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ORIGINAL ARTICLE

Influence of Flavonoids from *Galium verum* L. on the Activities of Cytochrome P450 Isozymes and Pharmacokinetic and Pharmacodynamic of Warfarin in Rats

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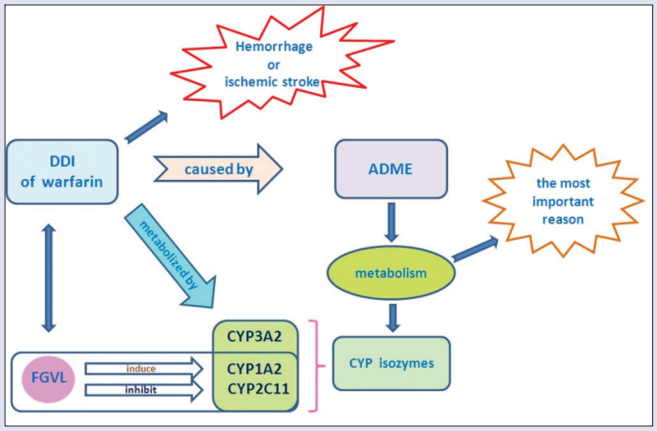
ABSTRACT

Background: *Galium verum* L., a traditional Chinese medicine, has been widely used in the folk in China. Preparations of *G. verum* L. were used to treat thromboembolic disease in clinic for many years and often combined with anticoagulants. Flavonoids from *G. verum* L. (FGVL) are the main active component. **Materials and Methods:** We assessed the potential influence of FGVL on the activities of five cytochrome P450 (CYP) enzymes and on the pharmacokinetic of warfarin in rats. The pharmacokinetics of five probe drugs and of warfarin was compared between control and FGVL-pretreated groups, which could estimate the effect on the activities of the five isozymes and warfarin metabolism. Moreover, the potential influence of FGVL on the pharmacodynamic of warfarin was investigated. **Results:** There was no significant difference in the pharmacokinetic parameters of chlorzoxazone and midazolam between control and FGVL-pretreated groups. However, the pharmacokinetic parameters of caffeine in every dose, metoprolol in middle dose, and tolbutamide in high dose were affected significantly ($P < 0.05$). It indicated that the metabolism of caffeine was markedly faster in FGVL-pretreated group but metoprolol in the middle dose and tolbutamide in the high-dose FGVL-pretreated group were markedly slower. The anticoagulation of combination group is better than warfarin group or FGVL group. This suggested that FGVL showed no effect on the enzyme activities of CYP2E1 and CYP3A2, induced the enzyme activities of CYP1A2, but inhibited the enzyme activities of CYP2D4 in the middle dose and CYP2C11 in high dose. **Conclusion:** This indicates that FGVL may increase the anticoagulation of warfarin in clinic dose. The dose of warfarin should be adjusted when combined with FGVL or preparations of *G. verum* L.

Key words: Cytochrome P450 isozymes, *Galium verum* L., herb–drug interaction, pharmacodynamic, pharmacokinetic, warfarin

SUMMARY

- Flavonoids from *G. verum* L. (FGVL) can affect the activities of cytochrome P450 isozymes and pharmacokinetic and pharmacodynamic of warfarin in rats. The dose of warfarin should be adjusted when combined with FGVL or preparations of *Galium verum* L. in clinic.



Abbreviations used: FGVL: Flavonoids from *G. verum* L.; TCM: Traditional Chinese medicine.

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INTRODUCTION

Flavonoids from *Galium verum* L. (FGVL) are the ethanol extracts of *G. verum* L. which is used in China for the treatment of circulatory system diseases, such as arteriosclerosis obliterans,[1,2] deep venous thrombosis,[3,4] and diabetic foot.[5,6] It has been suggested that some Chinese herb extracts may be associated with a number of clinically important herb–drug interactions that can cause adverse action.[7-9] Interactions mainly come from the impact on cytochrome P450 (CYP) enzyme activities.[10-12] However, no systematic study has reported on the impact of FGVL on CYP enzyme activities.

Warfarin is the widely used oral anticoagulants for the prevention and treatment of thromboembolic diseases.[13] Although it is effective, the adverse actions were reported constantly for hemorrhage (upon overdosing) or ischemic stroke (underdosing).[14-16] The main reasons are the narrow therapeutic window and difference of interindividual response. Drug–drug/herb–drug interactions of warfarin can be caused by any of absorption, distribution, and metabolism.[17-20] The metabolic interaction of warfarin is the most important.[20-22]

The aims of the present study were to evaluate the effects of FGVL on the activities of CYP enzymes (CYP1A2, CYP2D4, CYP2E1, CYP2C11, and

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CYP3A2) and to investigate the impact of FGVL on the pharmacokinetic and pharmacodynamic of warfarin in rats.

MATERIALS AND METHODS

Chemical and reagents

FGVL were made by our laboratory (FGVL: 75%), and caffeine (M1082) was obtained from Sigma Inc. (USA). Tolbutamide (J1010AS), metoprolol (D0406AS), chlorzoxazone (20130615), warfarin (J0206AS), naproxen (S0322AS), and diazepam (1605271) were obtained from Meilun Biotech Co. Ltd. (Liaoning, China). Midazolam (20160308) was obtained from Jiangsu Nhwa Pharma Co. Ltd. (Jiangsu, China). Methanol was from Dikma Technologies Inc. (USA). All other reagents were of analytical grade and were obtained from Weiye Biotech Co. Ltd. (Heilongjiang, China).

Animals and treatments

The experiment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Experiment Animal Center of Heilongjiang University of Chinese Medicine. Male Sprague Dawley rats (200 ± 20 g) were purchased from Qingdao Laboratory Animal Co. Ltd. All rats were maintained in a room under controlled temperature and humidity with standard laboratory food and water.

Cytochrome P450 probe–drug groups

The experimental animals were divided into the blank control group (BCG) and CYP probe group (low-dose group [LD-G], middle-dose group [MD-G], and high-dose group [HD-G]). BCG received 0.5% carboxymethyl cellulose (CMC) solution for 7 days. FGVL was suspended in a 0.5% aqueous solution of CMC and administered orally for 7 days at the once daily dose of 45, 90, and 180 mg/kg (LD-G, MD-G, and HD-G groups, respectively).

On the 8th day, the rats of every group were treated with probe cocktail solution (5.0 mL/kg) containing caffeine (2.5 mg/kg), metoprolol (10 mg/kg), chlorzoxazone (5.0 mg/kg), tolbutamide (2.5 mg/kg), and midazolam (5.0 mg/kg), through caudal vein.

Influence on pharmacokinetic of warfarin

The experimental animals were divided into combination group (FGVL 90 mg/kg + warfarin 0.2 mg/kg, $n = 6$) and warfarin group (0.2 mg/kg, $n = 6$).

The combination group: FGVL was suspended in a 0.5% aqueous solution of CMC and administered orally for 7 days. Warfarin group received 0.5% CMC solution for 7 days. Similarly, on either the 8th day, the rats of the combination group and warfarin group were administered warfarin (0.2 mg/kg).

Influence on pharmacodynamic of warfarin

The experimental animals were divided into the BCG (0.5% CMC, $n = 6$), FGVL group (90 mg/kg, $n = 6$), warfarin group (0.2 mg/kg, $n = 6$), and combination group (FGVL 90 mg/kg + warfarin 0.2 mg/kg, $n = 6$). The rats were administered orally for 7 days.

Sample preparation

Cytochromes P450 probe–drug groups

Blood samples were collected in Eppendorf tubes coated with heparin sodium from rats at 0.083, 0.167, 0.250, 0.333, 0.5, 1, 2, 4, 8, 12, and 24 h after dosing and were immediately centrifuged at room temperature for 10 min (3500 r/min). The samples were then stored at −20°C.

All samples (100 μL) spiked with internal standard diazepam 50 μL (8 μg/mL) were mixed with 2 mL of chloroform. The mixture was centrifuged at 3500 r/min for 10 min. The organic phase (1.6 mL) was transferred to a fresh tube and concentrated under nitrogen at 45°C. The residues were reconstituted with 100 μL initial mobile phase for high-performance liquid chromatography (HPLC), followed by vortexing for 1 min, and centrifuged at 12,000 r/min at 4°C for 10 min.

Influence on pharmacokinetic of warfarin

Blood samples were collected in Eppendorf tubes coated with heparin sodium from rats at 0.167, 0.5, 1, 3, 5, 12, 24, 48, 72, 120, and 144 h after dosing and were immediately centrifuged at room temperature for 10 min (3500 r/min). The samples were then stored at −20°C.

All samples (100 μL) spiked with internal standard naproxen 50 μL (4 μg/mL) were mixed with 20 μL of acetic acid and 2 mL ethyl acetate. The mixture was centrifuged at 3500 r/min for 10 min. The organic phase (1.6 mL) was transferred to a fresh tube and concentrated under nitrogen at 45°C. The residues were reconstituted with 100 μL mobile phase for HPLC, followed by vortexing for 1 min, and centrifuged at 12,000 r/min at 4°C for 10 min.

Influence on pharmacodynamic of warfarin

Blood samples of every group were collected before the first administration, on the 3th, 5th, and 7th days before administration and then on the 8th day. Blood samples were collected in Eppendorf tubes coated with sodium citrate and were immediately centrifuged at room temperature for 10 min (3500 r/min). The samples were then stored at −20°C.

Analytical methods

Cytochrome P450 probe–drug groups

HPLC analysis was performed in a gradient elution mode on Topsil C_{18} column (250 mm × 4.6 mm 5 μm, Welch, China) at 35°C. The mobile phase consisted of methanol (A) and ammonium dihydrogen phosphate buffer solution (B) (pH 3.4). A linear gradient at a flow rate of 1.0 mL/min was run at 40% A over 0.01–8.00 min, 40%–50% A over 8.01–10.00 min, 50% A over 10.01–28.00 min, 50%–55% A over 28.01–29.00 min and 55% A over 29.01–45.00 min, λ = 230 nm. 10 μL of samples was injected into the HPLC system. The HPLC chromatograms are presented in Figure 1.

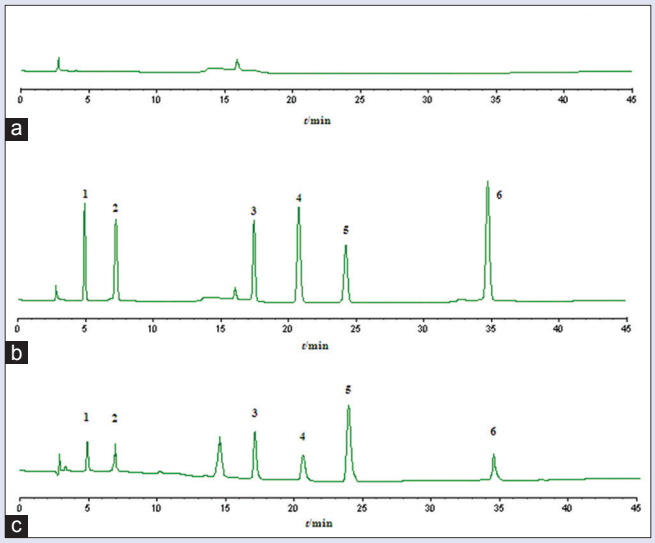

Figure 1: HPLC chromatograms of five probe drugs. (a) Blank plasma. (b) Blank plasma + probe–drug + internal standard. (c) Plasma samples + internal standard. (1) Caffeine. (2) Metoprolol. (3) Chlorzoxazone. (4) Midazolam. (5) Tolbutamide. (6) Diazepam

Influence on pharmacokinetic of warfarin

HPLC analysis was performed on Topsil C_{18} column (250 mm × 4.6 mm 5 μm, Welch, China) at 40°C. The mobile phase consisted of methanol–0.2% acetic acid (62:38), λ = 284 nm. Flow rate was 1.0 mL/min, 10 μL of samples was injected into the HPLC system.

Influence on pharmacodynamic of warfarin

The prothrombin time (PT) value of the blood samples was measured by PT reagent kit (Shanghai Sun Biotech Co. Ltd.).

Statistical analysis

Data were presented as mean ± standard deviation (SD); pharmacokinetic parameter calculations were carried out by DAS 2.0 pharmacokinetic program (Chinese Pharmacological Society, Beijing, China) and generated using a noncompartmental model (statistical moment). Statistically significant differences in the pharmacokinetic parameters between the treatment groups and BCG were assessed using one-way analysis of variance, followed by Dunnett's test, with the level of statistical significance set at 0.05.

RESULTS

Effect of flavonoids from *Galium verum* L. on cytochromes P450 1A2 in rats

The effects of FGVL in the different treatment groups on pharmacokinetic parameters of caffeine in rats are presented in Table 1. The mean plasma concentration–time curves of caffeine in different groups in rats are presented in Figure 2. Area under the curve $(AUC)_{(0-t)}$, $AUC_{(0-\infty)}$, mean residence time $(MRT)_{(0-t)}$, $MRT_{(0-\infty)}$, and $t_{1/2}$ decreased significantly. CL is increasing. These results indicate that FGVL may induce CYP1A2 activity *in vivo*.

Effect of flavonoids from *Galium verum* L. on cytochromes P450 2D4 in rats

The effects of FGVL in the different treatment groups on pharmacokinetic parameters of metoprolol in rats are presented in Table 2. The mean plasma concentration–time curves of metoprolol in different groups in rats are presented in Figure 3. $t_{1/2}$ increased and CL is decreasing appreciably in MD-G. These results imply that FGVL revealed the influence on CYP2D4 activity *in vivo,* and the influence on activity of CYP2D4 was different from dose.

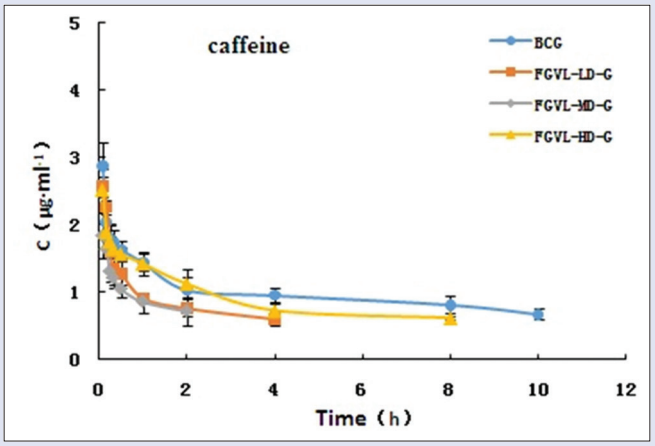

Figure 2: The mean plasma concentration–time curves of caffeine in different groups

Effect of flavonoids from *Galium verum* L. on cytochromes P450 2E1 in rats

The effects of FGVL in the different treatment groups on pharmacokinetic parameters of chlorzoxazone in rats are presented in Table 3. The mean plasma concentration–time curves of chlorzoxazone in different groups in rats are presented in Figure 4. No significant difference in the pharmacokinetic parameters was observed in rats between FGVL and

Table 1: Effects of flavonoids from *Galium verum* L. on pharmacokinetic parameters of caffeine in rat

Parameters	BCG	FGVL		
		LD-G	MD-G	HD-G
$t_{1/2}$ (h)	9.96±2.67	2.74±0.89*	1.40±0.61*	4.12±1.00*
AUC_{0-t} (mg/L·h)	10.03±0.95	3.69±0.40*	1.97±0.24#	7.49±0.75*
$AUC_{0-\infty}$ (mg/L·h)	19.84±3.37	5.68±0.57*	3.03±0.50#	10.26±2.17
MRT_{0-t} (h)	4.18±0.09	1.54±0.06#	0.82±0.01#	3.10±0.13*
$MRT_{0-\infty}$ (h)	14.01±3.58	4.20±0.65*	2.38±0.46*	7.25±0.28
CL (L/h/kg)	0.12±0.02	0.44±0.04#	0.83±0.12#	0.25±0.05

Values are represented as mean±SD (n=6). *P<0.05 when compared with related parameters of BCG, #P<0.01 when compared with related parameters of BCG. SD: Standard deviation; FGVL: Flavonoids from *Galium verum* L.; AUC: Area under the curve; LD-G: Low-dose group; MD-G: Middle-dose group; HD-G: High-dose group; BCG: Blank control group; MRT: Mean residence time

Table 2: Effects of flavonoids from *Galium verum* L. on pharmacokinetic parameters of metoprolol in rat

Parameters	BCG	FGVL		
		LD-G	MD-G	HD-G
$t_{1/2}$ (h)	10.72±0.88	10.25±3.15	15.92±2.75*	10.51±3.13
AUC_{0-t} (mg/L·h)	45.30±1.41	33.87±6.84*	33.19±1.36#	24.20±6.33*
$AUC_{0-\infty}$ (mg/L·h)	57.93±4.42	42.54±13.02	50.35±6.63	30.42±7.96*
MRT_{0-t} (h)	9.14±0.27	8.68±0.24	10.11±1.05	9.14±0.33
$MRT_{0-\infty}$ (h)	15.70±1.61	16.10±1.95	26.76±9.77*	17.83±4.04
CL (L/h/kg)	0.17±0.01	0.24±0.06	0.20±0.28	0.34±0.09

Values are represented as mean±SD (n=6). *P<0.05 when compared with related parameters of BCG, #P<0.01 when compared with related parameters of BCG. SD: Standard deviation; FGVL: Flavonoids from *Galium verum* L.; AUC: Area under the curve; LD-G: Low-dose group; MD-G: Middle-dose group; HD-G: High-dose group; BCG: Blank control group; MRT: Mean residence time

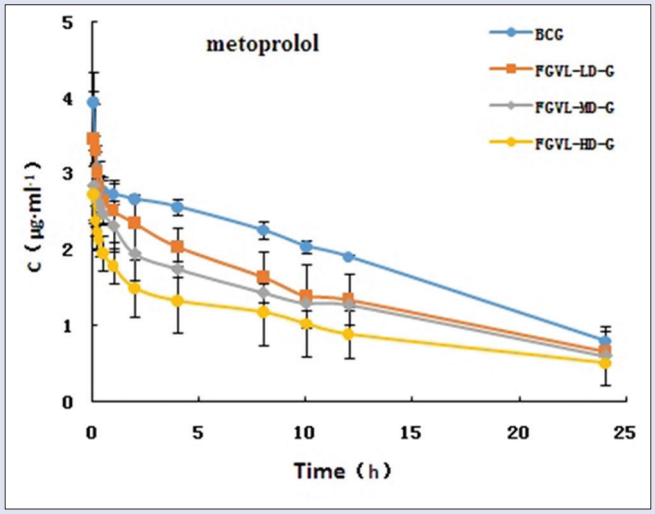

Figure 3: The mean plasma concentration–time curves of metoprolol in different groups

BCG. These results imply that FGVL revealed little influence on CYP2E1 activity *in vivo*.

Effect of flavonoids from *Galium verum* L. on cytochromes P450 2C11 in rats

CYP2C11 activity was evaluated by assessment of tolbutamide pharmacokinetic behavior [Table 4]. The mean plasma concentration–time curves of tolbutamide in different groups in rats are presented in Figure 5. It has no significant difference between LD-G, MD-G, and BCG. Otherwise, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, and $t_{1/2}$ increased significantly in HD-G. CL is decreasing. These results indicate that FGVL in high dose may inhibit CYP2C11 activity *in vivo*.

Effect of flavonoids from *Galium verum* L. on cytochromes P450 3A2 in rats

The effects of FGVL in the different groups on pharmacokinetic parameters of midazolam in rats are presented in Table 5. The mean plasma concentration–time curves of midazolam in different groups in rats are presented in Figure 6. Little changes in pharmacokinetic parameters of midazolam were observed in rats pretreated with FGVL. This indicates that FGVL had no influence on CYP3A2 activity *in vivo*.

Effects of flavonoids from *Galium verum* L. on pharmacokinetic of warfarin

To determine the effect of FGVL on pharmacokinetic of warfarin, time–course plasma of warfarin was monitored in rats with and without pretreatment with FGVL. The mean plasma concentration–time curves are presented in Figure 7, and the pharmacokinetic parameters are summarized in Table 6. Pretreatment with FGVL for 7 days in rats decreased the AUC and $t_{1/2}$ and increased the C_{max} and CL of warfarin.

Table 3: Effects of flavonoids from *Galium verum* L. on pharmacokinetic parameters of chlorzoxazone in rats

Parameters	BCG	FGVL		
		LD-G	MD-G	HD-G
$t_{1/2}$ (h)	0.96±0.05	0.90±0.35	1.04±0.17	1.15±0.36
AUC_{0-t} (mg/L·h)	6.97±0.49	6.25±1.57	5.81±0.53	6.43±1.09
$AUC_{0-\infty}$ (mg/L·h)	9.02±0.77	8.01±2.80	7.79±1.31	8.71±0.73
MRT_{0-t} (h)	0.70±0.01	0.72±0.03	0.70±0.02	0.74±0.04
$MRT_{0-\infty}$ (h)	1.31±0.05	1.38±0.20	1.39±0.26	1.72±0.18*
CL (L/h/kg)	0.55±0.04	0.67±0.23	0.65±0.10	0.57±0.05

Values are represented as mean±SD (n=6). *P<0.05 when compared with related parameters of BCG, #P<0.01 when compared with related parameters of BCG. SD: Standard deviation; FGVL: Flavonoids from *Galium verum* L.; AUC: Area under the curve; LD-G: Low-dose group; MD-G: Middle-dose group; HD-G: High-dose group; BCG: Blank control group; MRT: Mean residence time

Table 4: Effects of flavonoids from *Galium verum* L. on pharmacokinetic parameters of tolbutamide in rat

Parameters	BCG	FGVL		
		LD-G	MD-G	HD-G
$t_{1/2}$ (h)	5.41±1.65	6.26±1.29	7.10±1.59	10.47±1.50*
AUC_{0-t} (mg/L·h)	77.02±3.31	72.03±4.69	55.97±7.25*	88.97±13.41
$AUC_{0-\infty}$ (mg/L·h)	81.33±6.65	77.33±2.05	61.37±6.03*	111.08±10.36*
MRT_{0-t} (h)	5.62±0.21	6.70±0.46*	6.03±0.25	8.04±0.31*
$MRT_{0-\infty}$ (h)	7.30±0.87	9.22±0.96	8.61±1.29*	14.42±1.56*
CL (L/h/kg)	0.03±0.002	0.03±0.001	0.04±0.004	0.02±0.002

Values are represented as mean±SD (n=6). *P<0.05 when compared with related parameters of BCG, #P<0.01 when compared with related parameters of BCG. SD: Standard deviation; FGVL: Flavonoids from *Galium verum* L.; AUC: Area under the curve; LD-G: Low-dose group; MD-G: Middle-dose group; HD-G: High-dose group; BCG: Blank control group; MRT: Mean residence time

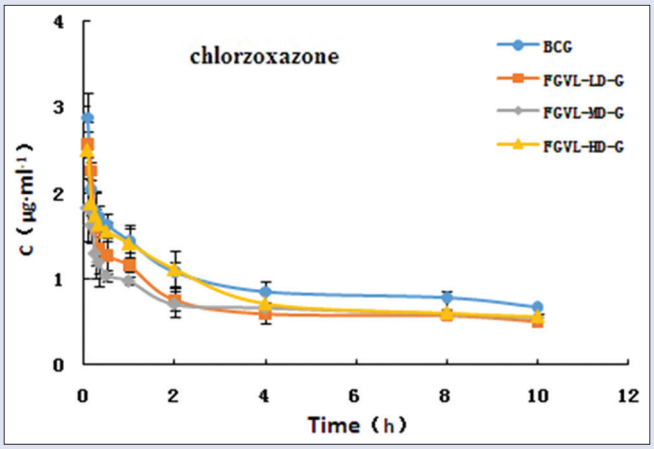

Figure 4: The mean plasma concentration–time curves of chlorzoxazone in different groups

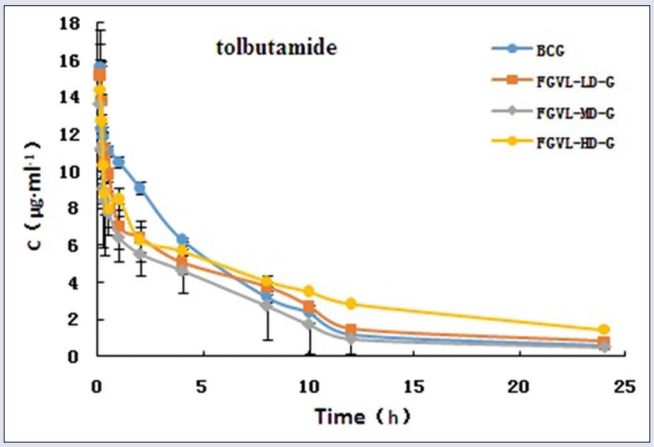

Figure 5: The mean plasma concentration–time curves of tolbutamide in different groups

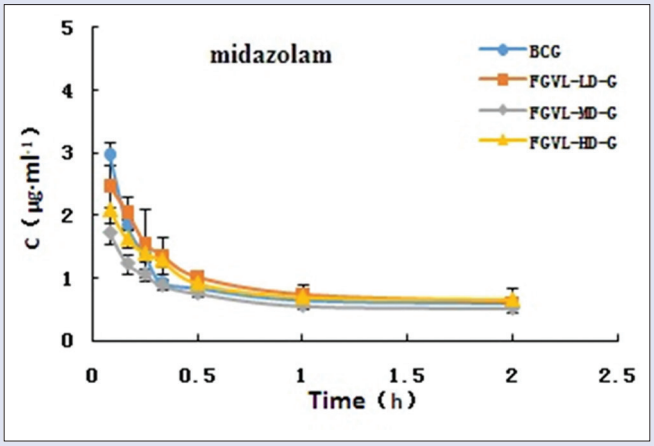

Figure 6: The mean plasma concentration–time curves of midazolam in different groups

Effects of flavonoids from *Galium verum* L. on pharmacodynamic of warfarin

Effects of FGVL on pharmacodynamic of warfarin are presented in Figures 8 and 9. It has no difference from 1st to 8th of PT and international normalized ratio (INR) in CMC-Na group ($P > 0.05$). The difference is statistically significant from 1st, 3rd, 5th, to 7th ($P < 0.05$) but statistically insignificant between 7th and 8th in FGVL group ($P > 0.05$). The difference is statistically significant from 3rd, 5th, 7th, to 1st ($P < 0.05$) but statistically insignificant between 7th and 8th in warfarin group ($P > 0.05$). The tendency in combination group is similarity to warfarin group. On the other hand, result of analysis between different groups is that anticoagulation of combination group is better than warfarin group or FGVL group. This indicates that FGVL may increase anticoagulation of warfarin.

DISCUSSION

Morbidity rate and mortality rate of thromboembolic diseases are higher in clinic. Many thromboembolic diseases may be treated with warfarin, such as cardiovascular and pulmonary embolism.[23,24] Although good anticoagulant effect, bleeding is an important adverse drug response due to the narrow therapeutic index. Moreover, among them, drug–drug interaction is one of the important reasons.[24-27] Warfarin is often used in combination with some traditional Chinese medicine (TCM) preparations possess activating blood circulation to dissipate blood stasis in China, such as Shuxuetong injection,[28] Ginkgo biloba preparation,[29] compound Danshen dripping pills.[30] However, more and more interactions about warfarin combining with TCM were reported.[21,31,32] Kangmai capsule is the preparations of *G. verum* L. to treat thromboembolic disease in clinic.[2] FGVL was the main anticoagulant component of *G. verum* L.[33] Whether the combination is safety between FGVL and warfarin, this drew our attention to FGVL-mediated pharmacokinetic and pharmacodynamic behavior alteration of warfarin, especially the effect of FGVL on CYP enzymes.

Table 5: Effects of flavonoids from *Galium verum* L. on pharmacokinetic parameters of midazolam in rat

Parameters	BCG	FGVL		
		LD-G	MD-G	HD-G
$t_{1/2}$ (h)	1.01±0.60	0.78±0.22	0.81±0.04	1.04±0.69
AUC_{0-t} (mg/L·h)	1.25±0.13	2.01±0.31*	0.92±0.06*	1.17±0.28
$AUC_{0-\infty}$ (mg/L·h)	2.20±0.83	2.36±0.41	1.57±0.10	2.20±0.70
MRT_{0-t} (h)	0.33±0.01	0.73±0.01#	0.38±0.01*	0.38±0.01*
$MRT_{0-\infty}$ (h)	1.31±0.62	1.54±0.16	1.12±0.05	1.43±0.86
CL (L/h/kg)	1.29±0.62	1.08±0.21	1.58±0.10	1.21±0.39

Values are represented as mean±SD (n=6). *P<0.05 when compared with related parameters of BCG, #P<0.01 when compared with related parameters of BCG. SD: Standard deviation; FGVL: Flavonoids from *Galium verum* L.; AUC: Area under the curve; LD-G: Low-dose group; MD-G: Middle-dose group; HD-G: High-dose group; BCG: Blank control group; MRT: Mean residence time

Table 6: Effects of Flavonoids from *Galium verum* L. on pharmacokinetic parameters of warfarin (n=6, $\bar{x}$±s)

Parameters	Warfarin group	Combination group
$t_{1/2}$ (h)	26.56±2.16	18.98±2.89*
t_{max} (h)	4.33±1.15	3.67±1.15
C_{max} (mg/L)	0.49±0.06	0.60±0.09
AUC_{0-t} (mg/L·h)	19.81±1.84	15.23±2.81*
$AUC_{0-\infty}$ (mg/L·h)	20.36±1.94	15.31±2.76*
CL (L/h/kg)	0.009±0.001	0.013±0.002*

Values are represented as mean±SD (n=6). *P<0.05 when compared with related parameters of warfarin group. SD: Standard deviation; AUC: Area under the curve

In this study, the rats were chosen as the experimental animal. Although rats differ from humans with regard to isoform composition, expression, and catalytic activity of drug metabolizing enzymes, rats are often as animal models for metabolism studies. Rat CYP1A2, CYP2D4, CYP2E1, CYP2C11, and CYP3A2 are homolog to human CYP1A2, CYP2D6, CYP2E1, CYP2C9, and CYP3A4, respectively.[34] We investigated the potential influence of FGVL on the activities of different isoforms CYP1A2, CYP2D4, CYP2E1, CYP2C11, and CYP3A2 in rats, by examining pharmacokinetic parameters of their probe drugs – caffeine, metoprolol, chlorzoxazone, tolbutamide, and midazolam, respectively. Furthermore, the assessment of the potential

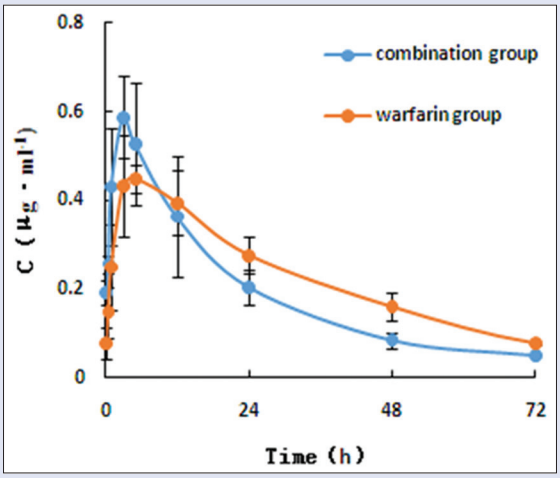

Figure 7: C-t curve of warfarin and combination groups in rat plasma

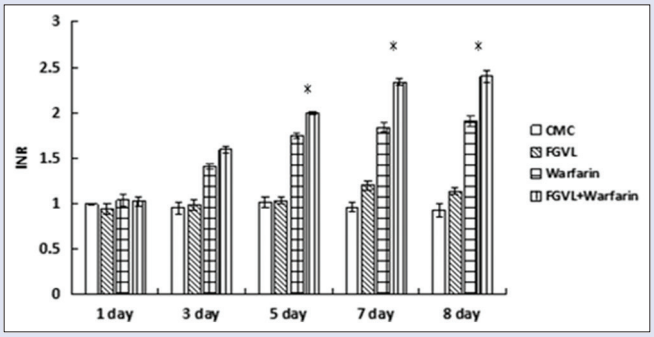

Figure 8: Prothrombin time of rats in different drug groups; *P < 0.05, significant different from the warfarin group (Dunnett's test)

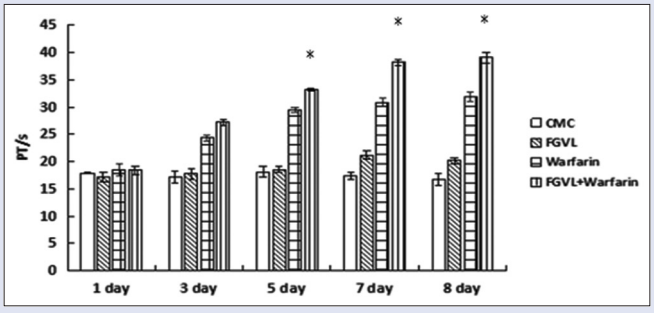

Figure 9: International normalized ratio of rats in different drug groups: *P < 0.05, significant different from the warfarin group (Dunnett's test)

impact on pharmacodynamic of warfarin from FGVL was based on the measurement of PT and INR. The anticoagulation mechanism of warfarin is inhibiting Vitamin K to take part in the composing of coagulation factors II, VII, IX, and X.[35] The anticoagulant effect of FGVL was not significant from 1st to 5th and increasing from 7th to 8th. Whether the anticoagulation mechanism of FGVL includes inhibiting Vitamin K to take part in the composing of coagulation factor needs further investigation.

Antioxidant mechanism of FGVL was reported to promote endogenous antioxidant enzymatic activities[36] and to inhibit the expression of the genes of apoptosis.[37]

FGVL includes diosmin, diosmetin, rutin, quercetin, luteoloside, and diosmentin-7-O-β–D-xyl-(1 → 6)-β–D-glc.[33,38] Recent studies demonstrated that diosmin showed inhibitory effects on CYP3 enzymes.[10] Diosmetin showed inhibitory effects on CYP1A2 enzymes.[39] No information is available for the effect of other flavonoids of *G. verum* L. on CYP450 activity *in vivo*. It needs further investigation, whether the observed increase in CYP1A2 activity by FGVL results from other flavonoids of *G. verum* L.

Previously reported studies showed that warfarin (R- and S-) is metabolized through CYP3A4, CYP1A2, and CYP2C9 in humans, which are homolog to CYP3A2, CYP1A2, and CYP2C11 in rats.[27,40] The effect of FGVL on CYP isoform is complex. The present study demonstrated that co-administration of FGVL could inhibit the activity of CYP2C11 and induce the activity of CYP1A2. This result may be an important factor lead to the blood concentration of warfarin in rats increase first and then decrease when combined with FGVL. The increasing blood concentration may inhibit vitamin K to take part in the composing of coagulation factor. Therefore, the anticoagulant effect was not increased with the concentration of warfarin. On the other hand, the anticoagulant effect was increasing from 3th to 8th, which may come from the induction on CYP1A2. Summing up, herb–drug interaction from metabolism between FGVL and warfarin is complex. Further study is needed to evaluate the relativity of pharmacodynamic and pharmacokinetics of warfarin combining with FGVL.

CONCLUSION

FGVL can affect the activity of CYP isozymes. It can induce the activity of CYP1A2 and inhibit the activity of CYP2C11 in rats. Pharmacokinetic parameters will change and the anticoagulant effect will be increasing when combined with warfarin. Hence, the authors recommend that the dose of warfarin should be adjusted when combined with FGVL or preparations of *G. verum* L. in clinic.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Zhao G, Bochuan LV, Sun Q. Kangmai soft capsule in treating 40 cases of arteriosclerosis obliterans. Chin J Integr Tradit Western Med 2009;15:117-9.
2. Lv B, Zhang B, Wang Z, Zhao G. Clinical observation of Kangmai soft capsule in treating arteriosclerosis obliterans. J Tradit Chin Med 2014;42:90-2.
3. Yin Z, Wang K, Xie L, Kan L. Treatment of 30 cases of deep venous thrombosis of lower extremity with Pengziye injection. Chin J Tradit Med Sci Technol 2001;8:365.
4. Lv B, Xia L, Li L, Gao J. Therapeutic effect of Kangmai 2 capsule on 31 cases of deep venous thrombosis of lower extremity. Chin Med Inf 2014;31:128-30.
5. Tao Y. Clinical Study on Kangmai III Capsule in Treating Diabetic Foot. Heilongjiang University of Traditional Chinese Medicine; 2007.
6. Gao J, Zhao G, Chen W, Li L. Therapeutic effect and mechanism of Kangmai 3 capsule on diabetic foot. Chin J Integr Tradit Western Med 2006;12:521-4.
7. Lim JW, Chee SX, Wong WJ, He QL, Lau TC. Traditional Chinese medicine: Herb-drug interactions with aspirin. Singapore Med J 2018;59:230-9.
8. Balap A, Lohidasan S, Sinnathambi A, Mahadik K. Herb-drug interaction of *Andrographis paniculata* (Nees) extract and andrographolide on pharmacokinetic and pharmacodynamic of naproxen in rats. J Ethnopharmacol 2017;195:214-21.
9. Zhang XS, Zhao ZQ, Qin ZS, Wu K, Xia TF, Pang LQ. Herb-drug interaction between irinotecan and psoralidin-containing herbs. Eur J Drug Metab Pharmacokinet 2015;40:481-4.
10. Bedada SK, Neerati P. Modulation of CYP3A enzyme activity by diosmin and its consequence on carbamazepine pharmacokinetics in rats. Naunyn Schmiedebergs Arch Pharmacol 2018;391:115-21.
11. Siu YA, Hao MH, Dixit V, Lai WG. Celecoxib is a substrate of CYP2D6: Impact on celecoxib metabolism in individuals with CYP2C9*3 variants. Drug Metab Pharmacokinet 2018;33:219-27.
12. Volpe DA, Xu Y, Sahajwalla CG, Younis IR, Patel V. Methadone metabolism and drug-drug interactions: *In vitro* and *in vivo* literature review. J Pharm Sci 2018;107:2983-91.
13. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G, *et al.* Pharmacology and management of the Vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th edition). Chest 2008;133:160S-98S.
14. Linkins LA, Choi PT, Douketis JD. Clinical impact of bleeding in patients taking oral anticoagulant therapy for venous thromboembolism: A meta-analysis. Ann Intern Med 2003;139:893-900.
15. Green L, Alikhan R, Curry N, Maclean R, Saja K, Stanworth S, *et al.* Oral anticoagulant agent-associated bleeding events reporting system (ORANGE) study. Br J Haematol 2014;167:274-6.
16. Schaufele MK, Marciello MA, Burke DT. Dosing practices of physicians for anticoagulation with warfarin during inpatient rehabilitation. Am J Phys Med Rehabil 2000;79:69-74.
17. Hashikata T, Yamaoka-Tojo M, Kakizaki R, Nemoto T, Fujiyoshi K, Namba S, *et al.* Ezetimibe enhances and stabilizes anticoagulant effect of warfarin. Heart Vessels 2017;32:47-54.
18. Yamasaki K, Sato H, Minagoshi S, Kyubun K, Anraku M, Miyamura S, *et al.* The binding of silibinin, the main constituent of silymarin, to site I on human serum albumin. Biol Pharm Bull 2017;40:310-7.
19. Nutescu EA, Shapiro NL, Ibrahim S, West P. Warfarin and its interactions with foods, herbs and other dietary supplements. Expert Opin Drug Saf 2006;5:433-51.
20. Leite PM, Martins MA, Castilho RO. Review on mechanisms and interactions in concomitant use of herbs and warfarin therapy. Biomed Pharmacother 2016;83:14-21.
21. Zhang X, Zhang X, Wang X, Zhao M. Influence of andrographolide on the pharmacokinetics of warfarin in rats. Pharm Biol 2018;56:351-6.
22. Shaik AN, Bohnert T, Williams DA, Gan LL, LeDuc BW. Mechanism of drug-drug interactions between warfarin and statins. J Pharm Sci 2016;105:1976-86.
23. Tapanainen JM, Braunschweig F, Schwieler J, Insulander P, Bastani H, Drca N, *et al.* Continuous warfarin therapy is safe and feasible in catheter ablation of atrial fibrillation. Scand Cardiovasc J 2013;47:109-13.
24. Liu MY, Ballard DW, Huang J, Rauchwerger AS, Reed ME, Bouvet SC, *et al.* Acute pulmonary embolism in emergency department patients despite therapeutic anticoagulation. West J Emerg Med 2018;19:510-6.
25. Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, *et al.* Systematic overview of warfarin and its drug and food interactions. Arch Intern Med 2005;165:1095-106.
26. Vranckx P, Valgimigli M, Heidbuchel H. The significance of drug-drug and drug-food interactions of oral anticoagulation. Arrhythm Electrophysiol Rev 2018;7:55-61.

27. Pourgholi L, Goodarzynejad H, Mandegary A, Ziaee S, Talasaz AH, Jalali A, *et al.* Gene polymorphisms and the risk of warfarin-induced bleeding complications at therapeutic international normalized ratio (INR). Toxicol Appl Pharmacol 2016;309:37-43.
28. Zhao HF, Sun JH, Liu S, Liu Y, Liu GF. Effect of shuxuetong injection on anticoagulant effect of warfarin in rats. Zhongguo Zhong Yao Za Zhi 2017;42:982-8.
29. Stoddard GJ, Archer M, Shane-McWhorter L, Bray BE, Redd DF, Proulx J, *et al.* Ginkgo and warfarin interaction in a large veterans administration population. AMIA Annu Symp Proc 2015;2015:1174-83.
30. Dan YI, Xiao-bo LUO, Xiang-hong LU, Sheng-ping LUO, Yu-lu ZHOU. The effects of compound Danshen dripping pills on the pharmacodynamics and pharmacokinetics of warfarin in humans. Chin J Pharmacovigil 2013;10:65-7.
31. Li H, Zhang C, Fan R, Sun H, Xie H, Luo J, *et al.* The effects of chuanxiong on the pharmacokinetics of warfarin in rats after biliary drainage. J Ethnopharmacol 2016;193:117-24.
32. Milić N, Milosević N, Golocorbin Kon S, Bozić T, Abenavoli L, Borrelli F, *et al.* Warfarin interactions with medicinal herbs. Nat Prod Commun 2014;9:1211-6.
33. Ma Y, Lu W, Yu X, Shi G, Yang F. Study on the chemical constituents of *Pleurotus ostreatus*. Chin Herb Med 2005;36:1464-65.
34. Martignoni M, Groothuis GM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2006;2:875-94.
35. Takahashi H, Hanano M, Hayashi S, Arai Y, Yoshino N, Takakuwa E, *et al.* Plasma levels of protein C and Vitamin K-dependent coagulation factors in patients on long-term oral anticoagulant therapy. Tohoku J Exp Med 1986;149:351-7.
36. Zhang Z, Ma Y, Dong J, Li R, Dong J, Ma L. Effects of total flavonoids from *Phyllostachys pubescens* L. on NF-κB/Iκ B signaling pathway in human umbilical vein endothelial cells induced by oxidative injury. Chin J Exp Formulaol 2015;21:107-11.
37. Shi L, Ling Q, Yang X, Li H, Ma L. Protective effect of total flavonoids from *Phyllostachys pubescens* L. on human umbilical vein endothelial cells injured by hydrogen peroxide. Chin New Drug J 2012;21:1523-7.
38. Zhao C, Shao J, Cao D, Zhang YW, Li X. Study on chemical constituents of *Pleurotus ostreatus*. Chin J Tradit Chin Med 2009;34:2761-4.
39. Chen JJ, Zhang JX, Zhang XQ, Qi MJ, Shi MZ, Yang J, *et al.* Effects of diosmetin on nine cytochrome P450 isoforms, UGTs and three drug transporters *in vitro*. Toxicol Appl Pharmacol 2017;334:1-7.
40. Kaminsky LS, Zhang ZY. Human P450 metabolism of warfarin. Pharmacol Ther 1997;73:67-74.