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ORIGINAL ARTICLE

# Effect of Total Flavonoids of *Hippophae rhamnoides* L. on the Activity and mRNA Expression of CYP450 in Rats

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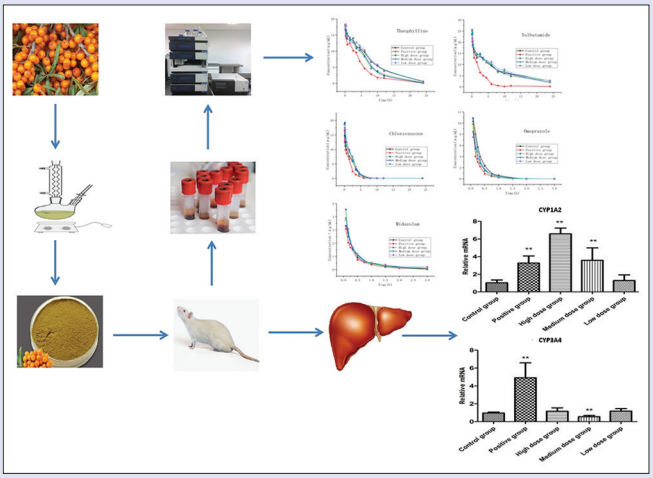
## ABSTRACT

**Objectives:** In this study, we aimed to study the effect of total flavonoids extracted from *Hippophae rhamnoides* L. (TFH) on the activity of five CYP450 enzymes in rats by high-performance liquid chromatography (HPLC). We also studied the effect of TFH on the expression of CYP450 enzyme mRNA in rat liver using quantitative real-time PCR (qRT-PCR). **Materials and Methods:** TFH was orally administered to male rats once daily at doses of 100, 200, and 400 mg/kg/day for 14 days. We established HPLC method to detect the concentration of the analytes in the rat plasma. We used DAS software to calculate the pharmacokinetic parameters. Then, qRT-PCR was used to study the effect of TFH on the expression of CYP450 mRNA. **Results:** The pharmacokinetic analysis of theophylline in the TFH group revealed a short half-life ($t_{1/2}$), a decrease in the area under the curve (AUC), and an increase in the value of plasma clearance (CL) ($P < 0.05$, $P < 0.01$). In the case of midazolam, $t_{1/2}$ was prolonged, $C_{max}$ was increased ($P < 0.05$). Results from the PCR analysis indicate that TFH downregulated the expression of CYP3A4 mRNA and upregulated the expression of CYP1A2 mRNA. **Conclusion:** In conclusion, TFH might induce the activity of CYP1A2 and inhibit the activity of CYP3A4. Herb–drug interaction should be carefully considered when TFH is being used in combination with drugs metabolized by CYP1A2 and CYP3A4.

**Key words:** Cocktail, CYP450, herb–drug interaction, high-performance liquid chromatography, *Hippophae rhamnoides* L., total flavonoids

## SUMMARY

- Seabuckthorn is a kind of drug and food homologous substance. Care should be taken when using drugs metabolized by CYP1A2 and CYP3A4 in combination.




**Abbreviations used:** TFH: Total flavonoids of *Hippophae rhamnoides* L.; $t_{1/2}$: Half-life; CYP450: Cytochrome P450; IS: Internal standard; AUC: Area under the curve; qRT-PCR: Quantitative Real-time PCR; CL: Plasma clearance; DDIs: Drug-drug interactions; $C_{max}$: Maximum plasma concentration.

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## INTRODUCTION

Traditional herbal medicines are widely used throughout the world. In addition, an increasing number of herb–drug interactions are related to the use of herbal or natural products. For example, *Hypericum perforatum* is a cytochrome P450 (CYP) 3A4 inducer that inhibits or induces other subtypes of enzymes. *H. perforatum* reduced plasma concentrations of digoxin, warfarin, cyclosporine, amitriptyline, indinavir, and midazolam.[1] In several cases, *H. perforatum* has been reported to reduce the concentration of cyclosporine in the blood, leading to organ rejection.[2] Alnaqeeb confirms that pomegranate peel or guava leaf extract can enhance the anticoagulant activity of warfarin. These interactions may be caused by inhibition of CYP3A4, CYP2C8, and CYP2C9 enzymes by guava leaves, quercetin, and ellagic acid.[3] Numerous studies have shown that Chinese and Mongolian medicines can affect the metabolism of other drugs by inducing or inhibiting drug-metabolizing enzymes, thus leading to various risks. Therefore, studying the effect of Chinese and Mongolian medicines on CYP450 enzyme activity is of great significance to better guide the rational application of Chinese and Mongolian medicine.

*Hippophae rhamnoides* L. (Seabuckthorn), known as "Shaji" in China, is mainly distributed in Asia and Europe and belongs to the *Elaeagnaceae* family. It is a medicinally important food source. The health properties of *H. rhamnoides* has been recorded in the medical classics of the Tang, Ming, and Qing Dynasties, such as Si bu Yi dian, Ben Cao Gang



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Mu, and Jing Zhu Ben Cao.[4] Seabuckthorn has a long history of therapeutic usage in Tibet and Mongolia.[5] According to the Chinese Pharmacopoeia, seabuckthorn facilitates blood circulation and removes blood clots; it relieves cough and eliminates phlegm; it invigorates the spleen and dissipates food (National Pharmacopoeia Commission, 2015).[6] Seabuckthorn protects the liver; decreases hypertension; protects cells against reactive oxygen species; shows anticancer activity; prevents the formation of ulcers; shows wound healing activity, regulates immune response; and shows antibacterial, antioxidant, cardioprotective activity.[7-10] Flavonoids in seabuckthorn berries are the active ingredients that exhibit most of the biological activity.[11] The primary components of the total flavonoids extracted from *H. rhamnoides* (TFH) are quercetin, isorhamnetin, kaempferol, and rutin.[12] TFH is used as a candidate functional food ingredient due to its biological activity. However, whether TFH can affect the metabolic processes of other drugs in combination by affecting the activities of drug-metabolizing enzymes remains unknown.

Hepatic CYP450 enzymes are mainly responsible for the metabolism of drugs. They metabolize many endogenous and exogenous substances in living organisms.[13] In this study, five important isoenzymes of CYP450 were studied: CYP3A4, CYP2C19, CYP2E1, CYP2C9, and CYP1A2. Each of these enzymes accounts for a high percentage of the total CYP activity (CYP1A2 is approximately 13%, CYP2C is approximately 20%, CYP2E1 is approximately 7%, and CYP3A is approximately 30%).[14] The activities of these enzymes are sometimes inhibited or induced by other foreign substances, such as drugs, phenobarbital, phenytoin sodium, rifampicin *et al.*

In clinical applications, inhibition or induction of CYP450 activities may affect the pharmacokinetic properties of various drugs, causing an unexpected or severe clinical drug–drug interactions (DDIs).[15,16] In particular, enzyme inhibitors may slow down the metabolism of another drug and increase the concentration of the drug, leading to adverse drug reactions and toxic effects.[17] A common method for evaluating DDIs *in vivo* is the cocktail method. Compounds catalyzed specifically by each CYP450 isoenzyme are called probe drugs, and they are often used when studying the activity of CYP450.[18] Studies have shown that rat CYP (3A1/2, 2D1, 2E1, 2C11, and 1A2) are homologous to human CYP (3A4, 2C19, 2E1, 2C9, and 1A2).[19,20] Therefore, the results in rats can be inferred for clinical applications in humans.

In this study, the safety of TFH in clinical combination was studied using five CYP450 subtype enzymes (CYP3A4, CYP2C19, CYP2E1, CYP2C9, and CYP1A2) and their specific probe drugs (midazolam, omeprazole, chlorzoxazone, tolbutamide, and theophylline respectively). The changes of pharmacokinetic parameters of these probes and the mRNA expression of CYP450 can reflect the effect of TFH. In this experiment, two high-performance liquid chromatography (HPLC) methods were established: One was to determine the concentration of midazolam and omeprazole in blood, and the other was to determine the concentration of chlorzoxazone, tolbutamide, and theophylline in blood. Both methods were very sensitive and specific.

# MATERIALS AND METHODS

## Drug, standard chemicals, and reagent

Seabuckthorn was purchased from Qinghai Kangpu Biotechnology Co. Ltd. and was identified as the dried and mature fruit of *H. rhamnoides* L. in the *Elaeagnaceae* family by Professor Wang Zhenwang of Baotou Medical College. Theophylline, tolbutamide, chlorzoxazone, midazolam, omeprazole, and tinidazole (all >98%) were purchased from Dalian Meilun Biotechnology Co. Ltd., (Dalian, China). Primers for the quantitative Real-time PCR(qRT-PCR) were synthesized by Kingsley Biotechnology Co. Ltd., (Nanjing, China). Total RNA rapid extraction kit-RNAfast200 was obtained from Feijie Biotechnology Co. Ltd., (Shanghai, China). First-strand cDNA and Master Mix (second-generation) Kits were obtained from Boldi Biotechnology Co. Ltd., (Nanjing, China).

## Animals

Beijing Sibefu Biotechnology Co. Ltd. provided us with male Wistar rats. The animals were kept at the Experimental Animal Research Center of Baotou Medical College, and the temperature was controlled at 22°C. Animal experiment procedures were approved by the Experimental Animal Management Committee of Baotou Medical College (Baoyuan zi 20181120).

## Instrumentation and conditions

Compounds were analyzed using an UltiMate3000 HPLC, and the column used was ZORBAX SB-C18 (4.6 × 250 mm, 5 μm, Agilent, USA), and the column temperature was 35°C. Then, aqueous solution of 0.1% phosphoric acid and acetonitrile were used as mobile phases. Table 1 shows the gradient programs of theophylline, tolbutamide, chlorzoxazone, and tinidazole (the internal standard [IS]). For omeprazole, midazolam, and IS, we performed gradient elution, and the acetonitrile content changed from 15% to 40% between 0 and 12 min.

## Preparation and determination of total flavonoids of *Hippophae rhamnoides* L.

According to the "Chinese Pharmacopoeia" (National Pharmacopoeia Commission, 2015),[6] TFH was extracted as follows: First, 1600 mL of 70% ethanol was added to 200 g of seabuckthorn pomace and heated to reflux for 2 h. After cooling and filtering, 1600 mL of 70% ethanol was added to the residue and heated to reflux twice for 1 h and each time. Then, it was filtered, and the combined the filtrate was evaporated, take out the dry residue for use.

Reference substances (quercetin, kaempferol, and isorhamnetin) were weighed and dissolved in methanol to respectively obtain 0.0362%, 0.0098%, and 0.0846% solution.

The content of quercetin, kaempferol, and isorhamnetin in the extract was detected by HPLC. The following chromatographic conditions were applied: Chromatographic column: ZORBAX SB-C18 (4.6 mm × 250 mm, 5 μm), Agilent, USA; flow rate: 1.0 mL/min; detection wavelength: 370 nm; column temperature: 25°C; sample volume: 20 μL; mobile phase: Methanol (A) –0.4% phosphoric acid (B), gradient elution, 0–10 min, A rises from 50% to 60%, 10–15 min, A rises from 60% to 69%, 15–20 min, A drops from 69% to 50%.

## Preparation of standard solutions

Tinidazole (IS), omeprazole, midazolam, chlorzoxazone, theophylline, and tolbutamide were prepared at a concentration 0.5 mg/mL methanol. A series of working standard solutions were prepared by taking proper quantities of each original solution and continuously diluting the stock solution. The prepared solutions were stored in a refrigerator at 4°C.

The standard curves of chlorzoxazone, tolbutamide, and theophylline were prepared in the range of 0.025–25 μg/mL (25, 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, and 0.025 μg/mL). Furthermore, the standard curve of

**Table 1:** Gradient elution procedures for tolbutamide, theophylline and chlorzoxazone

| Time/min | Acetonitrile/% | 0.1% phosphoric acid/% |
|---|---|---|
| 0 | 10 | 90 |
| 9 | 30 | 70 |
| 10 | 40 | 60 |
| 20 | 55 | 45 |

omeprazole was prepared in the range of 0.025–12.5 μg/mL (12.5, 5, 2.5, 1.25, 0.5, 0.25, 0.125, 0.05, and 0.025 μg/mL), and the standard curve of midazolam was prepared in the range of 0.015–7.5 μg/mL (7.5, 3, 1.5, 0.75, 0.3, 0.15, 0.075, 0.03, and 0.015 μg/mL).

## Pharmacokinetic study

In this study, 80 male Wistar rats (220 ± 20 g) were randomly divided into the control group, positive group, and administration group (including high-dose TFH group, medium-dose TFH group, and low-dose TFH group) ($n$ = 16). The control and TFH groups were orally administered with physiological saline and TFH, respectively, for 14 days (100, 200, and 400 mg/kg; distilled water was used as the solvent). In the positive group, phenobarbital (50 mg/kg) was injected intraperitoneally from the 7$^{th}$ day onward. After the last dose, half of the rats in each group were injected with a mixture of chlorzoxazone, tolbutamide, and theophylline from the tail vein. The remaining rats were injected with a mixture of midazolam and omeprazole through the tail vein.

After injection of the solution of mixed probe drugs, 0.5 mL of blood was collected respectively from the first batch of orbital vein of rats at 0. 08, 0.17, 0.33, 0.67, 1.5, 2.5, 3.5, 4. 5, 6, 10, 12, and 24 h. Then, 0.5 mL of blood was collected from the second batch of rats' orbital vein at 0.08, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, and 3 h separately. The blood samples were centrifuged immediately, and then 200 μL of the plasma was obtained from each sample and stored at −20°C.

The drug in the plasma was extracted with dichloromethane, dried, and reconstituted with methanol. HPLC was used to detect the drug extracted from the plasma samples. DAS software was used to calculate the pharmacokinetic parameters of each probe drug, including $t_{1/2}$, CL, $AUC_{0\sim t}$, $AUC_{0\sim\infty}$, and $C_{max}$.

## Effect of total flavonoids of *Hippophae rhamnoides* L. on CYP1A2 and CYP3A1 enzyme mRNA expression in rats

The grouping and administration methods are the same as above. After the last dose, the livers were excised quickly, rinsed with 0.9% (w/v) cold sodium chloride solution, and weighed and stored at −80°C. The instructions from the kit-RNAfast200 (Invitrogen) were followed to extract the total RNA from rat liver samples (15 mg). Then, we used NanoDrop 2000 spectrophotometer to detect the content/purity of the extracted total RNA. The 260/280 nm absorbance ratio (1.8–2.0 for high-purity samples) was used to judge the quality of the total RNA. Subsequently, the total RNA was reverse transcribed into cDNA in an amount of 500 ng per sample.

The concentration of total RNA was 25 μg/mL. Followings are the conditions used for the reverse transcription: 25°C for 10 min, 50°C for 45 min, and 85°C for 5 min. Followings are the conditions used for the amplification process: First, the HotStart Taq enzyme was activated at 95°C for 15 min and then denatured at 95°C for 20 s, annealed at 60°C for 30 s, and extended at 72°C for 1 min. A total of 45 cycles were performed. The reaction volumes for the PCR were as follows: 25 μL (12.5 μL 2 × RT-PCR Master Mix, 0.5 μL 10 μM forward primer, 0.5 μL 10 μM reverse primer, 1 μL template, 10.5 μL Nuclease-Free water). The PCR model is 5020 PCR instrument (ThermoFisher Scientific). The relative mRNA expression levels of five groups were calculated using the $2^{-\Delta\Delta CT}$ method. In this experiment, β-*actin* was used as the internal reference gene. By searching the National Biotechnology Information Center (NCBI), a nucleotide database, and some references, the mRNA sequence of CYP3A1, CYP1A2, and β-*actin* were identified. Table 2 lists the sequences of primers.

## Statistical analysis

DAS 3.0 software (version 3.0, Shanghai University of Traditional Chinese Medicine Center for Drug Clinical Research) was used to calculate the pharmacokinetic parameters of each rat, such as $t_{1/2}$, CL, AUC, and $C_{max}$. We performed calculations using the SPSS statistical software (version 17.0) was used to analyze the data. $P < 0.05$ was considered statistically significant. Data were expressed as mean ± standard deviation.

# RESULTS

## Methodological validation

### *High-performance liquid chromatography chromatograms and retention times of five probe drugs*

Figures 1 and 2 show HPLC chromatograms of probe drugs. As shown in Figure 1, the retention times of theophylline, tinidazole, chlorzoxazone, and tolbutamide were 5.283 min, 8.420 min, 14.815 min, and 18.373 min, respectively. As shown in Figure 2, the retention times of tinidazole, omeprazole, and midazolam were 6.833 min, 7.647 min, and 10.893 min, respectively. These samples had good separation effect.

### *Calibration curve*

Table 3 lists the regression equations for the calibration curve. The ratio of peak area to concentration for each probe drug showed a better

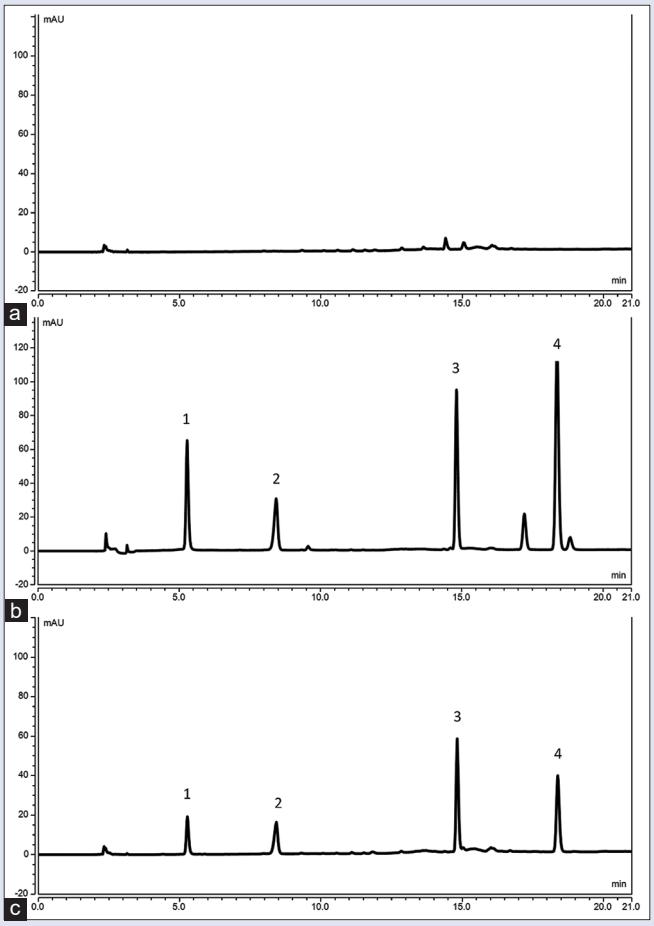


**Figure 1:** High-performance liquid chromatography chromatograms: (a) Blank plasma; (b) blank plasma spiked with the cocktail probe drugs and the internal standard; (c) plasma sample obtained from a rat after intravenous injection of the cocktail probe drugs spiked with the internal standard; (1) theophylline; (2) tinidazole; (3) chlorzoxazone; (4) tolbutamide

**Table 2:** Sequences of primers

| CYPs | Forward primer sequence | Reverse primer sequence |
|---|---|---|
| CYP1a2 | TGTGACAGAGCCCAAGGTGC | TGGTGAAGGGGACAAAGGAT |
| CYP3a1 | ATGGACCTGCTTTCAGCTCT | AATCCGTAGAGGAGCACCAG |
| β-actin | CATGTACGTTGCTATCCAGGC | CATGTACGTTGCTATCCAGGC |

CYP: Cytochrome P450

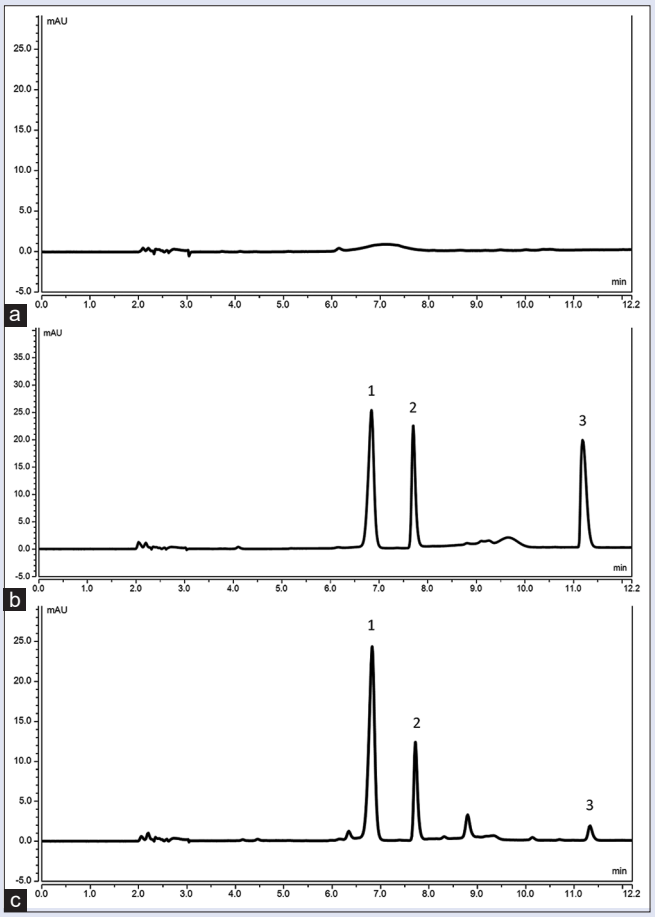


**Figure 2:** High-performance liquid chromatography chromatograms: (a) Blank plasma; (b) blank plasma spiked with the cocktail probe drugs and the internal standard; (c) plasma sample obtained from a rat after intravenous injection of the cocktail probe drugs spiked with the internal standard; (1) tinidazole; (2) omeprazole; (3) midazolam

linear relationship within the concentration range. Theophylline, tolbutamide, chlorzoxazone, and omeprazole have a quantitative limit of 0.025 μg/mL and midazolam has a quantitative limit of 0.015 μg/mL in plasma.

### *Precision and extraction efficiency*

The measured intraday precision was 9.67% or lower, and the interday precision was 9.45% or lower. The average extraction efficiency was 87.59%–109.26%. Table 4 lists the specific data.

### *Stability*

The plasma samples were placed at room temperature and frozen for 1 week and 3 weeks for stability studies. The data showed that the relative errors of these probe drugs in the plasma were < 10%, indicating that the plasma sample was stable.

### *Effects of total flavonoids of Hippophae rhamnoides L. on the activity of CYP450 isozymes in rats*

Figure 3 shows the average plasma concentration-time curve of the probe drug. Table 5 lists the main pharmacokinetic parameters of these probe drugs.

From the experimental results, the AUC, $t_{1/2}$, and CL of theophylline in the high-dose TFH group, the medium-dose TFH group, and the control group were significantly different ($P < 0.05$ or $P < 0.01$). For omeprazole, the AUC of the TFH groups has a tendency to decrease and the CL has a tendency to increase, but there was no significant difference. For midazolam, the AUC tended to increase, and CL tended to decrease; however, compared to the control group, the differences have not yet reached a significant level, but the $t_{1/2}$ was prolonged and showed a significant difference. For chlorzoxazone and tolbutamide, there was no significant difference in each index ($P > 0.05$). In summary, TFH might affect the activities of CYP3A4 and CYP1A2 but has no effect on the activities of CYP2E1, CYP2C9, and CYP2C19.

### *Effects of total flavonoids of Hippophae rhamnoides L. on the mRNA expression of CYP1A2 and CYP3A4 enzymes*

Figure 4 shows the effects of TFH on the mRNA expression of CYP3A4 and CYP1A2 enzymes. The expression of CYP1A2 mRNA in the large- and medium-dose TFH groups was increased after 14 days of continuous administration ($P < 0.01$); however, in medium-dose TFH group, the expression of CYP3A4 mRNA decreased.

### *Preparation and measurement of total flavonoids of Hippophae rhamnoides L.*

The active ingredients in TFH are isorhamnetin, kaempferol, and quercetin. In this study, we determined the content of the aforementioned compounds, as well as quercetin (1.8%), kaempferol (0.8%), and isorhamnetin (6.9%).

## DISCUSSION

CYP450 is an important enzyme required for the metabolism of many clinical drugs in the liver and intestines and is one of the most important family of phase-1 drug metabolism enzymes. However, in the presence of an inducer or inhibitor, the activities of the CYP450 enzymes may change, thereby altering the metabolism of the isozyme substrate and result in DDIs.[21] Therefore, it is important to know whether herbs induce or inhibit CYP450 isoenzymes. This information can be useful in predicting the interaction of herbal drugs and reduce the occurrence of adverse drug reactions.

The review of domestic and foreign literature revealed that there was no report on the influence of TFH on CYP450. Therefore, we studied the effects of TFH on the activities of CYP450 enzymes under *in vivo* conditions. The CYP450 family mainly includes CYP1, CYP2, and CYP3 subtypes. They are involved in the metabolic response of many clinical drugs.[22] CYP1A2 is an important subtype that participates in the metabolism of many medicinal drugs, for example, antiarrhythmic drugs (lidocaine), antipyretic drugs, analgesic and anti-inflammatory drugs (acetaminophen, nabumetone), heart-related vascular medications (triamterene, propranolol), antipsychotics (clozapine and

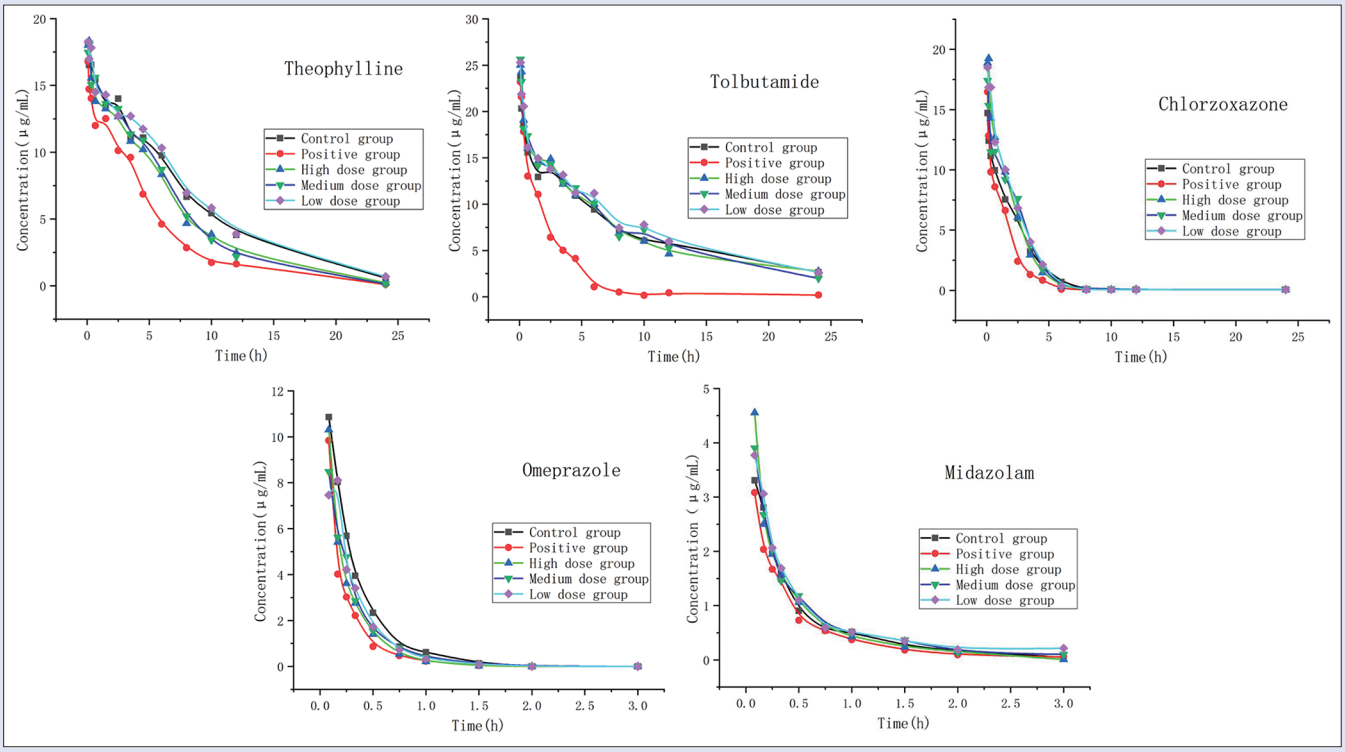


**Figure 3:** Mean plasma concentration-time curves for theophylline, tolbutamide, chlorzoxazone, omeprazole and midazolam

**Table 3:** Regression equation of five probe drugs (X=Concentration of probe drugs; Y=Peak area ratio of probe drugs vs. internal standard)

| Probe drugs | Regression equation | Correlation coefficient | Liner range (ug/mL) |
|---|---|---|---|
| Midazolam | Y=0.0117x+0.0001 | 0.9997 | 0.015-7.5 |
| Chlorzoxazone | Y=0.0979x-0.0045 | 0.9994 | 0.025-25 |
| Tolbutamide | Y=0.0727x+0.0158 | 0.9995 | 0.025-25 |
| Theophylline | Y=0.0371x+0.0069 | 0.9993 | 0.025-25 |
| Omeprazole | Y=0.02x+0.0013 | 0.9996 | 0.025-12.5 |

olanzapine), antidepressants (duloxetine), hypnotics, local anesthetics, muscle relaxants, and choline esterase inhibitors.[23,24] Other endogenous substrates such as melatonin, estrogen, and arachidonic acid require biological activation through CYP1A2.[25] Table 5 shows that the $t_{1/2}$ of theophylline in the high and medium TFH groups is shortened, the AUC is reduced, and the CL is increased. These pharmacokinetic parameters are significantly different. Figure 3 shows that the AUC of theophylline in the TFH group decreased, indicating that the activity of the CYP1A2 enzyme may be induced by TFH. Figure 4 shows that TFH might promote the expression of CYP1A2, thereby enhancing the activities of CYP1A2. This suggests that there is a metabolic interaction between TFH and the substrates of CYP1A2. This interaction leads to a decrease in the blood concentration of the drug metabolized by CYP1A2 and a decrease in the effect. Therefore, in clinical drug application, TFH should be used with caution when combined with drugs metabolized by CYP1A2. If combined use is required, then the drug dosing regimens need to be adjusted to ensure effectiveness without increasing the risk of adverse events. However, CYP1A2 participates in the activation of some carcinogens, such as 2-acetylaminofluorene and 2-napthylamine.[26] (b) Therefore, the activation of CYP1A2 may put the body at some risk.

CYP3A4 can metabolize 40%–50% of the drugs that are in clinical use, such as lipid-lowering statins (simvastatin, atorvastatin, and lovastatin), calcium channel blockers, anticoagulant factor Xa inhibitors (rivaroxaban and apixaban), and macrolide antibiotics.[27] Table 5 shows that the $t_{1/2}$ of midazolam in the middle- and low-TFH groups was prolonged and that the AUC revealed an increasing trend, whereas the CL revealed a decreasing trend. This indicates that TFH inhibits CYP3A4 enzyme activity. Figure 4 shows that the medium dose of TFH can inhibit the expression of CYP3A4. This shows that TFH can reduce the activity of CYP3A4, which in turn indicates the metabolic interaction between TFH and CYP3A4 substrates. Inhibition of CYP3A4 by TFH might increase the risk of adverse effects of rhabdomyolysis with certain statins.[28] Therefore, when TFH is used in combination with drugs metabolized by CYP3A4, the administration schedule needs to be adjusted, either by reducing the dose or by extending the time interval between doses to prevent serious adverse reactions. At present, it is known that CYP3A4 is also involved in the activation of some carcinogens, such as aflatoxin B1 and benzo(a) pyrene 7,8-diol.[26] TFH does not increase the consumer's risk of toxicity, mutagenesis, and carcinogenesis due to the toxicants activated by CYP3A4.

CYP2C19, CYP2E1, and CYP2C9 are also very important metabolic enzymes in the human body. Table 5 shows that the pharmacokinetic behavior of omeprazole, chlorzoxazone, and tolbutamide in the TFH group did not differ significantly from the control group. Figure 3 shows that TFH has no effect on CYP2C19, CYP2E1, and CYP2C9 enzymes. It is suggested that TFH is a safe drug and can be used in combination with drugs metabolized by CYP2C9, CYP2E1, and CYP2C19. It has potential guiding significance for clinical drug combination application.

Seabuckthorn is rich in flavonols (1500–2000 mg/kg), which mainly contains isorhamnetin, quercetin, and kaempferol (Wang *et al.* 2016).[29] Studies have shown that isorhamnetin has an inhibitory effect on CYP3A4.[30] Quercetin and kaempferol in *Ginkgo biloba* extracts showed no effect on CYP3A4 but could induce CYP1A2.[31] Another study reported that quercetin did not show any significant effect on CYP1A2 and CYP2E1 but showed a moderate inhibitory effect on CYP2C19 and

**Table 4:** Precision and recovery rate (*n*=5)

| Compound | Concentration (μg/mL) | Intraday precision | | Interday precision | | Extraction efficiency | RSD% |
|---|---|---|---|---|---|---|---|
| | | $\bar{X}$±S | RSD% | $\bar{X}$±S | RSD% | | |
| Theophylline | 10 | 10.33±0.29 | 2.81 | 10.28±0.32 | 3.11 | 103.27±2.95 | 2.86 |
| | 1 | 0.88±0.08 | 9.09 | 0.89±0.08 | 8.98 | 87.59±7.90 | 9.02 |
| | 0.25 | 0.24±0.02 | 8.33 | 0.19±0.01 | 5.26 | 97.44±9.13 | 9.37 |
| Tolbutamide | 10 | 9.93±0.55 | 5.54 | 9.70±0.42 | 4.33 | 99.28±5.51 | 5.55 |
| | 1 | 0.88±0.07 | 7.95 | 0.82±0.05 | 6.10 | 88.00±7.33 | 8.33 |
| | 0.25 | 0.31±0.03 | 9.67 | 0.28±0.02 | 7.14 | 108.54±8.87 | 8.17 |
| Chlorzoxazone | 10 | 9.59±0.33 | 3.45 | 9.55±0.40 | 4.19 | 95.85±3.33 | 3.47 |
| | 1 | 0.96±0.04 | 4.17 | 0.93±0.01 | 1.08 | 96.05±3.98 | 4.14 |
| | 0.25 | 0.26±0.02 | 7.69 | 0.26±0.02 | 7.69 | 102.48±9.12 | 8.90 |
| Omeprazole | 12.5 | 11.46±0.72 | 6.28 | 10.79±1.02 | 9.45 | 90.35±5.72 | 6.33 |
| | 2.5 | 2.73±0.21 | 7.69 | 2.83±0.21 | 7.42 | 109.26±3.27 | 2.99 |
| | 0.5 | 0.56±0.02 | 3.57 | 0.53±0.02 | 3.77 | 105.83±2.23 | 2.11 |
| Midazolam | 7.5 | 6.83±0.62 | 9.08 | 6.48±0.51 | 7.87 | 89.35±7.24 | 8.10 |
| | 1.5 | 1.30±0.09 | 6.92 | 1.29±0.07 | 5.43 | 87.82±4.56 | 5.19 |
| | 0.3 | 0.32±0.03 | 9.37 | 0.29±0.02 | 6.89 | 98.37±6.30 | 6.40 |

RSD: Relative standard deviation

**Table 5:** Pharmacokinetic parameters (mean±standard deviation, *n*=8)

| Probe drugs | Group | $T_{1/2}$ (h) | $C_{max}$ (μg/mL) | AUC0~t (μg/mL/h) | AUC0~∞ (μg/mL/h) | CL (L/h/kg) |
|---|---|---|---|---|---|---|
| Theophylline | Control | 5.09±0.73 | 19.61±3.49 | 141.24±28.20 | 150.85±30.78 | 0.07±0.01 |
| | High | 3.56±0.62** | 20.32±3.47 | 106.95±14.87* | 111.93±18.24* | 0.09±0.02* |
| | Medium | 4.01±0.41* | 19.44±1.74 | 107.09±17.19* | 126.77±18.57 | 0.08±0.01 |
| | Low | 4.96±0.94 | 19.20±1.84 | 139.06±24.95 | 153.06±24.15 | 0.07±0.02 |
| | Positive | 3.78±0.52 | 17.85±1.51 | 76.73±12.11 | 81.19±14.93 | 0.13±0.02 |
| Tolbutamide | Control | 8.46±1.17 | 24.07±5.00 | 152.19±18.59 | 206.82±17.99 | 0.014±0.002 |
| | High | 8.00±1.00 | 27.29±5.10 | 161.63±28.37 | 202.61±36.28 | 0.013±0.002 |
| | Medium | 7.46±1.38 | 27.83±2.47 | 132.98±13.89 | 184.81±31.01 | 0.014±0.002 |
| | Low | 10.2±1.42 | 25.32±4.12 | 166.99±25.68 | 225.88±46.93 | 0.012±0.002 |
| | Positive | 2.16±0.32 | 25.69±2.58 | 51.30±7.82 | 53.51±9.96 | 0.049±0.008 |
| Chlorzoxazone | Control | 2.57±0.25 | 16.37±2.87 | 36.00±7.16 | 38.44±5.52 | 0.28±0.05 |
| | High | 2.55±0.38 | 18.85±3.25 | 44.52±7.97 | 49.52±6.94* | 0.23±0.04 |
| | Medium | 2.30±0.28 | 18.37±3.60 | 38.51±5.51 | 38.83±5.67 | 0.25±0.03 |
| | Low | 2.39±0.10 | 19.32±3.76 | 43.28±7.10 | 44.34±7.49 | 0.24±0.05 |
| | Positive | 2.12±0.15 | 16.47±1.92 | 21.25±3.13 | 25.54±2.75 | 0.42±0.04 |
| Omeprazole | Control | 0.27±0.06 | 10.86±1.25 | 3.23±0.67 | 3.39±0.50 | 3.28±0.46 |
| | High | 0.24±0.03 | 10.30±1.82 | 3.38±0.65 | 3.41±0.65 | 3.20±0.64 |
| | Medium | 0.19±0.02 | 8.48±1.39 | 2.75±0.20 | 2.79±0.21 | 3.79±0.59 |
| | Low | 0.29±0.03 | 8.13±1.36 | 2.59±0.35 | 2.66±0.37 | 4.00±0.40 |
| | Positive | 0.16±0.02 | 10.83±1.67 | 2.12±0.27 | 2.48±0.24 | 3.78±0.82 |
| Midazolam | Control | 0.62±0.09 | 3.44±0.39 | 1.89±0.27 | 1.95±0.38 | 2.63±0.49 |
| | High | 0.63±0.08 | 4.55±0.63* | 2.10±0.37 | 2.11±0.35 | 2.43±0.49 |
| | Medium | 0.91±0.16* | 3.90±0.51 | 2.06±0.30 | 2.24±0.36 | 2.38±0.42 |
| | Low | 0.90±0.16* | 4.01±0.61 | 2.16±0.41 | 2.46±0.48 | 2.22±0.27 |
| | Positive | 0.42±0.08 | 3.63±0.72 | 1.36±0.26 | 1.42±0.17 | 3.64±0.36 |

*$P$<0.05, **$P$<0.01.[15] TFH groups versus control group. TFH: Total flavonoids of *Hippophae rhamnoides* L.; $T_{1/2}$: Half-life; AUC: Area under the curve; CL: Plasma clearance; $C_{max}$: Maximum plasma concentration

CYP3A4.[32] In the case of TFH, isorhamnetin and quercetin might be the primary components that inhibited the activity of CYP3A4, whereas kaempferol might be the primary component that induced the activity of CYP1A2.

## CONCLUSION

Two HPLC methods were established, which can quickly and accurately determine the concentration of the probe drugs in the plasma. Pharmacokinetic parameters and PCR results showed that TFH might inhibit the activity of CYP3A4, induce the activity of CYP1A2, and have no effect on the activity of CYP2E1, CYP2C9, and CYP2C19. When TFH is used with drugs metabolized by CYP3A4 and CYP1A2 enzymes, extra care should be taken as herb–drug interactions might occur. These results can be useful in rationalizing drug use in clinical practice and provide reference information for DDI.

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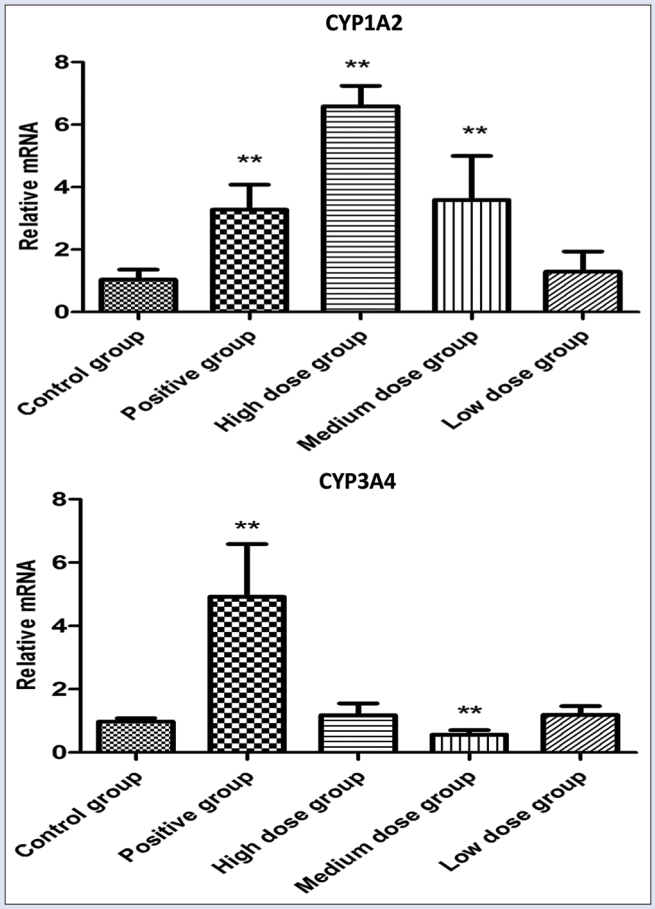


**Figure 4:** Effects of total flavonoids of *Hippophae rhamnoides* L. on mRNA expression of CYP enzymes in rats (values are means ± standard deviation, $n = 6$; *$P < 0.05$, **$P < 0.01$ vs. control group)

## Conflicts of interest

There are no conflicts of interest.

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