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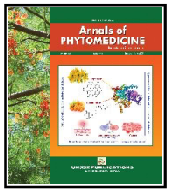

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Effects of ferulic acid and senkyunolide A on activities of cytochrome P450 1A2, 2C11, 2D1, and 3A1/2 in rats *in vivo* and *in vitro*

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Abstract

Ferulic acid and senkyunolide A are bioactive ingredients of herbal medicine *Chuanxiong rhizome* Hort., and ferulic acid also presents in more than 50 other herbal medicines. Effects of both ingredients on cytochrome P450 (CYP450) enzymes are important for predicting their influence on drugs metabolized by CYP450. This study aims to explore the effects of ferulic acid and senkyunolide A on CYP1A2, CYP2C11, CYP2D1, and CYP3A1/2 in rats *in vivo* and *in vitro*. For *in vivo* study, rats were administered ferulic acid (10 mg/kg) or senkyunolide A (20 mg/kg) and probe drugs of the four CYP450 isoforms including caffeine (10 mg/kg), tolbutamide (20 mg/kg), metoprolol (20 mg/kg), and dapsone (10 mg/kg). Blood samples were collected at predetermined time points and determined by UPLC-MS-MS. For *in vitro* study, liver microsomes were isolated and incubated with probe drugs phenacetin (50 µmol/l), tolbutamide (20 µmol/l), dextromethorphan (300 µmol/l), and testosterone (100 µmol/l). HPLC was used to quantify metabolite concentrations of the probe drugs. The *in vivo* study showed that ferulic acid and senkyunolide A accelerated the metabolism of dapsone but had no significant effects on other probe drugs. Compared with control group, C_{max}, $t_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$ decreased by 20.97% ($p<0.01$), 42.08% ($p<0.05$), 25.76% ($p<0.05$), 35.10% ($p<0.05$) in ferulic acid group, and 26.12% ($p<0.01$), 51.92% ($p<0.01$), 40.92% ($p<0.01$), 46.61% ($p<0.05$) in senkyunolide A group. Consistently, the *in vitro* study showed that the concentrations of 6β-hydroxytestosterone (the metabolite of testosterone) in the ferulic acid group (1.46 ± 0.43) and senkyunolide A group (1.29 ± 0.29) were higher than those in the control group (0.72 ± 0.48; $p<0.05$), but the concentrations of other metabolites were not significantly changed. Both the *in vivo* and *in vitro* results indicated that ferulic acid and senkyunolide A could induce activities of CYP3A1/2, but have no effects on CYP1A2, CYP2C11, CYP2D1 in rats. Therefore, attention should be paid on the potential risk that herbal medicines containing ferulic acid and senkyunolide A may decrease the curative effects of drugs metabolized by CYP3A4 in humans.

1. Introduction

Herbal medicine have been applied to complementary and therapeutic treatment for thousands of years benefiting from their efficacy and safety (Dolly *et al*., 2022; Lalitha *et al*., 2022; Moha *et al*., 2023; Zeenath, 2024). According to the report of WHO, approximately 70% of people worldwide use medicinal herbs for therapy and the number is increasing (Shaikh *et al*., 2020). Herbs are normally safe for the prevention and treatment of diseases, as long as being used rationally. Herbs and drugs are frequently used concurrently. The combination therapy helps to achieve multidrug ecology, but ideal therapeutic effects require excellent pharmacokinetic compatibility among co-administered medications (Li *et al*., 2019). Improper pharmacokinetic herb-drug interactions (HDI) may cause adverse clinical responses by decreasing efficacy or increasing toxicity (Cheng *et al*., 2023; Parvez *et al*., 2019).

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One of the major pathways of HDI involve the cytochrome P450 (CYP450)-mediated metabolism (Sharma *et al*., 2022). CYP450 isoforms are the most important drug-metabolizing enzymes in humans (Iversen *et al*., 2022; Zhao *et al*., 2021). Since the majority of drugs are metabolized by CYP450 enzymes, any induction or inhibition of the enzymes by herbal medicines can result in potential HDI and an increase of adverse events. Therefore, it is valuable and significant to gather information from studies on the effects of herbal medicines on CYP450 activities for clinical use to prevent undesirable HDI (Shaikh *et al*., 2020; Thikekar *et al*., 2021; Wang *et al*., 2020). As a traditional herbal medicine, *Ligusticum chuanxiong* refers to the rhizomes of *Ligusticum chuanxiong* Hort., a member of Umbelliferae family. It has various functions including cardioprotective effects, neuroprotective effects, antipyretic, analgesic and anti-inflammatory effects, *etc.* (Kong *et al*., 2024). Due to its diverse bioactivities, *Ligusticum chuanxiong* and its preparations are widely used in clinical practice (Ji *et al*., 2024; Liu *et al*., 2025).

Ferulic acid (Figure 1A) and senkyunolide A (Figure 1B) are important bioactive ingredients of *L. chuanxiong*. Ferulic acid, in particular, is used as a standard to assess the quality of *L. chuanxiong* (Pei *et al*., 2019; Hu *et al*., 2021). Ferulic acid possesses a variety of pharmacological actions including antiplatelet aggregation activity

(Zhang *et al*., 2020), anti-atherosclerosis activity (Neto-Neves *et al*., 2021; Wu *et al*., 2022) anti-inflammatory properties (Liu *et al*., 2022), antiviral activity (Ma *et al*., 2020) and protective effects on the liver and kidney (Shi *et al*., 2023; Niu *et al*., 2023), *etc.* Senkyunolide A is a significant component of the volatile oil found in *Chuanxiong rhizoma* and exhibits bioactivities in the treatment of cardiovascular diseases (Lan *et al*., 2022; Lei *et al*., 2019; Tang *et al*., 2021).

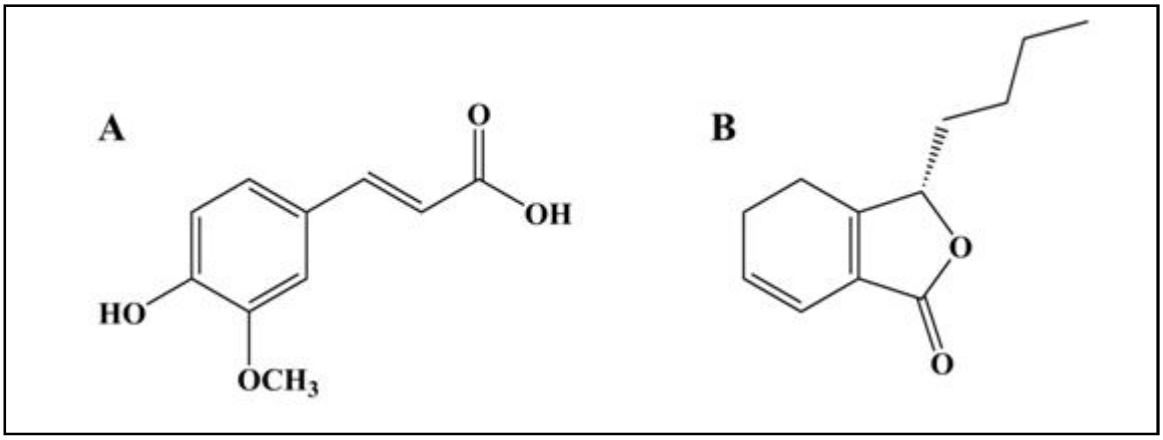

Figure 1: Chemical structures of ferulic acid (A) and senkyunolide A (B).

It was reported that the extracts of *L. chuanxiong* had significant effects on the activities of CYP450 enzymes (Wu *et al*., 2017; Sun *et al*., 2022). However, previous studies about *L. chuanxiong* mainly focused on ligustrazine, while no studies have been conducted on the potential CYP450-mediated HDI caused by ferulic acid and senkyunolide A (Feng *et al*., 2018; Sun *et al*., 2022). Ferulic acid and senkyunolide A are important bioactive components of *L. chuanxiong*, especially ferulic acid, which is specified as a quality standard for this herbal medicine. Therefore, it is crucial to investigate the effects of ferulic acid and senkyunolide A on CYP450 enzymes. The purpose of this study is to assess the effects of ferulic acid and senkyunolide A on activities of CYP1A2, CYP2C11, CYP2D1, and CYP3A1/2, which are the main CYP450 isoforms of drug metabolism. This study will provide reference for rational use of *L. chuanxiong* and herbal preparations containing ferulic acid and senkyunolide A, and avoid undesirable CYP450-mediated HDI. The results of this study could help clinicians make informed decisions when recommending *L. chuanxiong*, its preparations, or other herbal preparations containing either ingredients particularly in combination with other medications metabolized by the four CYP450 isoforms.

2. Materials and Methods

2.1 Chemicals and reagents

Dapsone (100114199101), metoprolol (101056642), phenacetin (100095-198904), caffeine (100364-200301), acetaminophen (10018-0107), dextromethorphan (013K1428), and dextrorphan (22K4610) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Testosterone (340362) was purchased from International Laboratory Limited (San Bruno, CA, USA). 6β-hydroxytestosterone (451012) was supplied by BD Biosciences Co. (Woburn, MA, USA). Tolbutamide (CDCT-C17589900) and 4-hydroxytolbutamide (Lot#1-PSB-27-2) were purchased from Dr. Ehrenstorfer GmbH (Shenzhen, China). Ferulic acid (10080301) and Senkyunolide A (11052002) were obtained from Munster Biotechnology Co., Ltd (Chengdu, China). NADPH (041939) was supplied by Roche Diagnostics GmbH (Mannheim, Germany). All other reagents were of HPLC or analytical grades.

2.2 Animals for experiments

Male Wistar rats (200 ± 20 g) were obtained from the Animal Experimental Center of the Second Affiliated Hospital of Harbin Medical University (Harbin, China), which is accredited by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental protocols were examined and approved by the Animal Ethics Committee of the Second Affiliated Hospital of Harbin Medical University (SYDW2023-075). All methods were carried out in accordance with the National Society for Medical Research and Guidelines for the Care and Use of Laboratory Animals. All methods are reported in accordance with Animals in Research: Reporting *in vivo* Experiments (ARRIVE) guidelines. After the experiment, rats were euthanized by intravenous injection of pentobarbital sodium (120 mg/kg) according to the standards of the American Veterinary Medical Association (AVMA).

2.3 Preparation of rat plasma samples

The rats were randomly treated with normal saline (0.2 ml), ferulic acid (10 mg/kg, 0.2 ml), or senkyunolide A (20 mg/kg, 0.2 ml) for 14 days (n = 8 in each group). On the 15th day, a compound mixed with caffeine (10 mg/kg, a probe drug for CYP1A2), tolbutamide (20 mg/kg, a probe drug for CYP2C11), metoprolol (20 mg/kg, a probe drug for CYP2D1), and dapsone (10 mg/kg, a probe drug for CYP3A1/2) was injected intraperitoneally into the rats. Venous blood samples (0.3 ml) were collected from the rat tails at setting time points: 0.17, 0.5, 0.83, 1.17, 1.5, 2, 3, 5, 8, 12, and 24 h. After centrifugation, 20 µl of internal standard (IS) phenacetin (150 ng/ml) was added to 100 µl of plasma taken from blood samples. To separate the probe drugs and IS from the plasma, 1 ml of acetonitrile was used as the extraction solution. The mixture was then treated and centrifuged, resulting in a 0.9 ml supernate obtained from the plasma samples. The supernate was evaporated under a 40° nitrogen flow, leaving a residue. The residue was then dissolved in 200 µl of acetonitrile and mixed, followed by decontamination through membrane filtration for analysis.

2.4 Preparation of rat microsome samples *in vitro*

Rat liver microsomes were prepared using differential ultra-centrifugation and subsequently packed and stored at –80°C for further analysis (Xiang *et al*., 2022). The Bradford method was used to measure the microsomes' protein concentrations (Karimi *et al*., 2022). The *in vitro* incubation system consisted of individual probe drugs (phenacetin, 50 µmol/l, for CYP1A2; tolbutamide, 20 µmol/l, for CYP2C11; dextromethorphan, 300 µmol/l, for CYP2D1; testosterone, 100 µmol/l, for CYP3A1/2) at concentrations determined by K_m (Michaelis constant of drugs), phosphate buffer (100 mM, pH 7.4), liver microsome protein (optimized concentration), and $MgCl_2$ (10 mM), in a final volume of 200 µl. After pre-incubation for 5 min, the incubation reaction was initiated by adding 40 µl of NADPH (1 mM) and incubated at 37°C for an optimized period. To terminate the reaction, 80 µl of ice-cold acetonitrile was added, followed by the addition of 20 µl corresponding IS drug for different isoforms. After centrifugation at 9000 rpm/min for 15 min, 20 µl of the supernate was taken for analysis. The incubation time, protein concentrations, probe drugs, and concentrations for different isoforms are shown in Table 1.

Table 1: Optimized incubation conditions *in vitro*

CYPs	Probe drug (*in vitro*)	Concentration (µmol/l)	Incubation time (min)	Protein concentration (g/l)
CYP1A	Phenacetin	50	50	1.0
CYP2C11	Tolbutamide	20	50	2.0
CYP2D1	Dextromethorphan	300	60	1.5
CYP3A1/2	Testosterone	100	60	0.75

2.5 Assays of plasma samples *in vivo*

The plasma samples were analyzed using an Agilent 6430 UPLC-QQQ-MS. The probe drugs were separated on an Agilent SB-C18 column (2.1 mm × 50 mm, 1.8 µm) with a column temperature of 40°C and an automatic sampler temperature of 10°C. A 5 µl plasma sample was injected into the UPLC system, with a mobile phase consisting of acetonitrile, water, phosphate, and formic acid (75:25:0.05, v/v/v) at a flow rate of 0.4 ml/min. The mass spectrometric conditions included an ESI^+ ion source, a 4 KV capillary voltage, an ion source temperature of 120°C , a sample room temperature of 10°C, an N_2 temperature of 350°C, and an N_2 velocity of 660 l/h. Parameters of parent ion, daughter ion, cone voltage, and collision energy are shown in Table 2.

Table 2: Mass spectrographic parameters

CYPs	Probe drug (*in vivo*)	Parent ion (m/z)	Daughter ion (m/z)	Cone voltage (V)	Collision energy (eV)
CYP1A2	Caffeine	195.1	110.2	130	25
CYP2C11	Testosterone	271.2	91.2	130	30
CYP2D1	Metoprolol	268.2	98.1	135	30
CYP3A1/2	Dapsone	249.3	108.1	125	23
–	Phenacetin (internal standard)	180.1	110.1	105	18

2.6 Assays of microsome samples *in vitro*

The supernatant (20 µl) was analyzed using the Waters HPLC system 2010 (Waters, USA), equipped with a 600 pump, 996PAD UV detector, and Millipore Systems. Metabolite concentrations resulting from the incubation were analyzed on a Diamonsil C18 reverse phase column (250 mm × 4.6 mm, 5 µm). The analysis conditions for the *in vitro* samples are shown in Table 3. The method of UPLC-MS-MS determination for *in vivo* study and HPLC determination for *in vitro* study have been validated and reported in our previous paper (Shi *et al*., 2019). In this study, the methods were performed with some modifications and validated.

Table 3: Analysis conditions *in vitro*

CYPs	Metabolite	Internal standard	Mobile phase	Detection wave-length (nm)
CYP1A2	Acetaminophen	Caffeine	Acetonitrile:water:ethylic acid = 15:85:0.020	254
CYP2C11	4-hydroxytolbutamide	Phenacetin	Carbinol:wanter:ethylic acid = 54:46:0.092	229
CYP2D1	Dextrorpha	Phenacetin	Carbinol:water:phosphoricacid: riethylamine = 40:60:0.12:0.20	280
CYP3A1/2	6β-hydroxytestosterone	Cortisone acetate	Carbinol:water=65:35	245

2.7 Statistical analysis

DAS2.0 software was applied for the analysis of pharmacokinetic parameters. The C_{max} and T_{max} were calculated using plasma drug concentration-time curves. The area under the plasma concentration-time curve AUC_{0-t} was calculated using the linear trapezoidal rule, and $AUC_{0-\infty}$ was calculated as follows: $AUC_{0-\infty} = AUC_{0-t}+C_t/k_e$, in which k_e was the slope of the linear regression of the log-transformed concentration-time curve and C_t was the last concentration obtained at time t. The half-time ($t_{1/2}$) was calculated as $0.693/k_e$.

The data were shown as mean ± SD and analyzed using SPSS 22.0 software. Significant differences between the groups were analyzed by the unpaired Student *t*-test, with *p<0.05 or **p<0.01 denoting statistical significance.

3. Results

3.1 Effects of ferulic acid and senkyunolide A on rat CYP450 isoforms *in vivo*

The plasma concentration-time profiles and pharmacokinetic parameters of caffeine, tolbutamide, metoprolol, and dapsone in different groups were shown in Figures 2-5 and Tables 4-7, respectively.

As shown in Figures 2-4 and Tables 4-6, compared to the control group, the pharmacokinetic parameters and concentration-time profiles of caffeine, tolbutamide, and metoprolol in ferulic acid treated groups were not changed significantly (p>0.05), indicating that ferulic acid had no effects on CYP1A2, CYP2C11, nor CYP2D1. Similarly, as shown in Figures 2-4 and Tables 4-6, in comparison to the control group, the concentration-time profiles and pharmacokinetic parameters of caffeine, tolbutamide, and metoprolol in senkyunolide A treated groups showed minimal changes (p>0.05), indicating that senkyunolide A has no significant effects on CYP1A2, CYP2C11 and CYP2D1.

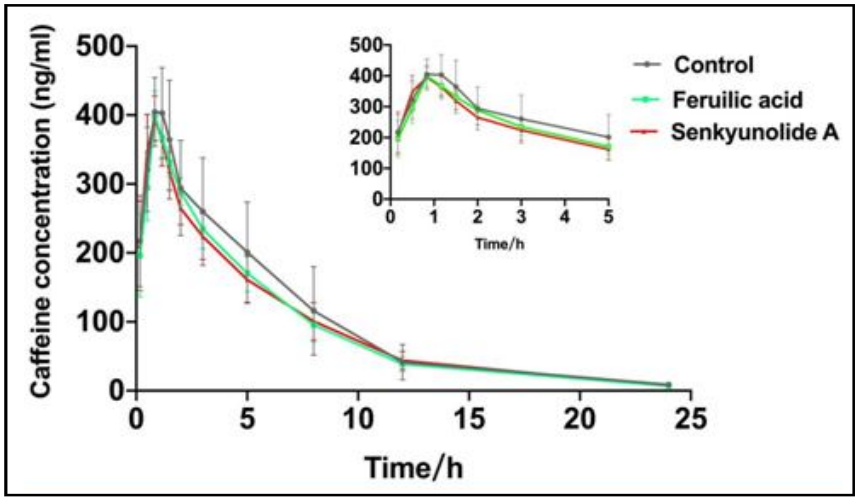

Figure 2: Mean plasma concentration-time profiles of caffeine.

Table 4: The pharmacokinetic parameters of caffeine in rats

Parameters	Control	Feruilic acid	*p*	Senkyunolide A	*p*
C_{max} /ng·ml^{-1}	428.27 ± 55.46	402.04 ± 24.56	0.139	395.24 ± 32.92	0.192
T_{max} /h	1.04 ± 0.18	0.91 ± 0.16	0.334	0.91 ± 0.16	0.149
$t_{1/2}$ /h	3.72 ± 0.71	4.13 ± 0.31	0.106	4.21 ± 0.39	0.112
AUC_{0-t} /ng·h·ml^{-1}	2505.78 ± 720.75	2233.25 ± 258.21	0.138	2250.00 ± 303.56	0.147
$AUC_{0-\infty}$/ng·h·ml^{-1}	2538.33 ± 749.44	2274.05 ± 260.10	0.131	2294.05 ± 304.56	0.139

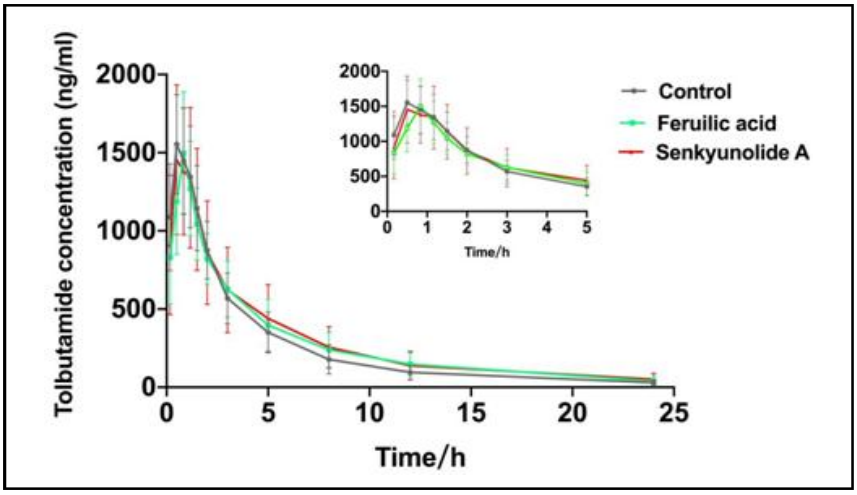

Figure 3: Mean plasma concentration-time profiles of tolbutamide.

Table 5: The pharmacokinetics parameters of tolbutamide in rats

Parameters	Control	Feruilic acid	*p*	Senkyunolide A	*p*
C_{max} /ng·ml^{-1}	1658.73 ± 344.33	1500.03 ± 391.54	0.404	1568.36 ± 479.66	0.672
T_{max} /h	0.75 ± 0.34	0.87 ± 0.12	0.366	0.70 ± 0.24	0.776
$t_{1/2}$ /h	4.42 ± 1.31	5.11 ± 1.32	0.310	4.19 ± 1.33	0.728
AUC_{0-t} /ng·h·ml^{-1}	6249.89 ± 1888.03	6823.77 ± 2354.64	0.599	7156.14 ± 3222.04	0.504
$AUC_{0-\infty}$ /ng·h·ml^{-1}	6497.93 ± 2023.26	7105.96 ± 2600.46	0.553	7362.71 ± 3447.02	0.506

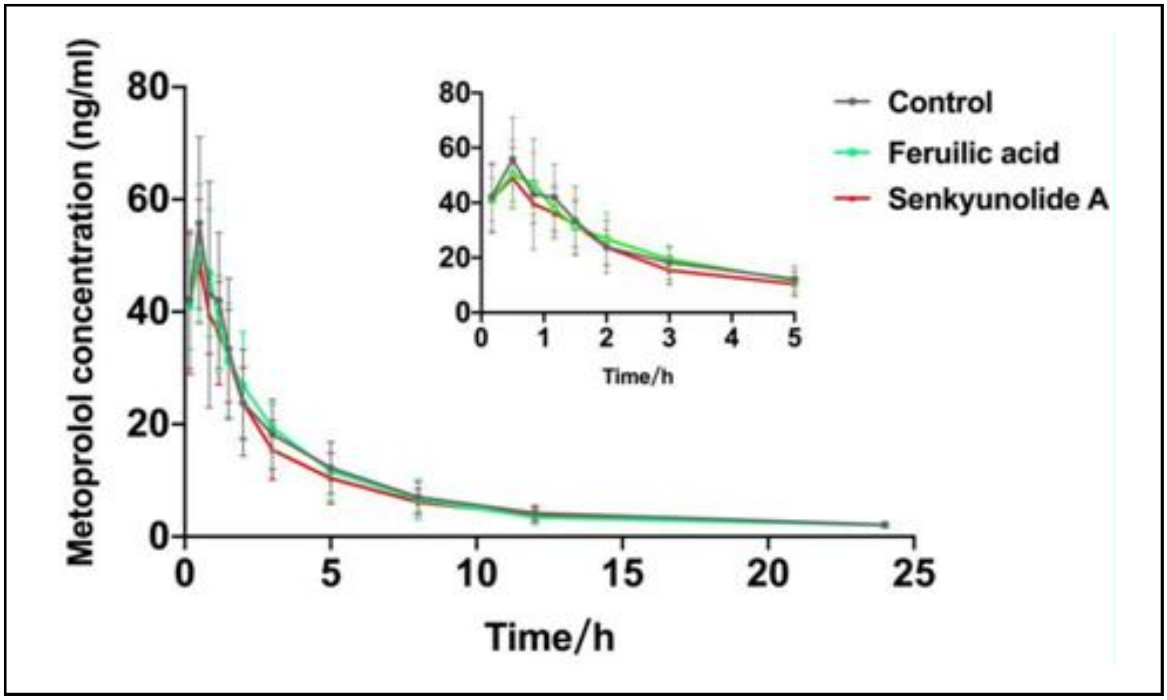

Figure 4: Mean plasma concentration-time profiles of metoprolol.

Table 6: The pharmacokinetics parameters of metoprolol in different groups of rats

Parameters	Control	Feruilic acid	*p*	Senkyunolide A	*p*
C_{max} /ng·ml^{-1}	56.89 ± 15.74	50.41 ± 12.33	0.375	48.66 ± 11.92	0.605
T_{max} /h	0.63 ± 0.25	0.54 ± 0.12	0.403	0.75 ± 0.35	0.417
$t_{1/2}$ /h	6.37 ± 3.03	4.84 ± 2.95	0.323	5.76 ± 1.57	0.620
AUC_{0-t} /ng·h·ml^{-1}	220.33 ± 56.51	232.26 ± 57.40	0.704	203.65 ± 49.08	0.539
$AUC_{0-\infty}$ /ng·h·ml^{-1}	209.42 ± 55.91	217.17 ± 56.80	0.605	214.39 ± 56.96	0.542

Note: (Mean ± SD, n = 8).

As shown in Figure 5 and Table 7, treatment with ferulic acid for 14 days significantly accelerated the metabolism of dapsone. In comparison to the control group, the pharmacokinetic parameters of the ferulic acid group, including C_{max}, $t_{1/2}$, $AUC_{0-\infty}$, and $AUC_{0-\infty}$ decreased by 20.97% (p<0.01), 42.08% (p<0.05), 25.76% (p<0.05), and 35.10% (p<0.05), respectively. T_{max} was not changed significantly. These results indicate that ferulic acid could stimulate CYP3A1/2 activity *in vivo* since dapsone functions as the probe drug for CYP3A1/2 activity. Similarly, senkyunolide A significantly accelerated the metabolism of dapsone. In comparison to the control group, the pharmacokinetic parameters of the senkyunolide A group, including C_{max}, $t_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$, decreased by 26.12% (p<0.01), 51.92% (p<0.01), 40.92% (p<0.01), and 46.61% (p<0.05), respectively. The *in vivo* experiment demonstrated that senkyunolide A had the potential to induce the activity of CYP3A1/2.

3.2 Effects of ferulic acid and senkyunolide A on rat CYP450 isoforms *in vitro*

The concentrations of relative metabolites were analyzed and shown in Table 8 and Figure 6. Compared to the control group, ferulic acid and senkyunolide A had no significant effects (p> 0.05) on the concentrations of the metabolites of phenacetin, tolbutamide and dextromethorphan, which were the *in vitro* probe drugs for CYP1A2,

CYP2C11 and CYP2D1, respectively. However, in the ferulic acid group and senkyunolide A group, the concentration of 6β-hydroxytestosterone (the metabolite of testosterone) increased by 102.8% (p<0.05) and 79.17% (p<0.05), respectively. Testosterone is the *in vitro* probe drug for CYP3A1/2. Therefore, the *in vitro* results indicated that both ferulic acid and senkyunolide A had the potential to induce the activity of CYP3A1/2, but had no effects on CYP1A2, CYP2C11, and CYP2D1.

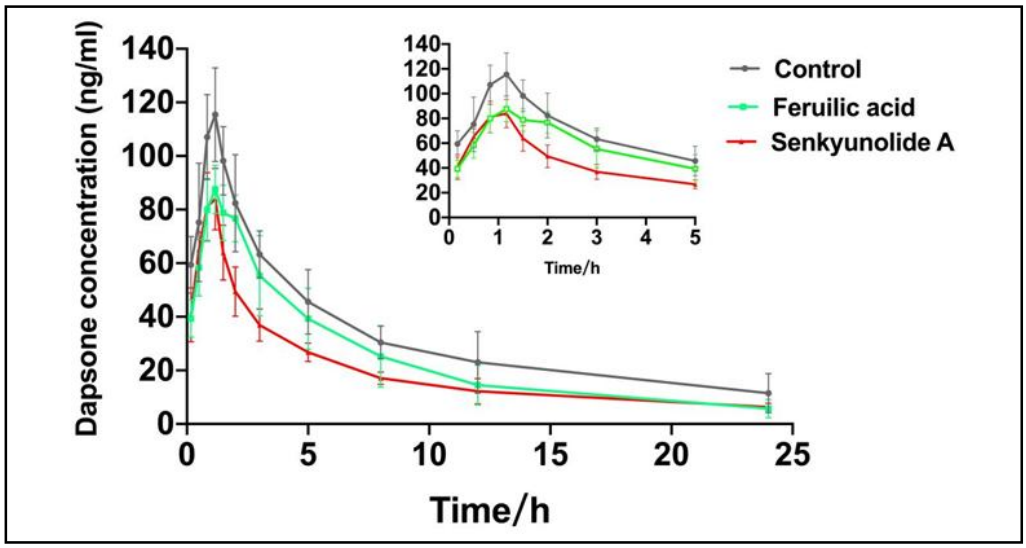

Figure 5: Mean plasma concentration-time profiles of dapsone.

Table 7: The pharmacokinetics parameters of dapsone in different groups of rats

Parameters	Control	Feruilic acid	*p*	Senkyunolide A	*p*
C_{max} /ng·ml^{-1}	115.51 ± 17.41	91.29 ± 8.20	0.005**	85.34 ± 11.74	0.002**
T_{max} /h	1.21 ± 0.12	1.31 ± 0.43	0.538	1.12 ± 0.16	0.225
$t_{1/2}$ /h	8.84 ± 4.39	5.12 ± 1.57	0.041*	4.25 ± 2.95	0.003**
AUC_{0-t} /ng·h·ml^{-1}	797.34 ± 184.80	591.94 ± 163.03	0.034*	471.09 ± 54.71	0.001**
$AUC_{0-\infty}$ /ng·h·ml^{-1}	965.23 ± 397.49	626.48 ± 183.31	0.046*	515.34 ± 65.69	0.015*

Note: *p<0.05, **p<0.01 compared with Control group. (Mean ± SD, n = 8)

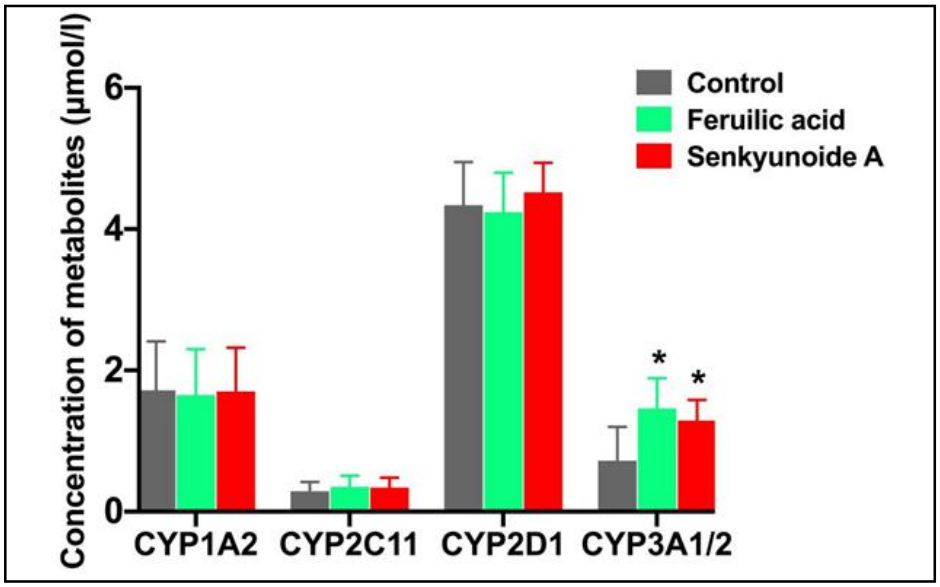

Figure 6: Effects of ferulic acid and senkyunolide A on the activities of the four CYP450 isoforms in rat liver microsomes.

Table 8: Concentration of metabolites *in vitro*

CYP	Probe drug	Metabolite	Control (μmol/l)	Ferulic acid (μmol/l)	*p*	Senkyunolide A (μmol/l)	*p*
1A2	Phenacetin	Acetaminophen	1.72 ± 0.69	1.65 ± 0.65	0.843	1.70 ± 0.62	0.958
2C11	Tolbutamide	4-hydroxytolbutamide	0.29 ± 0.13	0.35 ± 0.16	0.497	0.34 ± 0.14	0.545
2D1	Dextromethorphan	Dextrorphan	4.34 ± 0.61	4.24 ± 0.56	0.728	4.52 ± 0.42	0.514
3A1/2	Testosterone	6β-hydroxytestosterone	0.72 ± 0.48	1.46 ± 0.43	0.024*	1.29 ± 0.29	0.039*

Note: *$p<0.05$ compared with Control group. (Mean ± SD, n = 8).

4. Discussion

In this study, the effects of ferulic acid and senkyunolide A on the activities of the four CYP450 isoforms in rats were studied *in vivo* and *in vitro*. Enzyme-specific substrates are commonly used as probes to indicate CYP450 activities. The pharmacokinetic parameters of the probe drugs can be measured to evaluate the impacts of medicines on CYP450 activities *in vivo* (Chen *et al*., 2022). *In vitro* studies usually utilize changes of probe drug metabolites in liver microsomes to assess variations of CYP450 isoforms activities (Pai *et al*., 2023). In this study, the Cocktail probe method was utilized. Caffeine, tolbutamide, metoprolol and dapsone were chosen as the *in vivo* probe drugs for CYP1A2, CYP2C11, CYP2D1, and CYP3A1/2, respectively (Lu *et al*., 2022; Zhang *et al*., 2018). For the *in vitro* study, phenacetin, tolbutamide, dextromethorphan, and testosterone were used as the *in vitro* probe drugs for CYP1A2, CYP2C11, CYP2D1, and CYP3A1/2 (Neyshaburinezhad *et al*., 2020; Paudel *et al*., 2023; Wang *et al*., 2022; Wang *et al*., 2023), to assess the effects of ferulic acid and senkyunolide A on the activities of the four CYP450 isoforms *in vitro*.

Based on the *in vivo* results, both ferulic acid and senkyunolide A could induce the activity of CYP3A1/2 by increasing the metabolism of dapsone. Compared to the control group, the administration of ferulic acid resulted in a significant acceleration of dapsone metabolism. Similarly, senkyunolide A also decreased C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0-\infty}$ of dapsone, demonstrating an induction effect on CYP3A1/2. However, neither ferulic acid nor senkyunolide A showed significant changes in the pharmacokinetic parameters of caffeine, tolbutamide and metoprolol, indicating that they did not have significant effects on the activities of CYP1A2, CYP2C11 and CYP2D1. Furthermore, in the *in vitro* studies, compared to the control group, the metabolite of testosterone was increased by ferulic acid and senkyunolide A of 102.8% ($p<0.05$) and 79.17% ($p<0.05$), respectively implied induction on CYP3A1/2 by both ingredients. However, the concentrations of other three metabolites acetaminophen, dextrophan, and 4-hydroxytolbutamide were not significantly changed when compared to the control groups. Therefore, all the *in vivo* and *in vitro* results confirmed that both ferulic acid and senkyunolide A had an inducing effect on CYP3A1/2, but no effects on CYP1A2, CYP2C11, or CYP2D1.

Human CYP3A4 is the homologous enzyme to rat CYP3A1/2 (Xu *et al*., 2023). CYP3A4 is one of the most important isoforms involved in the metabolism of clinical drugs. It plays a crucial role in metabolizing about 50% of clinically used drugs, including but not limited to amitriptyline, clozapine, carbamazepine, diazepam, estazolam, ethosuximide, ketamine, methadone, cocaine, fentanyl, erythromycin, clarithromycin, itraconazole, cyclosporin A, nateglinide, pioglitazone, omeprazole, atorvastatin, simvastatin, nifedipine, verapamil, diltiazem, quinidine, lidocaine, digoxin, ticagrelor, chlorpheniramine, testosterone, dapsone, and cenobamate (Feng *et al*., 2023; Mulder *et al*., 2021). Therefore, the induction of CYP3A4 may result in lower plasma concentrations of the drugs which are CYP3A4 substrates, and weaken their therapeutic effects. The drugs with narrow therapeutic indexes, such as anti-heart failure drug digoxin, the antiepileptic drug carbamazepine, and the immunosuppressant cyclosporine A, should be closely monitored.

As a traditional herbal medicine, *L. chuanxiong* has active ingredients including ferulic acid and senkyunolide A which are present in many clinical drug formulations, such as *Guanxinning* injection, *Salvia chuanxiong* injection, Compound *Chuanxiong* tablet, and Compound *Chuanxiong* capsule (Liu *et al*., 2025; Chen *et al*., 2018). In addition, ferulic acid is not only an active ingredient of *L. chuanxiong,* but also present in more than 50 other herbal medicines, such as *Angelica sinensis*, *Leonurus japonicus*, *Sparganium stoloniferum*, *Cyathula officinalis, Codonopsis pilosula, Notopterygium incisum, Perilla frutescens, Equisetum hyemale, Smilax glabra, Taraxacum mongolicum,* and *Plantago asiatica*, *etc.* (Zhang *et al*., 2020; Li *et al*., 2021). Furthermore, ferulic acid itself also has been made into preparations, *e.g*., Sodium ferulate tablets and Sodium ferulate injections, which are used for treatment of cardiovascular and cerebrovascular diseases and Alzheimer's diseases (Shen *et al*., 2023; Thapliyal *et al*., 2021). All these herbal medicines have the potential to be used concurrently with other drugs, and the HDI should be concerned (Bernardo *et al*., 2024; Cheng *et al*., 2023; Shaikh *et al*., 2020).

Our study has indicated that both ferulic acid and senkyunolide A can induce CYP3A4 activity, which may potentially reduce the efficacy of the drugs metabolized by CYP3A4. Therefore, it is preferred to avoid concurrent use of CYP3A4-metabolized drugs with herbal medicines that containing ferulic acid or senkyunolide A. When co-administration is necessary, careful consideration is warranted to the potential interaction and the possible impact on the efficacy, and dosage adjustment of the drugs should be considered to ensure the desired therapeutic effect.

Increasing attention is being paid to the effects of herbal medicines on CYP450 enzymes and HDI that may result in adverse consequences (Cheng *et al*., 2023; Meng *et al*., 2021; Liu *et al*., 2023; Zhang *et al*., 2022; Shen *et al*., 2025). For example, St John's wort can decrease *AUC* and $t_{1/2}$ of warfarin by inducing CYP3A4 and CYP2C9, and attenuate its anticoagulant effect (Nicolussi *et al*., 2020). However, *Salvia miltiorrhiza* can increase the exposure of warfarin by inhibiting CYP450, enhance the efficacy and cause the risk of bleeding (Zhang *et al*., 2022). Cardiovascular disease is an important public health

problem in today's society. The combination use of herbal medicines and drugs is one of the main ways for prevention and treatment of cardiovascular diseases in some countries. However, the combination use of herbal medicines and drugs is complex and may cause occurrence of HDI and clinical adverse events. Another report indicates that it is very common for elderly diabetic patients to administer herbal medicines besides drugs. The use of herbs increases the rate of multiple medicines and the incidence of potentially inappropriate medication, which is easy to cause bleeding, hypoglycemia, hypokalemia and other adverse reactions (Shen *et al*., 2025).

The adverse HDI caused by improper combination use of herbal medicines and drugs are easy to be ignored, and appropriate measures should be taken to provide warning information. Metabolic process is an important stage for interactions of herbal medicines and drugs. CYP450-mediated inhibition and induction are the most common causes of drug interactions, with more enzyme inhibition than induction. Studies on HDI are scarce compared with drug-drug interactions, so it is essential to investigate the effects of herbal medicines on CYP450 enzymes, which is of great significance for their rational applications especially in combination use with other drugs, which require extensive and in-depth research in the field.

5. Conclusion

This study demonstrated that both ferulic acid and senkyunolide A could induce the activity of CYP3A1/2, but had no impacts on the activities of CYP1A2, CYP2C11, and CYP2D1 in rats. Given the known similarities between rat CYP3A1/2 and human CYP3A4, the result suggests that *L. chuanxiong*, its preparations or other herbal medicines containing ferulic acid or senkyunolide A may cause CYP450-mediated herb-drug interactions when combined with other drugs metabolized by CYP3A4 in humans, and decrease the curative effects of the drugs, which may result in therapeutic failure. In clinical practice, clinicians are suggested to avoid prescribing herbal medicines containing either ingredient such as *L. chuanxiong* or its preparations to patients already taking drugs metabolized by CYP3A4. Careful consideration is warranted regarding the concurrent use of *L. chuanxiong* or herbal preparations containing ferulic acid and senkyunolide A with other drugs, and avoids undesirable CYP3A4-mediated HDI. Understanding the potential interactions is important and essential for rational applications of herbal medicines and their combination use with other drugs. However, this study was based on animal experiments, so it should be noted that potential differences in enzyme regulation and expression between rats and humans due to species-specific differences. Further studies should be conducted to confirm the relevant interactions in humans.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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