Effects of Unprocessed Versus Vinegar-Processed *Schisandra chinensis* on the Activity of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 Enzymes in Rats

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**Abstract**

*Schisandra chinensis* (SC) is a well-known traditional Chinese herbal medicine that has been used in clinical practices for thousands of years. However, the differences between the effects of unprocessed and vinegar-processed *Schisandra chinensis* (VSC) on cytochrome P450 (CYP450) activities are poorly understood. To evaluate the differences between processed and unprocessed SC on the metabolism of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 substrates in rats using a cocktail method based on a developed and validated high performance liquid chromatography-mass spectrometry (HPLC-MS) method. Six probe substrates (coumarin (CYP2A6), bupropion (CYP2B6), paclitaxel (CYP2C8), tolbutamide (CYP2C9), omeprazole (CYP2C19) and metoprolol (CYP2D6)) were delivered simultaneously into rats treated with single or multiple doses of processed or crude SC. The plasma concentrations of the six probes were profiled by HPLC-MS, and their corresponding pharmacokinetic parameters were calculated. Treatment with single or multiple doses of either extract of SC or VSC induced CYP2A6, CYP2B6 and CYP2C9 enzyme activity and inhibited CYP2D6, CYP2C19 and CYP2C8 enzyme activity in rats. Furthermore, the inhibitory or induced effect of multiple doses of SC was more potent after vinegar processing than without vinegar processing. CYP2A6, 2B6 and 2C9 enzyme activity were induced significantly after treatment with multiple doses but not after a single dose. CYP2C19 enzyme activity was inhibited significantly after treatment with multiple doses but not after a single dose. These results provide useful scientific data for the safe clinical application of either extract of SC in combination with other drugs, which should lack the side effects induced by other herb-drug interactions.

**Keywords:** *Schisandra chinensis*, Vinegar-processed; CYP450; Pharmacokinetic; Cocktail

**Introduction**

*Schisandra chinensis* (Wuweizi in China), the dry ripe fruits of *Schisandra chinensis* (Turcz.) Baill., officially listed as a sedative and tonic in the China Pharmacopoeia, has been used as an important component in various prescriptions in traditional Chinese medicine (TCM).

In recent decades, SC has been shown to have some pharmacologically useful properties for the management of certain intractable diseases, such as hepatitis [1]. SC has the ability to restore injured hepatocytes and remove free radicals from the body [2]. The results of many studies showed that the chemical constituents of *Schisandra chinensis* were mainly lignans, polysaccharides, volatile oils, three terpenoids, sesquiterpen and organic acids [3-5]. Lignans with dibenzocyclooctadiene skeletons are regarded as the main bioactive components, these include schizandrin A, B and C, schizandrol A and B, schizandrin A and B, gomisin J, y-schisandrin, gomisin N and angelylgomisin H [6-9]. These compounds are known to possess a wide variety of biological activities, including anti-hepatotoxicity, anti-oxidation, detoxification and inhibition of hepatocarcinogenesis [10-12]. Recently, several studies have shown that some different types of lignan compounds present in SC are able to induce or inhibit CYP450 activity in human liver microsomes [13-16]. Thus far, most pharmacological and pharmacokinetic studies have examined single components of SC. However, no single component can be completely responsible for the effects of the complete SC extract. In addition, the effects of unprocessed versus vinegar-processed SC on CYP450 activities are poorly understood. The purpose of the present study was to evaluate the difference between unprocessed SC and SC processed with vinegar (VSC) on CYP450 activities.

It is well known that the liver is the main organ responsible for drug metabolism, nearly 60% of the most commonly prescribed drugs are metabolized by the CYP450 system [17]. CYP450 enzymes constitute the major drug metabolism enzyme system in humans [18,19], which participate in the formation of steroid hormones, bile acids and bile pigments, as well as in transformation of many drugs [20,21]. In liver cells, in addition to a small part of drug metabolizing enzymes in nucleus, cytoplasm, cell membrane and mitochondria, the vast majority of drug metabolism is involved in microsomal enzymes located in the endoplasmic reticulum, and metabolic enzyme CYP450 enzyme is one of the most important, there are more than 90% involved in the metabolism of drugs, as an important phase I metabolic enzyme. The CYP450 enzyme system plays a major role in drug metabolism in the body, it has 9 major isozymes enzymes of the three families included CYP1, CYP2 and CYP3, namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 [22-24]. All of them...
accounted for about 8.9%, 5%, 7.2%, 4.7%, 12.8%, 30.2%, 6.8%, 20%, 7% in liver cells, respectively [24,25].

CYP1A2, CYP2E1 and CYP3A4 are the three major enzyme subtypes in the CYP450 system. We have researched on the effects of SC and VSC on the activity of CYP1A2, CYP2E1 and CYP3A4 enzymes in rats in previous studies [26]. The 10 most significant drug metabolizing CYPs in the human liver include CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP2D6 and CYP3A4/5. So, in the present study, we will continue to focus on the activity of the other 6 CYP450 enzymes, such as CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6. Herbs that contain potent inhibitors of one or more CYP450 enzyme subtypes may cause herb–drug interactions with adverse effects. Therefore, the evaluation of potential herb-drug interactions is important for the selection of candidate drugs during preclinical evaluation. Here, we evaluated the effect of SC on the CYP450 system using the ‘cocktail’ approach, which involves multiple probe drugs. This approach has been widely used to monitor the influence of drugs on the P450 activities and potential drug-drug or herb–drug interactions [27-30]. The activity of drug metabolism enzymes can be assessed in vivo by the use of specific probe drugs [31]. For example, tolbutamide and ibuprofen are often used as the probe substrates of CYP2C9, dexamethasone, codeine and propranolol are used for CYP2D6, and omeprazole and amitriptyline are often used for CYP2C19 [32].

SC and VSC are commonly used in Chinese patent drugs and TCM prescriptions. In the Chinese pharmacopoeia, there are more than ten Chinese patent drugs containing SC or VSC, including Sheng-mai-yin and Hu-gan-pian [33-35]. With the aim of avoiding possible side effects induced by herb-drug interactions, we evaluated the effects of SC and VSC on the activities of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 enzymes in rats. We used a cocktail strategy of probes containing coumarin, bupropion, paclitaxel, tolbutamide, omeprazole and metoprolol and an approach based on a developed and validated HPLC-MS method to assess CYP450 activities in vivo. We predict that the results may be useful for the clinical safety evaluation of herb-drug interactions involving SC or VSC.

**Experimental**

**Chemicals and reagents**

HPLC-grade methanol and acetonitrile were purchased from Shandong Yunwang Industrial Co. Ltd. (Shandong, China) and Merck (Merck, Darmstadt, Germany), respectively. Ultra-pure water was purified using an Milli-Q Gradient A10 superpurification system (Millipore, Bedford, MA, USA). The distilled water was used for sample extraction and preparation. Omeprazole, tolbutamide, coumarin, paclitaxel and diazepam (internal standard, IS) were purchased from the Shanghai yuanye Biotechnology Co., Ltd. (Shanghai, China). Bupropion hydrochloride and chrysophanol (internal standard, IS) were obtained from China National Institute for drug and biological products (Beijing, China).

**Materials**

The crude SC herb was purchased from Anhui Pengyuan Tongling Herbal Pieces Co. Ltd.(China), Batch number: LOT#140524. All the raw materials were identified by the corresponding author. The voucher specimens (NO. 101215) were deposited in the Jiangsu Key Laboratory of Chinese Herbal Medicine Processing Research of the Nanjing University of Chinese Medicine.
the eighth day. Blood samples (0.25 mL) were then collected into heparinized tubes pre-dose (0 hour) and at 0.05, 0.15, 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 10, 12 and 24 hours post-dose. The plasma samples were collected after centrifugation at 12, 000 rpm for 10 min and then stored at -80°C.

**Preparation of plasma**

Ten µL of a reference substance and 10 µL of each internal standard solution (diazepam: 41.20 µg/mL; chrysophanol: 40.40 µg/mL) were added to a 100 µL plasma sample. The plasma sample was then spiked with 450 µL acetonitrile and vortexed for 3 min. The precipitated protein was removed by centrifugation at 12,000 rpm for 10 min. The organic layer (400 µL) was evaporated to dryness under nitrogen and stored at -20°C until analysis. For HPLC-MS analysis, the residue was dissolved in 100 µL acetonitrile-water (50:50, v/v), and a 10 µL aliquot was injected into the column. The standards were prepared in the same way.

**Preparation of the standard solutions and quality control samples**

The “Guidance for Industry-Bioanalytical Method Validation” document from the FDA was used as a guide for the assay validation, which is described below.

**Calibration curve:** A series of stock standard solutions were added to blank plasma to yield eight different concentrations: for metoprolol, they were 0.0424, 0.0848, 0.2120, 0.4241, 1.0602, 1.6963, 2.5445 and 3.3926 µg/mL; for coumarin, they were 0.2058, 0.4115, 1.0288, 2.0576, 5.1440, 8.3204, 12.3456 and 16.4608 µg/mL; for omeprazole, they were 0.1384, 0.2768, 0.6920, 1.3840, 3.4600, 5.5360, 8.3040 and 11.0720 µg/mL; for bupropion, they were 0.1014, 0.2029, 0.5072, 1.0144, 2.5360, 4.0576, 6.0864 and 8.1152 µg/mL; for paclitaxel, they were 0.1974, 0.3948, 0.9870, 1.9740, 4.9350, 7.8960, 11.8440 and 15.7920 µg/mL; and for tolbutamide, they were 0.1201, 0.2402, 0.6006, 1.2012, 3.0030, 4.8084, 7.2072 and 9.6096 µg/mL. Ten µL of two internal standards were then added, respectively. The samples were extracted and analyzed according to the sample preparation procedures described above. Each calibration curve was constructed by plotting the peak area ratio of diazepam and chrysophanol/IS versus the concentration analyzed according to the sample preparation procedures described. The extraction recoveries (ER) of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide were evaluated under conditions mimicking the situations during sample storage and the analytical process by analyzing three replicates of QC samples for each analysis. The freeze-thaw stability was determined after one freeze and thaw cycle. The QC samples were stored at -80°C for 30 days and thawed unassisted at room temperature for 24 hours.

**Data and statistical analysis:** The plasma concentrations of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide were evaluated using conditions described above. The freeze-thaw stability was determined after one freeze and thaw cycle. The QC samples were stored at -80°C for 30 days and thawed unassisted at room temperature for 24 hours.

**Stability:** The stability of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide were evaluated under conditions mimicking the situations during sample storage and the analytical process by analyzing three replicates of QC samples for each analysis. The mean recoveries were 97.35%, 107.35%, 98.85%, and 95.35%, respectively (Figures 1 and 2). These results indicate that the assay had adequate specificity.

**Linearitd lower limit of quantification**

Each calibration curve contained eight different concentrations of standard was constructed by plotting the peak area ratio of IS versus the concentrations of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide using linear regression. The regression equation was $Y = 147.9250 - 0.0849 (r=0.9996)$ for metoprolol, $Y = 0.5654 - 0.0849 (r=0.9991)$ for coumarin, $Y = 35.7308 - 0.0849 (r=0.9990)$ for omeprazole, $Y = 25.6944 - 0.0849 (r=0.9992)$ for bupropion, $Y = 2.0537 - 0.0849 (r=0.9993)$ for paclitaxel and $Y = 24.5452 - 0.0849 (r=0.9991)$ for tolbutamide, where $X$ was the concentration of the analyte in rat plasma and $Y$ was the ratio of the analyte peak area to that of the internal standard. Based on a signal-to-noise ratio (S/N=10), the lower limits of quantification (LLOQ) for metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide were $0.2402, 0.1384, 0.1014, 0.1974$ and $0.1201$ µg/mL, respectively.

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**Results and Discussion**

**Specificity**

Under optimized chromatographic and mass spectrometry conditions, the retention times of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide were evaluated under conditions mimicking the situations during sample storage and the analytical process by analyzing three replicates of QC samples for each analysis. The mean recoveries were 97.35%, 107.35%, 98.85%, and 95.35%, respectively (Figures 1 and 2). These results indicate that the assay had adequate specificity.

**Accuracy and precision**

To assess the intra-day precision, inter-day precision and accuracy of the assay, QC samples at three different concentrations were prepared under the same sample preparation procedures. The intra-day precision of the assay was assessed by calculating the relative standard deviation (RSD) for the analysis of samples for quality control in six replicates, and the inter-day precision was determined by analysis of samples for quality control in three consecutive days. The accuracy was expressed by the relative error (RE).

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at three concentrations and were lower than 15% for intra-day and inter-day assays, and the accuracy was within 10% for the QC samples. The results demonstrate that the precision and accuracy of this method were acceptable.

**Stability**

Table 3 shows the stability of the three probe drugs. The metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide stabilities were measured by analyzing QC samples at three concentrations that had been exposed to sample storage conditions. The results of these studies demonstrated that there was no significant degradation of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide occurred in plasma under our experimental conditions.

**Application of the method in pharmacokinetics studies**

A developed and validated HPLC-MS method was used to determine the levels of the six probe drugs (metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide) in rat plasma after single (Figure 3 and Table 4) and multiple (Figure 4 and Table 5) doses of the SC and VSC extracts.

Table 4 showed that after a single dose of SC or VSC, there was no change (\(P > 0.05\)) in \(t_{\text{1/2}}\), \(T_{\text{max}}\) and \(MRT_{0-t}\) for metoprolol; no change in \(t_{\text{1/2}}\) and \(MRT_{0-t}\) for coumarin; no change in \(t_{\text{1/2}}\), \(T_{\text{max}}\) and \(CL/F\) for omeprazole; no change in \(AUC_{0-\infty}\), \(C_{\text{max}}\), \(CL/F\) and \(MRT_{0-t}\) for bupropion; no change in \(T_{\text{max}}\) and \(CL/F\) for paclitaxel and no change in \(CL/F\) and \(MRT_{0-t}\) for tolbutamide. Compared with the control group, the SC and VSC groups displayed increases in \(AUC_{0-\infty}\), \(C_{\text{max}}\) and \(CL/F\) for metoprolol. When compared with the SC group, the VSC group had a higher \(AUC_{0-\infty}\) and \(C_{\text{max}}\) and a lower \(CL/F\) for metoprolol (\(P < 0.05\)), it was indicated that SC could inhibit CYP2D6 and the inhibitory effect was more pronounced in the VSC group. Compared with the control group, the SC and VSC groups displayed decreases in \(AUC_{0-\infty}\), \(AUC_{0-t}\), \(T_{\text{max}}\) and \(C_{\text{max}}\) (\(P < 0.05\)) and increases in \(CL/F\) for coumarin (\(P < 0.05\)), that was indicated that SC could induce the activity of CYP2A6. Compared with the control group, the SC and VSC groups displayed increases in \(AUC_{0-\infty}\), \(AUC_{0-t}\), \(t_{\text{1/2}}\), \(C_{\text{max}}\) and \(MRT_{0-t}\) for omeprazole (\(P < 0.01\)). Compared with the control group, the SC and VSC groups displayed decreases in \(AUC_{0-\infty}\), \(AUC_{0-t}\), \(t_{\text{1/2}}\) and \(MRT_{0-t}\) for bupropion (\(P < 0.05\)). Compared with the control group, the SC and VSC groups displayed increases in \(AUC_{0-\infty}\), \(AUC_{0-t}\), \(t_{\text{1/2}}\), \(C_{\text{max}}\) and \(MRT_{0-t}\) for paclitaxel (\(P < 0.05\) or \(P < 0.01\)). When compared with the SC group, the VSC group had a higher \(AUC_{0-\infty}\), \(AUC_{0-t}\), \(t_{\text{1/2}}\), \(C_{\text{max}}\) and \(MRT_{0-t}\) for paclitaxel (\(P < 0.05\) or \(P < 0.01\)). Compared with the control group, the SC and VSC groups displayed decreases in \(AUC_{0-\infty}\), \(AUC_{0-t}\), \(t_{\text{1/2}}\), \(T_{\text{max}}\) and \(MRT_{0-t}\) for tolbutamide (\(P < 0.05\) or \(P < 0.01\)).

In general, after a single dose of the SC or VSC extract, CYP2D6, CYP2C19 and CYP2C8 enzyme activities were inhibited and CYP2A6, CYP2B6 and CYP2C9 enzyme activity was induced. The inhibitory effect of CYP2D6 and CYP2C8 were more pronounced in the VSC group.

The results in Table 5 showed that after multiple doses of SC or VSC, there was no change in the \(CL/F\) for coumarin, omeprazole, bupropion, paclitaxel and tolbutamide and \(t_{\text{1/2}}\) for tolbutamide (\(P > 0.05\)); however, there were significant differences in the \(MRT_{0-t}\), \(T_{\text{max}}\) for metoprolol, \(MRT_{0-t}\), \(t_{\text{1/2}}\) for coumarin, \(t_{\text{1/2}}\), \(T_{\text{max}}\) for omeprazole, \(AUC_{0-\infty}\), \(C_{\text{max}}\), \(MRT_{0-t}\) for bupropion, \(T_{\text{max}}\) for paclitaxel and \(MRT_{0-t}\) for tolbutamide (\(P < 0.05\)).
Figure 2: Mass spectrogram in positive and negative ion modes of blank plasma with diazepam (+) and chrysophanol (-) IS+ (A), blank plasma spiked with six probe drugs and IS- (B) and plasma samples 2.5 h after oral administration of six probe substrates (C). 1. metoprolol 2. coumarin 3. tolbutamide 4. chrysophanol (IS-) 5. Omeprazole 6. bupropion 7. diazepam (IS+) 8. paclitaxel.
### Table 2: Precision and accuracy of the measurement of metoprolol, coumarin, omeprazole, bupropion, paclitaxel and tolbutamide levels in rat plasma (mean ± SD, n=5).

| Analytes   | Parameter | Control group | SC group | VSC group |
|------------|-----------|---------------|----------|-----------|
|            | AUC$_{0-t}$ (µg·h/mL) | 5.1497 ± 0.2786 | 8.7300 ± 0.2408 | 11.8568 ± 0.3074 |
| Metoprolol | AUC$_{0-∞}$ (µg·h/mL) | 5.2854 ± 0.3338 | 8.8647 ± 0.3044 | 12.2354 ± 0.4866 |
|            | C$_{max}$ (µg/mL) | 3.7377 ± 0.0473 | 3.7594 ± 0.0255 | 3.6700 ± 0.0777 |
|            | MRT$_{0-t}$ (h) | 4.32 ± 0.24 | 3.92 ± 0.09 | 4.00 ± 0.11 |
|            | C$_{max}$ (µg/mL) | 3.4960 ± 0.1971 | 5.0259 ± 0.3613 | 7.18 ± 0.90 |
|            | t$_{1/2}$ (h) | 5.06 ± 0.76 | 5.31 ± 0.72 | 5.47 ± 0.71 |
|            | T$_{max}$ (h) | 0.58 ± 0.20 | 1.42 ± 0.20 | 1.58 ± 0.20 |
|            | CL/F(L·kg/h) | 3.7960 ± 0.2300 | 2.5600 ± 0.0790 | 1.6370 ± 0.0650 |
| Coumarin   | AUC$_{0-t}$ (µg·h/mL) | 54.1152 ± 0.9331 | 38.1747 ± 2.0796 | 25.4855 ± 2.5606 |
|            | AUC$_{0-∞}$ (µg·h/mL) | 54.4397 ± 1.0428 | 38.5815 ± 2.1143 | 27.8311 ± 2.1492 |
|            | C$_{max}$ (µg/mL) | 16.6153 ± 0.1487 | 13.9790 ± 0.8891 | 12.9526 ± 1.2166 |
|            | MRT$_{0-t}$ (h) | 4.60 ± 0.04 | 5.17 ± 0.10 | 5.65 ± 0.46 |
|            | C$_{max}$ (µg/mL) | 3.4389 ± 0.2343 | 10.6567 ± 0.4039 | 11.4450 ± 0.2216 |
|            | MRT$_{0-t}$ (h) | 3.89 ± 0.51 | 4.55 ± 0.11 | 4.64 ± 0.02 |
|            | T$_{max}$ (h) | 10.96 ± 1.13 | 1.72 ± 0.09 | 1.42 ± 0.05 |
|            | t$_{1/2}$ (h) | 3.84 ± 0.54 | 4.14 ± 0.17 | 6.60 ± 0.80 |
|            | CL/F(L·kg/h) | 4.12 ± 0.20 | 1.42 ± 0.20 | 1.42 ± 0.20 |
| Omeprazole | AUC$_{0-t}$ (µg·h/mL) | 5.1456 ± 0.6941 | 14.2173 ± 1.5038 | 11.38 ± 9.90 |
|            | AUC$_{0-∞}$ (µg·h/mL) | 2.4245 ± 0.0880 | 2.2864 ± 0.1260 | 5.52 ± 9.47 |
| Bupropion  | C$_{max}$ (µg/mL) | 14.8215 ± 0.4832 | 4.8687 ± 0.3566 | 7.33 ± 8.57 |
|            | MRT$_{0-t}$ (h) | 5.0259 ± 0.3613 | 5.0353 ± 0.3613 | 7.18 ± 0.90 |
|            | C$_{max}$ (µg/mL) | 0.2429 ± 0.0101 | 1.2059 ± 0.0136 | 5.40 ± 9.17 |
|            | t$_{1/2}$ (h) | 7.1799 ± 0.7783 | 7.2219 ± 0.1126 | 1.56 ± 8.73 |
| Paclitaxel | AUC$_{0-t}$ (µg·h/mL) | 10.5056 ± 0.6941 | 14.2173 ± 1.5038 | 11.38 ± 9.90 |
|            | AUC$_{0-∞}$ (µg·h/mL) | 5.2445 ± 0.0880 | 5.2864 ± 0.1260 | 5.52 ± 9.47 |
|            | C$_{max}$ (µg/mL) | 3.0552 ± 0.2022 | 3.0535 ± 0.3613 | 7.18 ± 0.90 |
|            | MRT$_{0-t}$ (h) | 4.32 ± 0.24 | 3.92 ± 0.09 | 4.00 ± 0.11 |
|            | C$_{max}$ (µg/mL) | 0.2429 ± 0.0101 | 1.2059 ± 0.0136 | 5.40 ± 9.17 |
|            | t$_{1/2}$ (h) | 7.1799 ± 0.7783 | 7.2219 ± 0.1126 | 1.56 ± 8.73 |
|            | CL/F(L·kg/h) | 3.7960 ± 0.2300 | 2.5600 ± 0.0790 | 1.6370 ± 0.0650 |
| Tolbutamide| AUC$_{0-t}$ (µg·h/mL) | 69.5099 ± 2.6129 | 105.3187 ± 2.6830 | 126.1533 ± 4.8135 |
|            | AUC$_{0-∞}$ (µg·h/mL) | 68.4310 ± 2.9991 | 105.0422 ± 2.6293 | 118.8637 ± 3.9188 |
|            | C$_{max}$ (µg/mL) | 14.0298 ± 0.8402 | 15.1230 ± 0.4196 | 16.1395 ± 0.2970 |
|            | MRT$_{0-t}$ (h) | 5.00 ± 0.57 | 14.34 ± 0.72 | 12.41 ± 1.36 |
|            | C$_{max}$ (µg/mL) | 0.2429 ± 0.0101 | 1.2059 ± 0.0136 | 5.40 ± 9.17 |
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|            | CL/F(L·kg/h) | 3.7960 ± 0.2300 | 2.5600 ± 0.0790 | 1.6370 ± 0.0650 |

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| Analytes       | parameter | Groups                      |
|----------------|-----------|-----------------------------|
| Tolbutamide    | AUC∞ (µg · h/mL) | 30.5098 ± 0.9816 | 19.5317 ± 0.3343* | 13.9170 ± 0.2848** |
|                | AUC∞ (µg · h/mL) | 31.7177 ± 2.6762 | 21.1236 ± 1.1203** | 24.5130 ± 3.5061** |
|                | C∞ (µg/mL)    | 9.3479 ± 0.3533      | 7.6528 ± 0.2226*  | 6.3227 ± 0.6508**  |
|                | MRT∞ (h)      | 5.02 ± 0.14          | 5.49 ± 0.06       | 6.42 ± 0.12        |
|                | t1/2 (h)      | 6.21 ± 0.14          | 5.38 ± 0.44*      | 5.90 ± 0.80*       |
|                | T_max (h)     | 0.47 ± 0.07          | 0.32 ± 0.03**     | 0.26 ± 0.03*       |
|                | CL/F(L·kg/h)  | 0.6340 ± 0.0480      | 0.9168 ± 0.1248   | 0.9352 ± 0.0344    |

| Metoprolol     | AUC∞ (µg · h/mL) | 30.5098 ± 0.9816 | 19.5317 ± 0.3343* | 13.9170 ± 0.2848** |
| Coumarin       | AUC∞ (µg · h/mL) | 31.7177 ± 2.6762 | 21.1236 ± 1.1203** | 24.5130 ± 3.5061** |
| Omeprazole     | AUC∞ (µg · h/mL) | 30.5098 ± 0.9816 | 19.5317 ± 0.3343* | 13.9170 ± 0.2848** |
| Bupropion      | AUC∞ (µg · h/mL) | 31.7177 ± 2.6762 | 21.1236 ± 1.1203** | 24.5130 ± 3.5061** |
| Paclitaxel     | AUC∞ (µg · h/mL) | 30.5098 ± 0.9816 | 19.5317 ± 0.3343* | 13.9170 ± 0.2848** |
| Tolbutamide    | AUC∞ (µg · h/mL) | 30.5098 ± 0.9816 | 19.5317 ± 0.3343* | 13.9170 ± 0.2848** |

*Significantly different from control, P<0.05; **Significantly different from control, P<0.01; *Significantly different from SC group, P<0.05; **Significantly different from SC group, P<0.01

Table 3: Pharmacokinetic parameters of the six probe drugs after a single dose of SC or VSC (mean ± SD, n=6).

Table 4: Pharmacokinetic parameters of the six probe drugs after multiple doses of SC or VSC (mean ± SD, n=6).
Table 5: The analysis results for the six probe drugs.

| Analytes | Parameter | Single dose | Multiple doses |
|----------|-----------|-------------|---------------|
| Metoprolol | AUC<sub>0-t</sub>, AUC<sub>0-∞</sub> | ++ | ++ |
| Coumarin | AUC<sub>0-t</sub>, AUC<sub>0-∞</sub> | -- | -- |
| Omeprazole | AUC<sub>0-t</sub>, AUC<sub>0-∞</sub> | ++ | ++ |
| Bupropion | AUC<sub>0-t</sub>, AUC<sub>0-∞</sub> | -- | -- |
| Paclitaxel | AUC<sub>0-t</sub>, AUC<sub>0-∞</sub> | ++ | ++ |
| Tolbutamide | AUC<sub>0-t</sub>, AUC<sub>0-∞</sub> | -- | -- |

*: AUC increased; -: AUC decreased; ++: AUC significantly increased; --: AUC significantly decreased

Compared with the control group, the SC and VSC groups displayed increases in AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, C<sub>max</sub>, MRT<sub>0-t</sub>, t<sub>1/2</sub> and T<sub>max</sub> and decreases in CL/F for metoprolol. When compared with the SC group, the VSC group had a higher AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, C<sub>max</sub> and T<sub>max</sub> and a lower CL/F for metoprolol (P<0.05), it was indicated that SC could inhibit CYP2D6 and the inhibitory effect was more pronounced in the VSC group. Compared with the control group, the SC and VSC groups displayed decreases in AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, MRT<sub>0-t</sub>, t<sub>1/2</sub>, T<sub>max</sub> and C<sub>max</sub> for coumarin (P<0.05 or P<0.01). When compared with the SC group, the VSC group had a lower AUC<sub>0-t</sub>, C<sub>max</sub>, MRT<sub>0-t</sub>, t<sub>1/2</sub> and T<sub>max</sub> for coumarin (P<0.05 or P<0.01). Compared with the control group, the SC and VSC groups displayed decreases in AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, C<sub>max</sub>, MRT<sub>0-t</sub>, t<sub>1/2</sub> and C<sub>max</sub> for omeprazole (P<0.05 or P<0.01). When compared with the SC group, the VSC group had a higher AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, t<sub>1/2</sub>, MRT<sub>0-t</sub>, T<sub>max</sub> and C<sub>max</sub> for omeprazole (P<0.05 or P<0.01), it was indicated that SC could inhibit CYP2C19 and the inhibitory effect was more pronounced in the VSC group. Compared with the control group, the SC and VSC groups displayed decreases in AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, MRT<sub>0-t</sub>, t<sub>1/2</sub>, T<sub>max</sub> and C<sub>max</sub> for bupropion (P<0.05 or P<0.01). When compared with the SC group, the VSC group had a lower AUC<sub>0-t</sub>, C<sub>max</sub>, MRT<sub>0-t</sub>, t<sub>1/2</sub> and T<sub>max</sub> for bupropion (P<0.05 or P<0.01). Compared with the control group, the SC and VSC groups displayed increases in AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, t<sub>1/2</sub>, MRT<sub>0-t</sub>, T<sub>max</sub> and C<sub>max</sub> for paclitaxel (P<0.05 or P<0.01). When compared with the SC group, the VSC group had a higher AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, t<sub>1/2</sub>, MRT<sub>0-t</sub>, T<sub>max</sub> and C<sub>max</sub> for paclitaxel (P<0.05 or P<0.01), it was indicated that SC could inhibit CYP2C8 and the inhibitory effect was more pronounced.
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Figure 4: Time-concentration curves for metoprolol (A), coumarin (B), omeprazole (C), bupropion (D), paclitaxel (E) and tolbutamide (F) after a multiple dose of SC or VSC.

Discussion

Modern research has shown that CYP450 enzymes can be induced or inhibited by exogenous materials such as drugs or herbs. Changes in CYP450 levels or activities can affect the concentration of drug in the blood, the pharmacokinetic process and biological medicinal properties [16,36]. Currently, Europe and the United States require that drug screens and metabolic research based on the CYP450 system should be included in new drug evaluations. This methodology is also required by the Chinese SFDA for pharmacokinetic research in new chemical drug development.

SC and VSC are widely used in TCM practice. Previous research has suggested that vinegar processing enhances the increase or decrease in CYP450 enzyme (CYP1A2, CYP2E1 and CYP3A4) levels elicited by SC and VSC [26]. No new lignan compounds were found after vinegar processing, but the amounts of lignan components were changed [37]. Other studies showed that the hepatoprotective potency of SC is significantly increased after vinegar processing [38]. This effect may be attributable to the fact that vinegar-processed SC increases the dissolution rate of the active principles, thus enhancing the curative effects [39]. In view of their well-known effects, we aimed to investigate the effects of SC and VSC on metabolism of CYP2A6, CYP2B6 and CYP2C9 enzyme activity. And this induction effect was more potent in the VSC group. The analysis results for the six probe drugs are shown in Table 5.

In general, after multiple doses of the SC or VSC extract, CYP2D6, CYP2C19 and CYP2C8 enzyme activities were inhibited. This inhibitory effect was more potent in the VSC group. However, multiple-dose SC or VSC treatment increased CYP2A6, CYP2B6 and CYP2C9 enzyme activity. And this induction effect was more potent in the VSC group. The analysis results for the six probe drugs are shown in Table 5.

In the VSC group. Compared with the control group, the SC and VSC groups displayed decreased in AUC0–t, AUC0-∞, Cmax, Tmax and MRT0-t for tolbutamide (P<0.05 or P<0.01). When compared with the SC group, the VSC group had a lower AUC0–t, AUC0-∞, Cmax, Tmax and MRT0-t for paclitaxel (P<0.05 or P<0.01).

Interestingly, the inhibitory effect of SC on CYP2D6, 2C19 and 2C8 and the induced effect of SC on CYP2A6, 2B6 and 2C9 were more potent after vinegar processing, possibly due to changes in the pharmacological activities and levels of its components. However, the mechanisms underlying this effect are...
not yet documented. In this study, investigation of six CYP450 enzymes activity showed that, as the administration time increased, the effect of SC and VSC changed from weak to strong.

To investigate the metabolic mechanisms of SC and VSC treatment, further study is required. In vitro methods, such as liver microsome incubation, gene recombination CYP450 enzyme system, hepatocyte culture and liver tissue slice explant culture may be used. The data obtained will serve as a reference to evaluate possible herb–drug interactions when SC and VSC are prescribed in clinical. Interactions between SC and VSC and other drugs require further study.

Conclusions

This study investigated the potential differences between SC and VSC in their effects on CYP450 enzyme activities, including those of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 in rats after single and multiple doses of extract. SC and VSC significantly inhibited CYP2D6, 2C19 and 2C8 activity and induced CYP2A6, 2B6 and 2C9 activity. The effect of VSC was more potent than that of SC. The results provide a scientific basis for the safe clinical application of SC and VSC in combination with other drugs, potentially preventing possible side effects induced by herb-drug interactions.

Conflict of Interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All of the protocols on living animals used in this paper were come from the Experimental animal center of Nanjing University of Chinese Medicine, license No. SYXK (Su) 2014-0001.

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