on a C18 column and with a mobile phase of 20 mmol ammonium acetate : acetonitrile (30 : 70, V/V). The detection wavelength was set to 270 nm. For determination of fingerprint of the ethanol extract of danshen, a gradient mobile phase was used. The fingerprint was showed in Figure 1, and the contents of tanshinone IIA, cryptotanshinone, tanshinone I, and dihydrotanshinone I were 106.2 mg/g, 88.0 mg/g, 53.1 mg/g, and 13.5 mg/g, respectively.

Human liver microsomes (10-donor pool, mixed gender) and human cryopreserved hepatocytes (Lot ONO and JYM, male donors) used in this study were provided by the Research Institute for Liver Disease Co. (Shanghai, China).

2.2. Subjects. The clinical protocol and informed consent form were approved by the independent ethics committee of Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine. 12 healthy male volunteers were enrolled in the study after obtaining written informed consent.

All subjects were nonsmokers and judged to be healthy according to their medical histories, complete physical examinations, electrocardiograms, and routine laboratory test results. Subjects abstained from consuming herbal and citrus fruit products for 2 weeks before the study and from alcohol and medications for 2 weeks before and during the study period; caffeine-containing foods and beverages were also excluded during the study period.

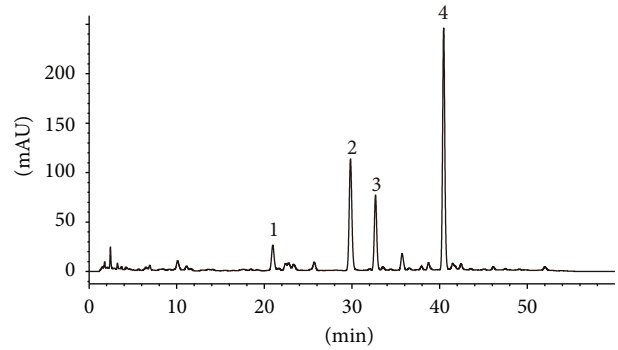

Figure 1: Representative chromatograms fingerprint of the danshen ethanol extract: (1) dihytdoxytanshinone I; (2) crytanshinone I; (3) tanshinone I; (4) tanshinone IIA.

2.3. Pharmacokinetics of Midazolam in Healthy Volunteers after Oral Administering of Midazolam. The study design was a sequential, open-label, and three-period trial [10] conducted at the Shuguang Hospital phase I clinical trial ward. On the morning of day 1, the volunteers took a single dose of 15 mg of midazolam. Beginning on day 2, they received the ethanol extract of danshen (1 g, three times a day) for 10 days. On day 12, the volunteers received 1 g danshen extract 0.5 h before taking 15 mg midazolam. After a 3-week washout period, a single dose of the danshen extract (1 g) was administered 0.5 h before taking 15 mg of midazolam. Midazolam pharmacokinetic study days followed an identical course: the volunteers were fasted overnight before each dosing. The subjects were provided a light standard meal 4 h after medication intake and at 6 PM on the three test days of taking midazolam. Smoking and consumption of alcohol, coffee, tea, and any drugs were prohibited during the study. Four milliliters of blood was sampled from the forearm veins at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after taking midazolam and kept in heparinized Eppendorf tubes. The blood samples were centrifuged, and plasma was separated and stored at −80°C until the time of analysis.

2.4. Analysis of Midazolam and 1-Hydroxymidazolam in Plasma Samples. Plasma samples were spiked with an internal standard (diazepam) and extracted with ethyl acetate. After evaporation of the organic solvent under nitrogen, reconstituted residues of the organic phase were analyzed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (API 4000, Applied Biosystems/SCIEX, CA). Chromatographic separation of the compounds was accomplished using a C_{18} column (5 μm, 4.6 mm × 150 mm, Agilent) with water phase (ammonium acetate 4 mmol/L and methanoic acid 0.08%) : methanol (10 : 90, v : v) as the mobile phase at a flow rate of 0.80 mL/min. The MS/MS system was operated in positive ion electrospray ionization. The multiple reaction monitoring (MRM) detection mode was applied to midazolam (m/z: 326.4 → 291.2), 1-hydroxylmidazolam (m/z: 341.8 → 324.0), and diazepam (m/z: 285.2 → 193.1). The collision energy (CE), declustering potential (DP), and collision cell exit potential (CXP) were set as follows:

midazolam: 35.88 V, 105.14 V, and 13.00 V, respectively; 1-hydroxymidazolam: 33.00 V, 91.05 V, and 17.94 V, respectively; and diazepam: 43.00 V, 98.06 V, and 11.45 V, respectively.

The quantitative range for measuring midazolam and 1-hydroxymidazolam was 0.1 ng/mL to 150 ng/mL. The accuracy, precision, recovery, and stability tests all met the requirements for quantitative determination in biological samples. No matrix effect existed in this LC-MS/MS method.

2.5. Analysis of Tanshinones in Plasma Samples. For tanshinones, tanshinone I, cryptotanshinone, tanshinone IIA, and dihydrotanshinone I levels were determined by LC-MS/MS as previously described [11–13]. The plasma extraction method, chromatographic column, mobile phase, and instruments were the same as those previously mentioned. The mass spectrometer was operated in the positive ion mode, and quantification was thus performed using the MRM of the transitions of m/z 277.1 → 249.0 for tanshinone I, 297.3 → 251.2 for cryptotanshinone, 295.2 → 249.2 for tanshinone IIA, 279.2 → 233.2 dihydroxytanshinone I, and 285.2 → 193.1 for diazepam. CE, DP, and CXP were set as follows: tanshinone I: 29.00 V, 98.06 V, and 16.32 V, respectively; cryptotanshinone: 43.00 V, 123.00 V, and 15.12 V, respectively; tanshinone IIA: 27.36 V, 103.66 V, and 15.00 V, respectively; dihydrotanshinone I: 28.32 V, 112.07 V, and 6.92 V, respectively; and diazepam: 43.00 V, 98.06 V, and 11.45 V, respectively.

2.6. Inhibition of CYP3A Activities by Danshen Ethanol Extract and Dihydrotanshinone I in Human Liver Microsomes [14]. HLMs used in this study were provided by the Research Institute for Liver Disease Co. (Shanghai, China). The microsomes were prepared from ten individual human donor livers.

The CYP3A enzymatic activities were characterized based on reaction of midazolam 1-hydroxylation. Incubation mixtures were prepared in a total volume of 200 μL as follows: HLMs (1 mg/mL) 40 μL, 1 mM NADPH 10 μL, 100 mM phosphate buffer (pH 7.4) 130 μL, 70 μM midazolam 10 μL, and a range of concentrations of tested compound 1 μL. There was a 5 min preincubation period at 37°C before the reaction was initiated by adding the NADPH. The reactions were conducted for 5 min. Triplicate samples were run to generate IC_{50} value by incubating midazolam at 3.5 μM in the presence of five concentrations of ethanol extract of danshen (final concentrations 0.2–100.0 μg/mL) and dihydrotanshinone I (final concentrations ranging from 0.5 to 100.0 μM) in the incubation mixture. Ethanol extract of danshen and dihydrotanshinone I were dissolved in DMSO (final concentration 0.5% in HLMs).

2.7. Induction of CYP3A4 mRNA by Tanshinones in Human Hepatocytes. Human cryopreserved hepatocytes were thawed in hepatocyte thawing medium and were seeded in collagen I precoated 24-well plates, with each well having a cell density of 3.0×10^5 viable cells in 0.25 mL of hepatocyte plating medium. Viability as determined by trypan blue exclusion was 85% or better for this study. The cells were maintained at 37°C in a humidified incubator with 90% atmospheric air and 5% CO_2. Twenty-four hours after isolation, plating, and incubation, hepatocytes were treated with vehicle, which contained the same amount of DMSO (0.1%), tanshinones (2 μM), and rifampicin (25 μM) for 72 hours. All drugs were dissolved in DMSO and then added to the culture medium (final DMSO concentration, 0.1%). Incubation medium containing 0.1% DMSO served as the vehicle control. After daily treatment for 3 days, the medium was removed, and the cells were washed with saline. Total RNA was isolated from cells using TRIzol reagent (Invitrogen) according to the manufacturer-supplied protocol. Quantitative real-time PCR was performed using gene-specific primers and the SYBR Green PCR kit (Invitrogen) in an ABI 7900 system (Applied Biosystems). The relative quantity of the target *CYP3A4* gene compared with the endogenous control (glyceraldehyde-3-phosphate dehydrogenase) was determined by the ΔΔCT method. The following primer sets were used in this study: CYP3A4 (5′-AGAAAGTCGCCTCGAAGATACA-3′ and 5′-GCTGGACATCAGGGTGAGTG-3′).

2.8. Pharmacokinetics and Statistical Analysis. The plasma concentration-time data of analytes were analyzed by compartment-independent approaches. The maximum plasma drug concentration (C_{max}) and time to C_{max} (T_{max}) were directly obtained from the plasma concentration-time data. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$, where K_e, the elimination rate constant, was calculated via semilog regression on the terminal phase of the plasma concentration-time curve. The AUC from time 0 to infinity ($AUC_{0-\infty}$) was estimated as $AUC_{0-t} + C_t/K_e$, where C_t is the plasma concentration of the last measurable sample, and AUC_{0-t} was calculated according to the linear trapezoidal rule. Total plasma clearance (CL/F) was calculated as dose/$AUC_{0-\infty}$. The AUC metabolic ratio was calculated by dividing the AUC_{0-t} of 1-hydroxymidazolam by the AUC_{0-t} of midazolam.

IC_{50} values (the concentration of inhibitor causing a 50% inhibition in enzyme activity) were calculated using Graphpad Prism 5.04 (GraphPad Prism, Inc. San Diego, CA, USA). For comparison of several groups against one control group, a one-way analysis of variance followed by Dunnett's test was performed. A P value < 0.05 was considered to be significant.

3. Results

All volunteers completed the study of three periods. 12 healthy male Chinese subjects with a mean age of 28 years (range, 26–38 years), a mean weight of 66.4 kg (range, 60–73.5 kg), and a mean height of 173 cm (range, 168–183 cm) participated in this study.

3.1. Effect of CYP3A Activities by Danshen Ethanol Extract in Healthy Volunteers. The mean plasma concentration-time profiles of midazolam and 1′-hydroxymidazolam before and after single or multidose administration of the danshen

Table 1: Pharmacokinetic parameters of midazolam and 1-hydroxymidazolam after the administration of a single dose of 15 mg of midazolam in 12 healthy volunteers before and after single- and multiple-dose coadministration of the ethanol extract of danshen.

PK Parameter	Control	Single dose	P value*	Multiple dose	P value*
Midazolam					
C_{max} (ng/mL)	95.17 ± 39.01	163.57 ± 86.36	0.00	39.55 ± 18.52	0.00
T_{max} (h)	0.69 ± 0.60	0.35 ± 0.13	0.07	0.79 ± 0.65	0.68
AUC_{0-12} (ng·h/mL)	219.86 ± 64.67	213.85 ± 86.31	0.70	42.24 ± 15.74	0.00
$AUC_{0-\infty}$ (ng·h/mL)	221.76 ± 63.78	218.15 ± 83.22	0.65	44.55 ± 17.68	0.00
$t_{1/2}$ (h)	4.20 ± 0.76	4.01 ± 1.08	0.61	2.20 ± 0.90	0.00
CL/F (L/h)	67.64 ± 20.05	75.78 ± 27.94	0.18	393.71 ± 157.14	0.00
1-Hydroxymidazolam					
C_{max} (ng/mL)	26.78 ± 11.00	45.04 ± 15.09	0.01	20.48 ± 13.70	0.18
T_{max} (h)	0.57 ± 0.20	0.61 ± 0.39	0.12	0.46 ± 0.13	0.74
AUC_{0-12} (ng·h/mL)	56.21 ± 22.75	66.62 ± 25.24	0.12	30.88 ± 15.09	0.00
$t_{1/2}$ (h)	3.30 ± 0.62	3.29 ± 0.78	0.16	1.72 ± 0.70	0.00
$AUC_{(1OHmdz)}/AUC_{(mdz)}$	0.26 ± 0.15	0.32 ± 0.15	0.08	0.77 ± 0.53	0.00

Data are presented as mean ± SD. *P values are given for the differences with respect to control. The data were analyzed using a one-way analysis of variance with post hoc Dunnett's procedure.

extract are presented in Figures 2 and 3. Table 1 summarizes the pharmacokinetic parameters of midazolam, 1′-hydroxymidazolam and metabolic ratio of midazolam before and after single- or multi-dose of the danshen extract treatment.

Regarding treatment with a single dose of the danshen extract, the C_{max} of midazolam and 1′-hydroxymidazolam was increased by 80.7% (163.57 ± 86.36 ng/mL versus 95.17 ± 39.01 ng/mL) and 68.2% (45.04 ± 15.09 ng/mL versus 26.78 ± 11.08 ng/mL), compared with the control, respectively.

After 10 days of the danshen extract intake, AUC_{0-12} of midazolam was decreased by 79.9% (42.24 ± 15.74 ng·h/mL versus 219.86 ± 64.67 ng·h/mL) compared with the control, and the clearance of midazolam was increased by 501.59% (393.71 ± 157.14 L/h versus 67.64 ± 20.05 L/h). After 10 days of treatment, the C_{max} of midazolam was decreased by 66.6% compared with the control; $t_{1/2}$ of midazolam was decreased by 43.8% (2.20 ± 0.90 h versus 4.20 ± 0.76 h). After 10 days of treatment, AUC_{0-12} of 1′-hydroxymidazolam was decreased by 44.50% (30.88 ± 15.09 ng·h/mL versus 56.21 ± 22.75 ng·h/mL) compared with the control, and $t_{1/2}$ of 1′-hydroxymidazolam was decreased by 47.97% (1.72 ± 0.40 h versus 3.30 ± 0.62 h). However, the C_{max} of of 1′-hydroxymidazolam was not significantly affected by 10-day treatment of the danshen extract.

After 10-day treatment with the danshen extract, AUC metabolic ratio of midazolam was increased by 35.64% compared with the control (0.77 ± 0.53 versus 0.26 ± 0.15).

3.2. Concentrations of Tanshinones in Human Plasma. After administration of a single dose (1 g) and multidose (1 g, three times each day) of the danshen extract, the pharmacokinetic parameters of tanshinones were listed in Table 2.

3.3. Inhibition of CYP3A Activities by Danshen Ethanol Extract in Human Liver Microsomes. To investigate whether the ethanol extract of danshen and which component(s) of the extract affected the catalytic activity of CYP3A, midazolam 1′-hydroxy reaction assays were conducted with various concentrations of ethanol extract of danshen and dihydrotanshinone I. The results showed that ethanol extract of danshen and dihydrotanshinone I had inhibition against CYP3A activities in HLMs with IC_{50} values of 8.6 μg/mL and 1.2 μM (Figure 4).

3.4. Induction of CYP3A4 mRNA by Danshen Components in Human Hepatocytes. Hepatocytes were treated with DMSO (0.1%), tanshinones (2 μM), and rifampicin (25 μM) for 72 hours. After treatment, expressions of *CYP3A4* mRNA were determined. Levels of *CYP3A4* transcripts were induced 18.2-fold by rifampicin (25 μM). At 2 μM, levels of *CYP3A4* transcripts by tanshinone I, cryptotanshinone, tanshinone IIA, and dihydrotanshinone I increased by 1.4-, 2.8-, 5.2-, and 1.5-fold, respectively (Figure 5).

4. Discussion

The ethanol extract of danshen in capsule dosage form represents a class of danshen product rich in lipophilic constituents in clinical practice. To our knowledge, this is the first report to evaluate the effect of the ethanol extract of danshen on the in vivo CYP3A activity in healthy volunteers. Midazolam (MDZ) is a widely accepted probe drug for CYP3A phenotype [15]. MDZ is rapidly metabolized by CYP3A to 1′-hydroxymidazolam (1′-OHMDZ) and, to a smaller extent, to 4-hydroxymidazolam (4-OHMDZ) and 1,4-dihydroxymidazolam (1,4-OHMDZ), and it is further metabolized to glucuronide conjugates by UDP-glucuronyltransferase (UGT) [16].

In this study, an 80.7% increase in the C_{max} of midazolam occurred with single-dose administration of the ethanol extract of danshen. This increase was not reflected in the AUC_{0-12}, CL/F, or $t_{1/2}$. Although midazolam is classified into BCS class I, with high membrane permeability [17], its oral bioavailability is only 24 to 46% in humans [18]. Gorski found

Table 2: Pharmacokinetic parameters of tanshinons in 12 healthy volunteers after single- and multiple-dose administration of the ethanol extract of danshen.

PK parameter	Tanshinone I	Cryptotanshinone	Tanshinone IIA	Dihydrotanshinone I
C_{max} (ng/mL)	11.52 ± 9.90	3.44 ± 1.32	4.00 ± 2.79	0.85 ± 0.59
C_{trough} (ng/mL)	3.09 ± 1.09	0.76 ± 0.23	1.36 ± 0.52	0.20 ± 0.15
C_{ssmax} (ng/mL)	18.10 ± 16.44	3.33 ± 1.85	3.98 ± 1.97	1.00 ± 0.56

Data are mean value ± SD.
C_{max}: peak plasma concentration after single-dose administration of the danshen extract; C_{trough}: plasma trough concentration at the end of a dosing interval after multiple-dose administration of the danshen extract; C_{ssmax}: peak plasma concentration at the end of a dosing interval after multiple-dose administration of the danshen extract.

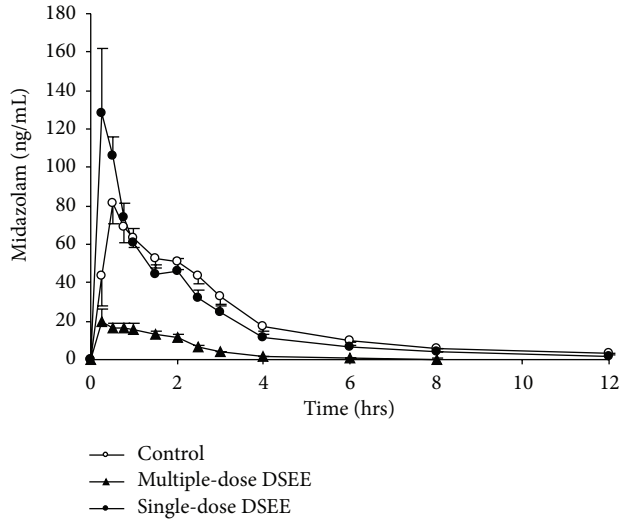

Figure 2: Mean (±SE, $n = 12$) plasma concentration of midazolam after the administration of a single dose of 15 mg of midazolam in 12 healthy volunteers before and after single- and multiple-dose co-administration of danshen ethanol extract (DSEE).

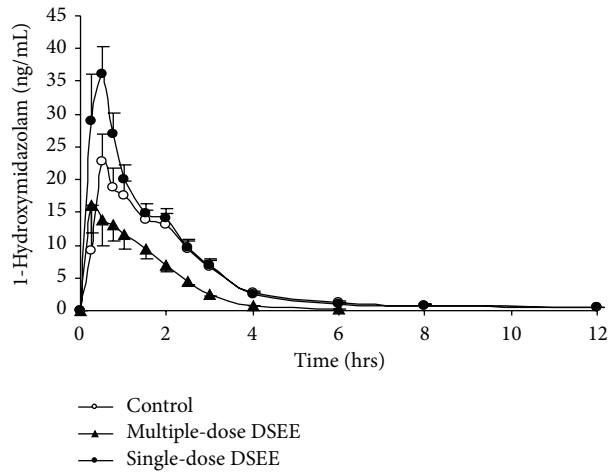

Figure 3: Mean (±SE, $n = 12$) plasma concentration of 1-hydroxymidazolam after the administration of a single dose of 15 mg of midazolam in 12 healthy volunteers before and after single- and multiple-dose coadministration of danshen ethanol extract (DSEE).

that the oral bioavailability of midazolam was almost entirely determined by CYP3A activity in the small intestine [19]. So, the increase in C_{max} could attribute to inhibition of CYP3A enzymes in the small intestine.

The danshen extract contains cryptotanshinone and dihydrotanshinone I, and the content of dihydrotanshinone I was 5 times lower than cryptotanshinone in the preparation. We reported that cryptotanshinone could activate midazolam 1-hydroxylation in human liver microsomes [20]. After administration of 1 g danshen extract, the concentration of cryptotanshinone in the liver and intestine can reach the concentration of 2 μM to activate midazolam 1-hydroxylation. In contrast, dihydrotanshinone I absorbed into liver cannot reach the concentration with inhibition against CYP3A4. Therefore, the inhibition of CYP3A4 in intestine can be offset by cryptotanshinone in liver activation effect. It can be one of reasons why the AUC of midazolam was not obviously changed after single-dose treatment of the danshen extract. An in vitro study found that the content of the ethanol extract of danshen had a significant inhibitory effect on CYP3A-mediated 1-hydroxymidazolam metabolism with IC_{50} 8.6 μg/mL in human liver microsomes. Although tanshinone IIA, tanshinone I, and cryptotanshinone in the danshen extract had no significant inhibitory effect individually [14, 20], dihydrotanshinone I (the danshen extract containing 13 mg dihydrotanshinone per 1 g) was a strong inhibitor of CYP3A4 with IC_{50} 1.20 μM. The results were consistent with Wang's report [9]. After treatment with 1 g of the danshen extract, there were higher concentrations of dihydrotanshinone I in the small intestine beyond inhibition concentration (IC_{50}) toward intestinal CYP3A.

By contrast, after 10-day intake of the danshen extract, the clearance was increased, and the C_{max}, $AUC_{0-\infty}$, and $t_{1/2}$ were decreased compared with baseline. It suggests that both presystemic processes and systemic elimination of midazolam are altered by prolonged intake of the ethanol extract of danshen. And it indicates that intestinal and hepatic CYP3A are induced by multi-dose of the extract administration. After 10 days of treatment, $t_{1/2}$ of 1-OHMDZ was also reduced by 54.1% compared with control, suggesting that UGTs could be induced to enhance the glucuronidation of 1-OH MDZ by the danshen extract. This is also evidenced by metabolic ratio of midazolam.

It was the first report to study induction of tanshinones on CYP3A4 using primary human hepatocytes. A cutoff value of 4-fold increase in mRNA levels for induction compared with those found in negative control was applied for assessing

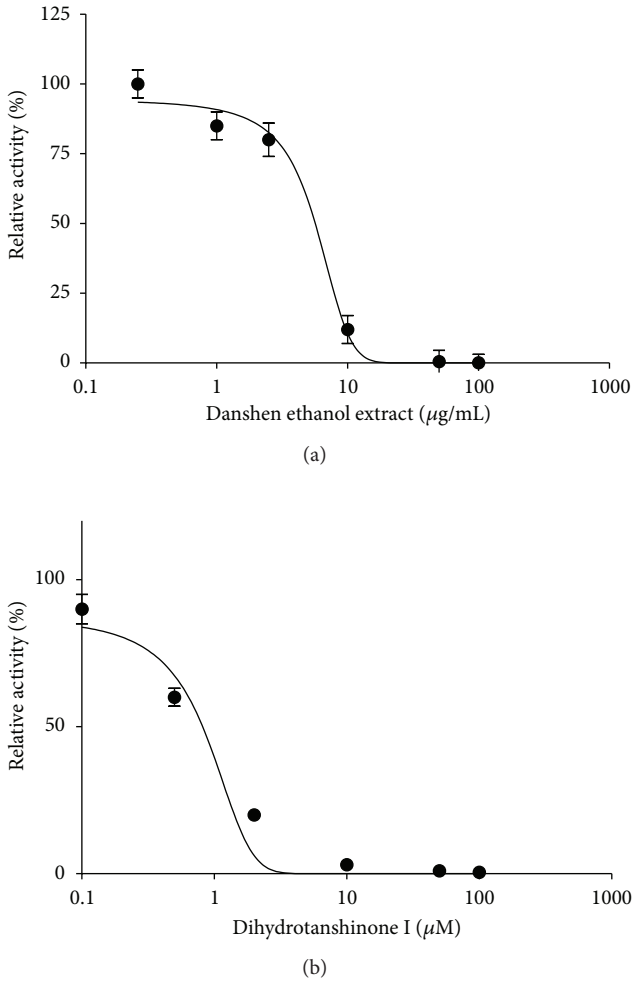

Figure 4: Inhibition of CYP3A4 activities by dihydrotanshinone I and danshen ethanol extract in human liver microsomes. Reactions were performed in the presence of midazolam (3.5 μM) at various concentrations of danshen ethanol extract (0.2–100.0 μg/mL) (a) and dihydrotanshinone I (0.5–100.0 μM) (b), in the microsomes (0.2 mg/mL) and NADPH in a 100 mM phosphate buffer, pH 7.4 in a final volume of 200 μL at 37°C for 5 min. Each point represents the mean of three separate experiments performed in triplicate, and the bar represents S.D.

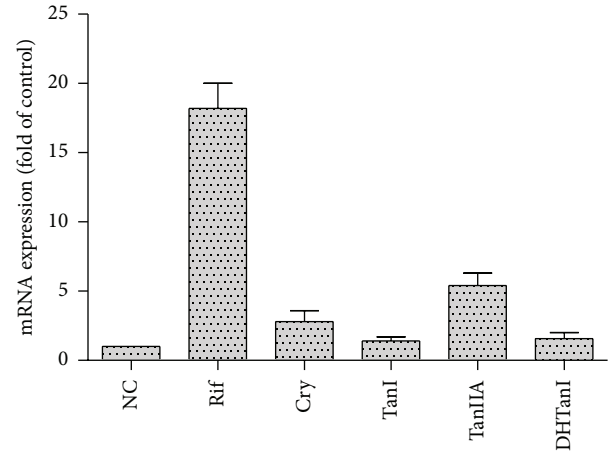

Figure 5: Induction of CYP3A4 mRNA by tanshinone I (TanI), cryptotanshinone (Cry), tanshinone IIA (Tan IIA), and dihydrotanshinone I (DHTanI). Human hepatocytes were exposed to tanshinone I (2 μM), cryptotanshinone (2 μM), tanshinone IIA (2 μM), dihydrotanshinone I (2 μM), or 25 μM rifampin (PC) for 3 days. CYP3A4 mRNA levels were measured with reverse transcription real-time PCR. These data were obtained from two independent experiments, and each experiment was performed in triplicate. Each column with bar represents the mean and S.D. The mean is expressed as fold induction over vehicle control (NC).

CYP3A4 mRNA expression [21]. The first in vitro finding in primary human hepatocytes demonstrates that tanshinone IIA can induce the expression of the CYP3A4 gene which was increased up to the cutoff value. Cryptotanshinone exhibits small increase (2.8-fold) of *CYP3A4* mRNA which does not reach the cutoff value. The cutoff value may vary among different laboratories because of the variability among hepatocyte lots. However, tanshinone I and dihydrotanshinone I at 2 μM do not cause significant increase of CYP3A4 mRNA. The results were consistent with Yu's findings in the reporter gene [8]. After pretreatment with higher dose of the danshen extract rich in tanshinone IIA and cryptotanshinone for 10 days, there were much higher tanshinones concentrations in the volunteers' guts to induce intestinal CYP3A, and there were also effective enough tanshinones concentrations in the liver which can induce the hepatic CYP3A even with less than 10 ng/mL of plasma concentration. This finding supports the hypothesis that relevant inhibition can only be achieved locally in the gut, whereas the concentration in the liver is sufficient for PXR activation and subsequent induction of metabolism. It is clear that dihydrotanshinone I can inhibit CYP3A, while other constituents such as tanshinone IIA and cryptotanshinone can mediate the inductive response in danshinone extract.

We reported that administration of danshen tablets for 2 weeks (4 tablets each time, 3 times a day) in healthy volunteers according to one-sequence crossover design caused 35.4% increase in apparent oral clearance. In this study, the clearance was increased by 501.5%. The extent CYP3A induction in intestine by the danshen extract rich in tanshinones in this study was 14.2 times higher than that of the danshen extract with low tanshinones content which contains cryptotanshinone 1.2 mg and tanshinone IIA 1.6 mg in each dose [22]. The contents of the three tanshinones in danshen tablet were 50 times lower than the danshen capsule. So, the extent of the induction of danshen extract toward CYP3A was shown to be dependent on dose of tanshinones.

In addition to CYP3A, the nuclear receptors of UGT are also targets of PXR [23–26]. So, tanshinone IIA and cryptotanshinone could activate PXR and consequently induce the expression of the *UGT* gene. Since more than 40% of clinically used drugs are catalyzed by CYP3A and with further biotransformation by UGT, the two drug metabolising enzymes can be induced by the danshen extract to promote their substrates conversion to more polar derivatives which can be readily excreted. PXR has also been shown to play key roles in the regulation of several other inducible *CYP2C9,*

CYP2C19, P-gp, MRP2, *sulfate transferase (ST),* and other drug metabolisming enzymes and transporter genes [23–26]. So, the inductive effect of tanshinones on these metabolic enzymes also should be researched.

Drugs that are substrates for CYP3A-mediated metabolism are likely to be potential candidates for drug-herb interactions [27]. The duration and dosage of exposure to the ethanol extract of danshen appear to be critical for drug-danshen interactions. An increase in the plasma drug concentration is possible during concomitant administration of the ethanol extract of danshen and prescribed drugs. By contrast, prolonged intake of the danshen extract followed by drug administration may result in subtherapeutic drug concentrations. Although we have shown that the ethanol extract of danshen has the potential to inhibit CYP3A4, particularly after single exposure at high concentrations, the inductive effect predominates with chronic exposure. It is suggested that caution should be taken when coadministrating the danshen extract rich in tanshinones with therapeutic drugs which are substrates for CYP3A4.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Review Article

Interaction of Carbamazepine with Herbs, Dietary Supplements, and Food: A Systematic Review

Sophia Yui Kau Fong, Qiong Gao, and Zhong Zuo

School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Correspondence should be addressed to Zhong Zuo; joanzuo@cuhk.edu.hk

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Background. Carbamazepine (CBZ) is a first-line antiepileptic drug which may be prone to drug interactions. Systematic review of herb- and food-drug interactions on CBZ is warranted to provide guidance for medical professionals when prescribing CBZ. *Method*. A systematic review was conducted on six English databases and four Chinese databases. *Results*. 196 out of 3179 articles fulfilled inclusion criteria, of which 74 articles were reviewed and 33 herbal products/dietary supplement/food interacting with CBZ were identified. No fatal or severe interactions were documented. The majority of the interactions were pharmacokinetic-based (80%). Traditional Chinese medicine accounted for most of the interactions ($n = 17$), followed by food ($n = 10$), dietary supplements ($n = 3$), and other herbs/botanicals ($n = 3$). Coadministration of 11 and 12 of the studied herbal products/dietary supplement/food significantly decreased or increased the plasma concentrations of CBZ. Regarding pharmacodynamic interaction, Xiao-yao-san, melatonin, and alcohol increased the side effects of CBZ while caffeine lowered the antiepileptic efficacy of CBZ. *Conclusion*. This review provides a comprehensive summary of the documented interactions between CBZ and herbal products/food/dietary supplements which assists healthcare professionals to identify potential herb-drug and food-drug interactions, thereby preventing potential adverse events and improving patients' therapeutic outcomes when prescribing CBZ.

1. Background

Introduced in 1960s, carbamazepine (CBZ) remains as one of the most commonly prescribed antiepileptic drugs worldwide and has established efficacy for the treatment of partial seizures, generalized tonic-clonic seizures, trigeminal neuralgia, and bipolar disorders [1–6]. Despite its clinical popularity, CBZ possesses several pharmacokinetic properties which make it prone to interaction with coadministered substances, including drugs, herbal products, and food [7]. CBZ is a potent inducer of CYP450 system and is subject to autoinduction. Its metabolism is exclusively hepatic and catalyzed by various enzymes including CYPs, UGTs, and SULTs [8]. CYP3A4 is the most important enzyme involved in the metabolism of CBZ as it leads to the formation of the active metabolite CBZ 10,11-epoxide, which appears to contribute to the toxicity and efficacy of CBZ [9, 10]. Furthermore, CBZ has a considerably narrow therapeutic index of 2-3 while there is a wide interindividual variation in tolerable doses and blood levels, making therapeutic drug monitoring and slow titration necessary [11, 12]. Many side effects associated with CBZ are concentration-related. Nausea, vertigo, dizziness, and blurred vision are examples of CBZ adverse effects which mostly are mild, transient, and reversible if the dosage is reduced or if initiation of treatment is gradual [13]. Signs of toxicity generally occur at plasma CBZ concentrations in excess of 10 to 12 mg/L, with diplopia, nystagmus, and aplastic anemia being the most characteristic ones [11]. Fatal cases of CBZ overdose were also recorded where patients were manifested with cardiac arrhythmias, abnormal movements, and seizures [14]. The occurrence of CBZ overdose is usually accidental, and in most times it is secondary to the coadministration of other substances [15–20].

Since antiepileptic regimens are normally given on a long-term basis, the opportunity of a clinical significant interaction between CBZ and coadministered substances is considerably

high. Herbal medicines, dietary supplements, and food may interact with CBZ pharmacokinetically and/or pharmacodynamically which leads to potential clinical consequences. One of the contributing factors towards increasing incidence of herb-drug interaction is the increased popularity of herbal medicines [21]. According to pharmacoepidemiologic surveys, the percentage of epileptic patients concurrently taking complementary and alternative medicines and antiepileptic drugs is considerably high in both developed and developing regions: United States (39%), Cambodia (36%), United Kingdom (34%), Taiwan (16%), Nigeria, (15%) and India (12%), while more than 60% of them did not inform their physicians [22–27]. In China, integrated medicine is a common practice where Western and traditional Chinese medicines are prescribed concurrently for the treatment of epilepsy [28]. Therefore, the opportunity of patients taking CBZ with herbal/dietary supplements is high, and it is necessary to address the safety issues of such combinational use.

When making clinical decisions on the use of herbal or dietary supplements, the review article is one of the major information sources for healthcare professionals [29]. In view of this, we tried to identify existing review articles that (1) summarized all the reports and studies on the pharmacokinetic and pharmacodynamic interactions of CBZ with herbs, dietary supplements, and food and (2) provided recommendations on their combinational use. It was found that there is a lack of well-conducted systematic review on CBZ and its herb, food, and dietary supplement interactions. The searching strategy adopted by these reviews is not comprehensive enough to identify all the relevant articles. Most of these reviews use general terms such as "herb-drug interaction" as searching keywords and do not focus on one particular drug (e.g., CBZ) [30–33]. This nondrug-specific searching method may result in missing CBZ-relevant papers if the paper does not contain the phrase of "herb-drug interaction". Besides, there is no single review that covers the interactions between CBZ and all the three aspects of herb, food, and dietary supplement. A systematic review is warranted to provide guidance for healthcare professions when prescribing and monitoring patients taking CBZ. From the review articles that report herb-drug interactions, we can see that herb-drug interactions are often less systematically documented and less familiar to medical practitioners compared to drug-drug interactions. The nonstandardized naming of herbals with several confusing generic names together with an unfamiliar Latin name may make it difficult for medical professionals to anticipate and monitor herb-drug interaction [34]. Furthermore, most herbal formulas (especially traditional Chinese medicine, TCM) contain multiple herbs, making it often impossible to know which herbs and at what doses they are present.

In view of all the constraints of the existing literature, a comprehensive systematic review focusing on CBZ and overcoming the mentioned hurdles is warranted for healthcare professionals to make proper decisions. In this current report, we conducted a systematic review on interactions between CBZ and herbs, dietary supplements, and food, summarizing the scientific evidence for such interactions and providing recommendations for the combinational use. In addition to the usual databases (e.g., Medline and Embase), we also included several Chinese databases to identify reports of interactions between CBZ and TCM which are written in Chinese. The aim of this review is to provide a clear and systematic presentation of herb and food interactions with CBZ to alert and provide guidance for medical professionals when prescribing CBZ.

Table 1: Keyword and MeSH search terms for herbs, food, and dietary supplements.

Keywords	MeSH terms
(i) alter∗ medic∗ (ii) botanical.tw. (iii) (chinese adj (herb$ or drug$ or formul$ or plant$ or presri$ or remed$ or materia medica)).ab,ti,ot. (iv) (drug∗ and chines∗ and herb∗).mp. (v) (herb or herbs or herbal).tw. (vi) herbal remed$.tw. (vii) ((herb$ or drug$ or formul$ or plant$ or presri$ or remed$ or materia medica) adj chinese).ab,ti,ot (viii) integrative medicin$.ab,ti,ot. (ix) Nutrition$ supplement or diet$ supplement.mp. (x) (phytodrug$ or phyto-drug$ or phytopharmaceutical$).tw. (xi) (plant∗ and extract∗).mp. (xii) (plant∗ and medic∗).mp. (xiii) (TCM or CHM).tw. (xiv) (tradition∗ and chines∗ and medic∗).mp. (xv) traditional chinese.tw.	(i) exp Chinese drug (ii) exp Chinese herb (iii) exp Chinese medicine (iv) exp diet supplementation (v) exp drugs, Chinese Herbal (vi) exp food (vii) exp food drug interaction (viii) exp herbal medicine (ix) exp herbaceous agent (x) exp medicine, Ayurvedic (xi) exp medicine, east asian traditional (xii) exp medicine, Chinese traditional (xiii) exp medicine, kampo (xiv) exp medicine, Korean traditional (xv) exp medicine, mongolian traditional (xvi) exp medicine, Oriental Traditional (xvii) exp medicine, tibetan traditional (xviii) exp phytotherapy (xix) exp plant extract (xx) exp Plants, Medicinal (xxi) exp shamanism

2. Materials and Methods

2.1. Data Sources and Literature Search. A computer-based search of the following English databases was conducted: AMED (1985–Oct. 2012), CINAHL Plus (1937–Oct. 2012), Cochrane Database of Systematic Reviews (2005–Dec. 2011), CENTRAL (Oct. 2012), Embase (1947–Oct. 2012), Medline (1946–Oct. 2012), and SciFinder Scholar (1907–Oct. 2012). The keyword search terms for carbamazepine ("Carbamazepine", "Tegretol", "Tegretol XR", "G-32883", "5H-Dibenz[b,f]azepine-5-carboxamide") were combined, using the combination term AND, with a comprehensive list of keywords and MeSH search terms for herbs, food, and dietary supplements (Table 1). Such search list was refined to include most of the relevant articles. No language restriction was imposed during the search, but non-English articles were included only if they contained an English abstract with sufficient information. As defined by the Dietary Supplement Health and Education Act of 1994 (DSHEA), "dietary supplement" refers to any dietary products containing one or

more of the following ingredients: vitamin, mineral, herb or other botanical, amino acid, and a dietary substance for use by man to supplement the diet by increasing the total dietary intake. In the current review, we separated "herb or other botanical" out and categorized this group as "herbs" while the remainings were referred to as "dietary supplement". The third category is "food" which includes any specific traditional food/fruit products or beverages. Paper containing only a general term of "food" without specifying any particular food item will be excluded. In addition to the English databases, four Chinese databases had been searched, including Chinese BioMedical Literature Database (1978–Oct. 2012), China Journal Net (1915–Oct. 2012), Traditional Chinese Medical Database System (1984–Oct. 2012), and Chinese Medical Academic Conference Database (1994–Oct. 2012). The MeSH headings and keywords used for the search were carbamazepine (Chinese name, Chinese common names, and chemical names) in combination with the Chinese equivalent terms of "interaction", "Chinese herbal medicines", and "Chinese and Western medicines" ("jie he", "xiang hu zuo yong", and "zong yao", "zong cao yao", "zong xi yi"). The bibliographies of every retrieved article were checked for any additional pertinent studies.

2.2. Inclusion Criteria and Data Extraction. The selection of relevant reports and evaluation of article eligibility was carried out by two reviewers independently (Fong and Gao). Articles were considered eligible for evaluation if they contained original data involving herb, food, or dietary supplement interactions with CBZ without restriction for *in vitro* studies, animal studies, clinical studies observational studies, or review articles. Any discrepancies were resolved by a third author (Zuo). All relevant literature fulfilling our inclusion criteria were extracted and complied, except for the interacting pairs that have beneficial effects.

We grouped the natural products into four categories: TCM, other herb/botanical, vitamin/mineral/amino acid, and food. We categorized the mechanisms for pairs of interactions into three types: pharmacokinetics, pharmacodynamics, and both. In order to standardize the names of the included TCMs, the official compendium Pharmacopoeia of the People's Republic of China 2010 (Chinese Pharmacopoeia) was consulted and their Latin names (for herbs) or Chinese pinyin names (for herbal formulae) were presented.

3. Results

3.1. Literature Search. A total of 3179 articles was initially found through database searches while an addition of 14 articles were obtained from scrutinizing the bibliographies of relevant literatures. 196 articles fulfilling the inclusion criteria were selected for further evaluation with perfect agreement between the two authors. Finally, seventy-four articles with full text, including 40 original articles and 34 review articles, were qualified to undergo an in-depth review (Figure 1); a total of 33 unique herbal products/dietary supplement/food-CBZ interacting pairs were identified from these articles. Summaries of the *in vitro*, animal, and clinical studies to retrieve information about interactions between CBZ and herbal products/dietary supplement/food for the original studies are listed in Tables 2 and 3, respectively. Among the original studies ($n = 40$), most are animal ($n = 24$) and human ($n = 14$) studies, with 2 mechanistic *in vitro*/*ex vivo* studies. Regarding the studied types of interaction, the majority ($n = 32$) are pharmacokinetic interactions followed by both pharmacokinetic and pharmacodynamic interactions ($n = 6$) and pharmacodynamic interactions ($n = 2$).

3.2. Interactions between Herbal Products and CBZ. Nineteen of the included original articles documented the interactions between 20 different herbal products and CBZ, where TCMs in the form of crude drug, extract, or single TCM compound were the major studied herbal products ($n = 17$). Among the 17 documented pharmacokinetic interactions between CBZ and TCMs, *Cassia auriculata* Linn., piperine (an active compound in *Piper longum* Linn.), Platycodonis Radix, and *Polygonum cuspidatum* were demonstrated to increase the plasma level/oral bioavailability of CBZ through decreasing the metabolism of CBZ or improving gastric solubility of CBZ [37, 47–49]. On the other hand, ginkgo biloba, Hu-gan-ning pian, Jia-wei-xiao-yao-san, and Xiao-yao-san decreased the plasma level/oral bioavailbaility of CBZ through increasing the metabolism of CBZ via CYP3A4 induction [40, 44, 53]. Ginsenoside (an active compound in *ginseng*) was also shown to activate CYP3A4 activity *in vitro* and thereby increased CBZ metabolism [39]. Xiao-qing-long-tang and Xiao-cha-hu-tang delayed the time for CBZ to reach peak plasma concentration through decreasing gastric emptying rate [38, 50], whereas Paeoniae Radix decreased the T_{max} of CBZ through possibly improving dissolution of CBZ [46]. *Acorus calamus* Linn., berberine (an active compound in Coptidis rhizome), *Cardiospermum halicacabum* Linn., Chai-hu-jia-long-gu-mu-li-tang, and *Hypericum perforatum* Linn. did not alter the plasma levels or other pharmacokinetic parameters of CBZ in animal or human studies [35–38, 42]. As regards the pharmacodynamic interactions between CBZ and TCMs, there were three articles reporting the effect of TCMs on the efficacy and/or side effects of CBZ. *Acorus calamus* Linn. was shown to have an additive antiepileptic activity with CBZ in an animal study [35]. Xiao-yao-san might increase the incidence of dizziness, blurred vision, skin rash, and nausea when coadministered with CBZ in a clinical study [53] while *Cardiospermum halicacabum* Linn. and *Cassia auriculata* Linn. did not potentiate CBZ-related toxicity in rats [37]. The three remaining herbal products categorized as "other herb/botanical" that had documented herb-drug interactions with CBZ were Ispaghula husk, mentat, and septilin. Ispaghula husk, also more commonly known as psyllium, decreased the oral bioavailability and absorption of CBZ in four healthy volunteers [43] while septilin also decreased the absorption of CBZ in rabbits possibly through interfering with the gastric emptying or intestinal transit time [50]. On the contrary, mentat (BR 16A) increased the bioavailability of CBZ in rabbits through an unknown mechanism [45].

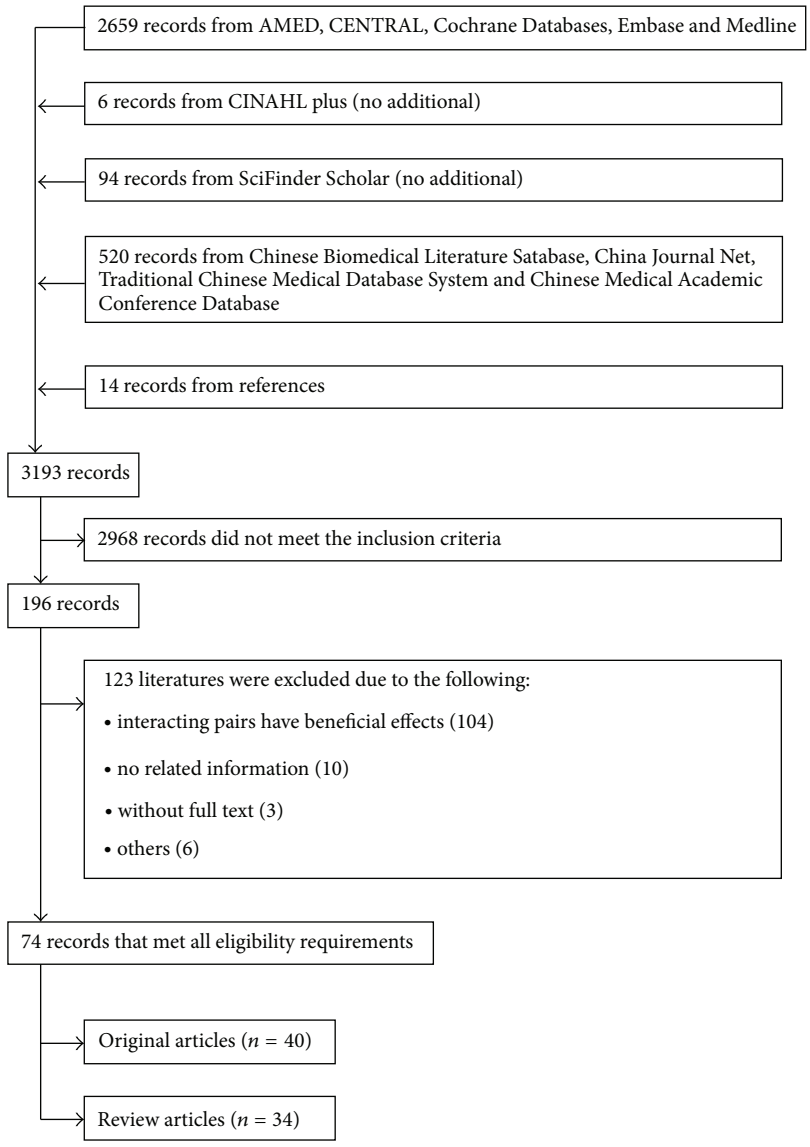

Figure 1: Flow chart of literature search.

3.3. Interactions between Dietary Supplement/Food and CBZ. A total of twenty-one original literatures covering 13 different dietary supplement/food-CBZ interaction studies were recorded in the current review. These included beverages (n = 7), food substances (n = 3), and dietary supplements (n = 3). Alcohol did not affect the pharmacokinetics of CBZ in healthy volunteers but increased the oral bioavailability and decreased the metabolism of CBZ in alcoholics [55]. Alcohol-CBZ combination also had an additive neurotoxicity in animals [54]. Another beverage Coca-Cola increased the oral bioavailability of CBZ in a clinical study which may be due to the enhanced dissolution of CBZ by its acidity [60]. Caffeine decreased the oral bioavailability as well as the antiepileptic efficacy of CBZ in human and animal studies, respectively [57–59]. Four juices, namely, grapefruit juice, kinnow juice, pomegranate juice, and star fruit juice, were demonstrated to increase the oral bioavailability of CBZ through inhibiting enteric CYP3A4 activity [62, 66, 71, 74] though an *ex vivo* study suggested that pomegranate juice might induce enteric CYP3A4 due to the decreased intestinal permeation of CBZ [72]. Pharmacokinetic interactions between food substances and CBZ were recorded: butter increased while soy bean decreased the oral bioavailability of CBZ in animal study. The former might improve dissolution of CBZ while the later might decrease the gastric emptying and enhance the metabolism of CBZ [56, 73]. Although honey was shown to decrease the oral bioavailability of CBZ in rabbits, it had no effect on the pharmacokinetic parameters of CBZ in human [63–65]. As regards the dietary supplement-CBZ interactions, folinic acid did not alter the plasma level of CBZ in rats [61] while nicotinamide increased CBZ plasma level and decreased its clearance in two children with epilepsy [70]. Melatonin did not interact with CBZ pharmacokinetically but potentiated the antiepileptic activity of CBZ in both animal and human studies [67–69].

Table 2: Summary of the included *in vitro*, animal, and clinical studies on interactions between carbamazepine and herbal products, dietary supplement, and food.

Types of herbal product*	Herbal products	Study type	Subject/model (number)	Study design	Outcome measures	Effect	Mechanism	References
TCM	*Acorus calamus* Linn.	Animal	Male Wistar rats with pentylenetetrazole-induced seizure model (6 in each group)	Randomized controlled study	PK and PD parameters	No effect on plasma level of CBZ Additive antiepileptic activity	Increased GABAergic activity	Katyal et al. 2012 [35]
TCM	Berberine (active compound in Coptidis rhizome)	Animal	Male Wistar rats (5 in each group)	Randomized parallel design	PK parameters	No effect on pharmacokinetic parameters of CBZ or CBZ 10,11-epoxide	Did not affect *in vivo* intestinal or hepatic CYP3A activity	Qiu et al. 2009 [36]
TCM	*Cardiospermum halicacabum* Linn.	Animal	Male Wistar rats (10 in each group)	Randomized crossover design	PK and PD parameters	No significant effect on CBZ plasma level No change in drug-related toxicity (including general behavior, liver function, haematological parameters, and kidney function)	N.D.	Thabrew et al. 2004 [37]
TCM	*Cassia auriculata* Linn.	Animal	Male Wistar rats (10 in each group)	Randomized crossover design	PK and PD parameters	Increased plasma level of CBZ No change in drug-related toxicity (including general behavior, liver function, haematological parameters and kidney function)	N.D.	Thabrew et al. 2004 [37]
TCM	Chai-hu-jia-long-gu-mu-li-tang	Animal	Wistar rats (5-6 in each group)	Randomized parallel design	PK parameters	No effect on pharmacokinetic parameters or protein binding of CBZ or CBZ 10,11-epoxide	Did not alter *in vivo* CYP3A activity	Ohnishi et al. 2001 [38]
TCM	Ginsenoside (active compound in *ginseng*)	*In vitro*	Human liver microsomes (3 in each group)	N/A	PK parameters	Increased CBZ metabolism	Activated CYP3A4 activity by interacting with CBZ in the active site	Haop et al. 2008 [39]
TCM	*Ginkgo biloba*	Animal	Rats (6 in each group)	Randomized parallel design	PK parameters	Decreased bioavailability and increased rate of elimination of CBZ	N.D.	Chandra et al. 2009 [40]
TCM	Hu-gan-ning pian	Animal	Male Sprague-Dawley rats (7 in each group)	Randomized parallel design	PK parameters	Decreased bioavailability No effect on C_{max}, $t_{1/2}$, t_{max}, CL, and elimination K of CBZ	Decreased absorption but not metabolism of CBZ	Zheng et al. 2009 [41]
TCM	*Hypericum perforatum* Linn.	Human	Healthy subjects (8)	Open label study	PK parameters	No effect on PK parameters of CBZ	Autoinduction or greater clearance by CBZ	Burstein et al. 2000 [42]
HP	Ispaghula Husk (Psyllium)	Human	Healthy male volunteer (4)	Open label study	PK parameters	Decreased bioavailability by reducing absorption and plasma levels of CBZ	Decreased amount of biological fluid in GI tract and thereby reduced dissolution rate of CBZ Also adsorb CBZ onto their surfaces	Etman 1995 [43]

Table 2: Continued.

Types of herbal product*	Herbal products	Study type	Subject/model (number)	Study design	Outcome measures	Effect	Mechanism	References
TCM	Jia-wei-xiao-yao-san	Human	Patients with major depression or bipolar disorder (61)	Randomized double-blinded control trial	PK parameters	Decreased plasma level of CBZ	Increased metabolism of CBZ by inducing CYP3A	Zhang et al. 2007 [44]
HP	Mentat	Animal	New Zealand white rabbits (8 in each group)	Randomized parallel design	PK parameters	Increased bioavailability of CBZ	N.D.	Tripathi et al. 2000 [45]
TCM	Paeoniae Radix	Animal	Male Sprague-Dawley rats (6 in each group)	Randomized parallel design	PK parameters	Decreased T_{max} of CBZ Decreased protein binding rate of CBZ No effect on AUC, C_{max}, $t_{1/2}$, CL, and F of CBZ	Improved dissolution of CBZ N.D.	Chen et al. 2002 [46]
TCM	Piperine (active compound in *Piper longum* Linn.)	Human	Patients with epilepsy (10 in each group)	Open label, crossover study	PK parameters	Increased bioavailability of CBZ Increased elimination rate and decreased elimination $t_{1/2}$	Decreased metabolism/elimination and/or increased absorption of CBZ	Pattanaik et al. 2009 [47]
TCM	Platycodonis Radix	Animal	Rabbits (4 in each group)	Randomized parallel design	PK parameters	Increased plasma level of CBZ	Improve CBZ absorption by increasing its solubility and stimulating bile secretion	Liu and Wei 2008 [48]
TCM	*Polygonum cuspidatum*	Animal	Male Sprague-Dawley rats (6 in each group)	Randomized crossover design	PK parameters	Increased level of CBZ and CBZ 10,11-epoxide in plasma, brain, liver, and kidney Decreased formation rate of CBZ 10,11-epoxide	Inhibited CYP3A in intestine and MRP2 in the kidney	Chi et al. 2012 [49]
HP	Septilin	Animal	Male rabbits (8 in each group)	Randomized crossover study	PK parameters	Decreased absorption of CBZ	Affected gastric emptying time or intestinal transit time	Garg et al. 1998 [50]
TCM	Xiao-cha-hu-tang	Animal	Female Sprague-Dawley rats (4 in each group)	Randomized parallel design	PK parameters	Increased T_{max}, decreased C_{max} of CBZ and AUC of CBZ 10,11-epoxide No effect on $t_{1/2}$, and $MRT_{0-\infty}$ of CBZ	Decreased GI absorption of CBZ by decreasing gastric emptying rate	Ohnishi et al. 2002 [51]
TCM	Xiao-qing-long-tang	Animal	Male Wistar rats (4–6 in each group)	Randomized parallel design	PK parameters	Increased T_{max}, elimination K of CBZ and decreased $t_{1/2}$ $MRT_{0-\infty}$ of CBZ No effect on C_{max} and AUC of CBZ and CBZ 10,11-epoxide	Decreased gastric emptying rate and accelerated metabolism of CBZ	Ohnishi et al. 1999 [52]
TCM	Xiao-yao-san	Human	Patients with major depression or bipolar disorder	Randomized double-blinded control trial	PK and PD parameters	Decreased plasma level of CBZ and increased incidence of dizziness, blurred vision, skin rash, and nausea	N.D.	Li et al. 2005 [53]

*Types of herbal product: traditional Chinese medicines (TCM)/other herbal products (HP); N.D.: not determined by authors; N/A: not applicable.

Table 3: Summary of the included *in vitro*, animal and clinical studies on interactions between carbamazepine and dietary supplement/food.

Dietary supplement (DS)/food	Dietary products	Study type	Subject/model (number)	Study design	Outcome measures	Effect	Mechanism	References
Food	Alcohol	Animal	Male CD-1 mice (10 in each group)	Randomized parallel design	PD parameters	Additive neurotoxicity (ethanol-induced motor incoordination and loss of righting reflex potentiated)	Nonadenosinergic action	Dar et al. 1989 [54]
Food	Alcohol	Human	Healthy volunteers (8)	Open label crossover study	PK parameters	No effect on pharmacokinetics of CBZ	Low ethanol level in subjects	Sternebring et al. 1992 [55]
Food	Alcohol	Human	Alcoholics (7)	Open label crossover study	PK parameters	Increased $AUC_{0-12\,h}$ of CBZ and decreased $AUC_{0-12\,h}$ of CBZ 10,11-epoxide	Acute inhibition of CBZ metabolism and/or accelerated CBZ metabolism in abstinence phase due to enzyme induction by previous ethanol abuse	Sternebring et al. 1992 [55]
Food	Butter	Animal	New Zealand white rabbit (8 in each group)	Crossover study	PK parameters	Increased bioavailability of CBZ	Improved solubility and dissolution of poorly soluble CBZ	Sidhu et al. 2004 [56]
Food	Caffeine	Human	Healthy male volunteers (6)	Open label crossover study	PK parameters	Decreased bioavailability and increased V_d of CBZ	Involving metabolism by mixed function oxidase	Vaz et al. 1998 [57]
Food	Caffeine	Animal	Albino Swiss male mice with maximal electroshock seizure model (7 in each group)	Randomized controlled parallel study	PK and PD parameters	Acute caffeine decreased antiepileptic efficacy of CBZ but had no effect on plasma level of CBZ	N.D.	Czuczwar et al. 1990 [58]
Food	Caffeine	Animal	Swiss male mice with maximal electroshock seizure model (8 in each group)	Randomized controlled study	PK and PD parameters	Chronic caffeine dose-dependently decreased anti-epileptic efficacy of CBZ but had no effect on plasma level of CBZ	May induce changes in neurotransmitter system causing sensitization effect	Gasior et al. 1996 [59]
Food	Coca-Cola	Human	Healthy male volunteers (10)	Randomized two-way crossover design	PK parameters	Increased bioavailability of CBZ; no change in elimination $t_{1/2}$	Enhanced dissolution of CBZ by its acidity	Malhotra et al. 2002 [60]
DS	Folinic acid	Animal	Male Sprague-Dawley rats (4 in each group)	Randomized parallel controlled design	PK parameters	No effect on plasma or brain level of CBZ	N.D.	Simth and Carl 1982 [61]
Food	Grapefruit juice	Human	Patients with epilepsy (10)	Randomized crossover study	PK parameters	Increased bioavailability of CBZ	Inhibited CYP3A4-mediated intestinal and hepatic metabolism of CBZ	Garg et al. 1998 [62]
Food	Honey	Animal	Angora grey rabbit (6 in each group)	Nonrandomized design	PK parameters	Decreased bioavailability of CBZ	Decreased metabolism of CBZ by inducing CYP enzymes	Koumaravelou et al. 2002 [63]

Table 3: Continued.

Dietary supplement (DS)/food	Dietary products	Study type	Subject/model (number)	Study design	Outcome measures	Effect	Mechanism	References
Food	Honey	Human	Healthy volunteers (10)	Randomized crossover study	PK parameters	Single dose of honey has no effect on pharmacokinetics of CBZ	N.D.	Malhotra et al. 2003 [64]
Food	Honey	Human	Healthy male volunteers (12)	Open label crossover study	PK parameters	Multiple doses of honey have no effect on pharmacokinetics of CBZ	Flavanoids in honey may not affect human CYP3A4 activity	Thomas et al. 2007 [65]
Food	Kinnow Juice	Human	Healthy male volunteers (9)	Randomized crossover study	PK parameters	Increased bioavailability of CBZ	Inhibited CYP3A activity	Garg et al. 1998 [66]
DS	Melatonin	Animal	Female Swiss mice (12 in each group)	Randomized parallel design	PK and PD parameters	Potentiated the anticonvulsant activity of CBZ but impair long-term memory but no effect on plasma and brain levels of CBZ	Enhanced GABAergic transmission in CNS	Borowicz et al. 1999 [67]
DS	Melatonin	Human	Children with epilepsy (28)	Double-blind randomized control study	PK and PD parameters	Increased glutathione reductase (antioxidant) activity but no effect on plasma level of CBZ and its metabolite	Antagonized CBZ-triggered reactive oxygen species accumulation	Gupta et al. 2004 [68]
DS	Melatonin	Animal	Male Swiss albino mice with maximal electroshock seizure model (7 in each group)	Randomized parallel design	PK and PD parameters	Synergistic anti-epileptic effect but no effect on plasma level of CBZ	N.D.	Gupta et al. 2004 [69]
DS	Nicotinamide	Human	Children with epilepsy (2)	Case report	PK parameters	Increased plasma level of CBZ and decreased clearance of CBZ	N.D.	Said et al. 1989 [70]
Food	Pomegranate juice	Animal	Male Wistar rats (5-6 in each group)	Randomized parallel design	PK parameters	Increased C_{max} and AUC of CBZ; no change in elimination $t_{1/2}$ and AUC ratio of CBZ 10,11-epoxide to CBZ	Inhibited enteric but not hepatic CYP3A activity	Hidaka et al. 2005 [71]
Food	Pomegranate juice	*Ex vivo*	Male Wistar rats (3 in each group)	*In vitro* everted and noneverted sac method	PK parameters	Decreased intestinal transport of CBZ	Induced enteric CYP3A4	Adukondalu et al. 2010 [72]
Food	Soybean	Animal	Albino Wistar rats (6 in each group)	Randomized parallel design	PK parameters	Decreased bioavailability of CBZ, increased plasma clearance and V_d of CBZ	Decreased gastric emptying and enhanced elimination of CBZ	Singh and Asad 2010 [73]
Food	Star Fruit Juice	Animal	Male Wistar rats (6 in each group)	Randomized parallel design	PK parameters	Increased C_{max} and AUC of CBZ; no change in elimination $t_{1/2}$ and AUC ratio of CBZ 10,11-epoxide to CBZ	Inhibited enteric but not hepatic CYP3A activity	Hidaka et al. 2006 [74]

N.D.: not determined by authors.

4. Discussion

Patients on antiepileptic therapy are usually on a long-term basis. Several antiepileptic drugs require therapeutic drug monitoring and are prone to drug interactions which may lead to serious consequences. CBZ is one of the antiepileptic drugs that are on the "watch-list". With the increased popularity of herbal products as well as dietary supplement, prescribers may need to be aware of the potential herb-drug or food-drug interactions when prescribing and monitoring CBZ therapy. In this study, we had conducted a systematic review and summarized the up-to-date evidence of the interactions between CBZ and herbal products/food/dietary supplements that have been reported in primary literature.

In order to achieve a comprehensive literature search, a total of eleven databases were searched. These included two conventional databases (EMBASE and MEDLINE), five other English databases (AMED, CINAHL Plus, Cochrane Database of Systematic Reviews, CENTRAL, and SciFinder Scholar) four Chinese databases. We had also consulted some relative tertiary literatures including Stockley's Herbal Medicines Interactions and Natural Medicines Comprehensive Database in case of any additional information. The keywords used for the search were optimized and refined in an attempt to include most of the relevant literatures (Table 1). We suggest that this search strategy could be applied on the search of other drugs—herb/food/dietary supplement interactions by substituting the drug name. It is interesting to note that, although a total of 100 clinical trials or case reports involving the concurrent use of TCMs with CBZ were identified from the Chinese databases, more than 90 of them focus on the beneficial effects or the antagonism of the side effects of CBZ of such combinational use and fallout from our inclusion criteria.

There were altogether 33 different herbal products/food/dietary supplements identified from literature in which their effects on CBZ were studied. These included 17 TCMs, 3 other herbs/botanicals, 10 foods, and 3 dietary supplements. The large number of studies involving TCM-drug interactions implies that TCM warrants special attention when coadministered with CBZ. However, the nonstandardized naming and multiple constituents of TCMs often confuse prescribers when anticipating such interaction. After extracting the herbal names from the original articles, we standardized the herbal names in Latin according to the Chinese Pharmacopoeia 2010 (Table 2). In order to raise the prescribers' awareness to the different names of the TCM products, we also provide the synonyms of the included TCMs in Table 4. For herbal formulae, their composition and content were also listed (Table 5).

No fatal or severe interactions between CBZ and herbal products/food/dietary supplement were found from the literature search. Majority of the studied interactions were pharmacokinetic-based, where the oral bioavailability or plasma level of CBZ was significantly altered by the natural products (Table 6). Twelve natural products/food, elevated the oral bioavailability/plasma level of CBZ with six of them demonstrating clinical evidence, namely, piperine, alcohol, Coca-Cola, grapefruit juice, kinnow juice, and nicotinamide (highlighted in bold in Table 6). In most cases the authors suggested that the increase in plasma CBZ concentrations was due to the inhibition of CYP3A4-mediated metabolism of CBZ by these natural products. Since CBZ has a narrow therapeutic index, and the side effects of CBZ are concentration-dependent, the increment of CBZ plasma level may result in serious adverse effects such as diplopia and nystagmus [75]. Therefore, it is advised to avoid the consumption of the food/herbal products which could elevate the CBZ plasma level as listed in Table 6.

Table 4: Synonyms of the included TCM products.

Herbal products	Synonyms
Acorus calamus Linn.	Sweet flag, Zhang-chang-pu
Cardiospermum halicacabum Linn.	Ballon vine, Winter cherry, Heartseed, Dao-di-ning
Cassia auriculata Linn.	Avaram, Senna auriculata, Tanner's Cassia, Er-ye-fan-xie
Hypericum perforatum Linn.	St John's wort
Paeoniae Radix	Peony, Shao-yao
Platycodonis Radix	Jie geng, Platycodon Root, Balloon flower
Polygonum cuspidatum	Japanese knotweed, Hu-zhang

On the other hand, nine natural products diminished the oral bioavailability/plasma level of CBZ significantly with four of them having clinical evidences: Ispaghula husk, Xiao-yao-san, Jia-wei-xiao-yao san, and caffeine (Table 4). Multiple mechanisms may contribute to the decrease of CBZ plasma level by these natural products, one of which is the increase in the metabolism of CBZ by induction of CYP3A4 (by Jia-wei-xiao-yao-san, ginkgo biloba, and soybean) and mixed function oxidase (by caffeine) activity. Ispaghula husk, septilin, soybean and Xiao-cha-hu-tang reduced the plasma level of CBZ by affecting its gastric absorption. Coadministration of CBZ with herbal products/food which are enzyme inducers entails the possibility of a clinically significant drug interaction. The reduction of CBZ plasma level may imply that less CBZ is present in the target site to assert its antiepileptic activity; a worsened seizure control may follow unless the dosage of CBZ is adjusted accordingly [76]. Since enzyme induction is a reversible phenomenon, particular caution is required when an enzyme-inducing agent is discontinued because the serum concentration of concurrently administered CBZ may rebound to potentially toxic levels. Patients are therefore not recommended to take the food/herbal products which could decrease the CBZ plasma level listed in Table 6.

Furthermore, CBZ has poor water solubility; consequently, its absorption time and extent are thus easily affected by coadministration of substances that may alter gastric conditions. For example, Xiao-cha-hu-tang and Xiao-qing-long-tang delayed the time for CBZ to reach peak plasma concentration by decreasing the gastric emptying rate. By improving the dissolution of CBZ, Paeoniae Radix

Table 5: Composition of individual herbs in the included herbal formulae.

Herbal formula	Other name	Herbs	Content
Chai-hu-jia-long-gu-mu-li-tang	Saiko-ka-ryukostsu-borei-to	Bupleuri Radix	5 parts
		Pinelliae Tuber	4 parts
		Cinnamomi Cortex	3 parts
		Hoelen	3 parts
		Scutellariae Radix	2.5 parts
		Zizyphi Fructus	2.5 parts
		Ginseng Radix	2.5 parts
		Ostreae testa	2.5 parts
		Fossilia Ossis Mastodi	2.5 parts
		Zingiberis Rhizoma	1 part
Hu-gan-ning pian	Huganning tablet	Sedi Herba	850 g
		Polygoni Cuspidati Rhizoma et Radix	500 g
		Salviae Miltiorrhizae Radix et Rhizoma	250 g
		Ganoderma	200 g
Jia-wei-xiao-yao-san	Free and easy wanderer plus	Bupleuri Radix	12.5%
		Scutellariae Radix	12.5%
		Zingiberis Rhizoma	11.2%
		Angelicae sinensis Radix	9.7%
		Zizyphi Fructust	9.7%
		Moutan Cortex	9.7%
		Paeoniae Radix Alba	9.7%
		Atractylodis Macrocephalae Rhizoma	8.3%
		Poria	6.9%
		Menthae Haplocalycis Herba	5.6%
		Glycyrrhizae Radix	4.2%
Mentat	BR 16A	*Bacopa monnieri* Linn.	Not known
		Centella asiatica Linn.	
		Withania somnifera Linn.	
		Evolvulus alsinoides Linn.	
		Nardostachys jatamansi Linn.	
		Acorus calamus Linn.	
		Celastrus paniculatus Linn.	
		Zingiber officinale Linn.	
		Valeriana wallichii	
		Prunus amygdalus	
		Orchis mascula Linn.	
		Syzygium aromaticum Linn.	
		Mukta pishti	
Xiao-cha-hu-tang	Sho-saiko-to	Bupleuri Radix	7 parts
		Pinelliae Tuber	5 parts
		Scutellariae Radix	3 parts
		Zizyphi Fructus	3 parts
		Ginseng Radix	3 parts
		Glycyrrhizae Radix	2 parts
		Zingiberis Rhizoma	1 part
Xiao-qing-long-tang	Sho-seiryu-to extract	Pinelliae Tuber	6 parts
		Glycyrrhizae Radix	3 parts
		Cinnamomi Cortex	3 parts
		Schisandrae Fructus	3 parts
		Asiasari Radix	3 parts
		Paeoniae Radix	3 parts
		Ephedrae Hebra	3 parts
		Zingiberis Siccatum Rhizoma	3 parts

Table 5: Continued.

Herbal formula	Other name	Herbs	Content
Xiao-yao-san	Free and easy wanderer	Bupleuri Radix	2 parts
		Angelicae sinensis Radix	2 parts
		Paeoniae Radix Alba	2 parts
		Atractylodis Macrocephalae Rhizoma	2 parts
		Poria	2 parts
		Zingiberis Rhizoma	2 parts
		Glycyrrhizae Radix	1 part
		Menthae Haplocalycis Herba	1 part

Table 6: Pharmacokinetic and pharmacodynamic interactions of herbal products/food/dietary supplements with CBZ by direction of interactions.

(a)

Pharmacokinetic interactions with CBZ		
Oral bioavailability/plasma level of CBZ		
Increased	Decreased	No effect
Piperine	Septilin	***Hypericum perforatum* Linn.**
Mentat	*Ginkgo biloba*	Paeoniae Radix
Polygonum cuspidatum	Hu-gan-ning pian	*Cardiospermum halicacabum* Linn.
Butter	**Ispaghula husk**	Berberine
Grapefruit juice	*Cassia auriculata* Linn.	Xiao-qing-long-tang
Platycodonis Radix	Hu-gan-ning pian	Chai-hu-jia-long-gu-mu-li-tang
Pomegranate juice	Xiao-cha-hu-tang	*Acorus calamus* Linn.
Star fruit juice	**Xiao-yao-san**	**Honey**
Kinnow juice	**Jia-wei-xiao-yao-san**	**Melatonin**
Alcohol*	Soybean	Folinic acid
Coca-cola	**Caffeine**	
Nicotinamide		

*In alcoholics, not healthy volunteers; study type: human study (**bold**), animal study (regular).

(b)

Pharmacodynamic interactions with CBZ					
Antiepileptic efficacy of CBZ			Side effects related to CBZ		
Potentiation	Inhibition	No effect	Potentiation	Reduction	No effect
Acorus calamus Linn.	Caffeine		**Xiao-yao-san**		*Cassia auriculata* Linn.
Melatonin			Melatonin		*Cardiospermum halicacabum* Linn.
			Alcohol		

Study type: human study (**bold**), animal study (regular).

allowed faster absorption while butter and Platycodonis Radix increased the extent absorption of CBZ.

Any changes of the plasma level of CBZ-10,11 epoxide caused by the simultaneous administration of herbal products/food/dietary supplements with CBZ should also be noted. Formed through the CYP3A4-mediated metabolism in intestine and liver, CBZ-10,11 epoxide is the principle metabolite of CBZ which is pharmacologically active and may contribute to the toxicities of CBZ [77]. Neurotoxic symptoms including ataxia, dizziness, nausea, and diplopia had been observed in patients in which lamotrigine or loxapine was added to CBZ therapy, with elevated blood levels of CBZ-10,11-epoxide [78, 79]. *Polygonum cuspidatum*, a widely used TCM indicated for menstrual and postpartum difficulties, traumatic burns, and acute viral hepatitis, was shown to increase CBZ and CBZ-10,11-epoxide levels in plasma, brain, liver, and kidney in an animal study [49]. On the other hand, Xiao-cha-hu-tang decreased the oral bioavailability of CBZ-10,11-epoxide in rats [51]. Although there was no clinical evidences of these two TCMs causing an elevated/decreased plasma level of CBZ-10,11-epoxide or showing their linkage to pharmacodynamic outcome, it is rational to pay necessary cautions and avoid their combinational use with CBZ.

Compared to pharmacokinetic interactions, there were fewer studies reporting pharmacodynamic-based interactions between herbal products/food/dietary supplements and CBZ. Pharmacodynamic interaction refers to the alteration of efficacy (antiepileptic activity) and/or the adverse effects of CBZ in the presence of natural products. Melatonin and *Acorus calamus* Linn. potentiated the anticonvulsant activity of CBZ but had no effect on the plasma levels of CBZ in animal studies so such interactions are mainly

pharmacodynamic-based. Despite the apparent efficacy-boosting effect, it is best to avoid the use of melatonin or *Acorus calamus* Linn. with CBZ until there further clinical evidence on the safe usage of such combination. Caffeine, on the other hand, decreased the plasma level of CBZ in human while decreased the antiepileptic efficacy of CBZ in mouse. Although there are no clinical studies on whether the antiepileptic activity of CBZ is influenced by caffeine, advice should be given to patients on CBZ therapy not to take caffeine. Caution should also be paid for beverage containing alcohol. Alcohol was demonstrated to have additive neurotoxicity with CBZ in mouse, including a potentiated motor incoordination and loss of righting reflex. Together with the fact that alcohol caused an increase in the oral bioavailability of CBZ in alcoholics, it is advised not to consume any alcohol while patients take CBZ. In a randomized double-blinded control trial, Xiao-yao-san increased the incidence of CBZ-related side effects including dizziness, blurred vision, skin rash, and nausea in patients with major depression or bipolar disorder. Though the mechanism is unknown, patients should be warned about the potential risks when taking this TCM with CBZ.

In this study, the documented evidence of interactions between CBZ and herbal products/food/dietary supplements was systematically reviewed from the published literature. The intention of this review was to provide guidance to assist healthcare professionals in identifying patients taking CBZ that are more susceptible to these interactions and make proper actions. A total of 33 unique herbal products/dietary supplement/food-CBZ interacting pairs were identified from this review. Considering the popularity and frequent usage of both CBZ (as first-line epilepsy regimen) and herbal products/food/dietary supplements, the number of studied interactions is considerably small. More evidence and reports are needed from research studies and, preferably, from adverse report system in clinical setting. Of course, the importance of therapeutic drug monitoring of CBZ is again emphasized while most pairs of natural products-CBZ interactions remained unknown. On the other hand, the amount of documented CBZ-herbal products/food/dietary supplements interactions might be underreported in this review due to several limitations, including publication bias and language restrictions. We had attempted to reduce language bias by including four evidence-based Chinese databases. However, the evidence regarding complementary alternative medicine or folk therapies, which were published in other languages (e.g., Japanese, Indian, and French), might be missing. Another limitation of this review was that it included all relevant information identified in the literature, regardless of the evidence types or quality of the studies. Such arrangement aimed to gather as much useful information regarding studies on interactions between CBZ and the natural products. Although species differences existed, human pharmacokinetic parameters and pharmacodynamic behavior could be successfully extrapolated from animal studies [80, 81]. Therefore, the data from animal studies are considered to be valuable, and hence the results should not be neglected.

5. Conclusion

This review provides a structured summary of the evidence of the documented interactions between CBZ and herbal products/food/dietary supplements. These findings should be helpful for healthcare professionals to identify potential herb-drug and food-drug interactions while prescribing CBZ and would also facilitate them to communicate these documented interactions to their patients, thus preventing potential adverse events and improving patients' therapeutic outcomes.

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