INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–224/2019/21–5–1036–1042
DOI: 10.17957/IJAB/15.0991
http://www.fspublishers.org

Full Length Article

Effects on Expression of Hepatic Phase-1 and Phase-2 Metabolism and Transporter Genes by *Swertia mussotii* Extract, A Hepatoprotective Herb from Tibetan Traditional Medicine

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Abstract

To study the effects of *Swertia mussotii* extract on the expression of drug processing genes, including Phase-1 (cytochrome P450 enzyme genes), Phase-2 (glucuronidation and sulfation genes) and Phase-3 (drug transporters) in mouse liver. Mice were orally administered *S. mussotii* extract at the dose of 0.1, 0.3, 1.0, 3.0 g/kg for consecutive eight days. Blood and livers were collected at the time of 24 h after dosing. RT-PCR was used to analysis of genes of interest. *S. mussotii* extract ingestion induced Cyp2b10 and inhibited Cyp3a11 at all doses, while increased the expressions of Cyp2e1 at the dose of 0.1 and 0.3 g/kg and decreased the expression gradually at the dose of 1.0 and 3.0 g/kg. For phase-2 enzyme genes, *S. mussotii* extract increased sulfotransferase (Sult1a1), heme oxygenase-1 (HO-1) and quinone oxidoreductase 1 (Nqo1), but had no effects on glucuronyltransferases (Ugt1a1 and Ugt1a6) and sulfotransferase (Sult1b1). It is very interesting that *S. mussotii* extract inhibited mRNA levels in the hepatic organic anion-transporting polypetides (Oatp1a1 and Oatp2b1) and induced multidrug resistance-associated proteins, such as Mrp1 and Mrp4 were especially induced at 3.0 mg/kg. The metabolism of *S. mussotii* extract includes the induction of cytochrome P450 enzyme genes, hepatic transporters and phase-2 enzyme genes. The *S. mussotii* extracts influenced all types of metabolic drug processing genes and therefore could play a role in producing herb-drug interactions. © 2019 Friends Science Publishers

Keywords: Tibetan traditional medicine; *Swertia mussotii*; Cytochrome P450; Hepatic transporters

Introduction

Herbal medications have been broadly utilized for centuries, and they are still used by 75–80% of the global population based on World Health Organization (WHO) statistics (Sieniawska *et al.*, 2013). And with the progress, there would up to 7000 chemical compounds used in phytotherapy are derived from herbal medicines (Sieniawska *et al.*, 2013). Because herbal medications have multiple pharmacologically active ingredients, more complex pathways are involved in absorption, distribution, metabolism, and excretion (*e.g*., activating or suppressing metabolic enzymes).

The Gentianaceae herb *Swertia mussotii* Franch has hepatoprotective (Hase *et al.*, 1999; Karan *et al.*, 1999; Li *et al.*, 2005; Sadeghi *et al.*, 2006; Kotoky and Das, 2008), anticancer (Saha *et al.*, 2004; Balasundari *et al.*, 2005), antibacterial (Ramesh *et al.*, 2002) and antioxidant properties (Scartezzini and Speroni, 2000; Tripathi *et al.*, 2007) because of the existence of many therapeutically active phytoconstituents, including flavonoids, xanthones, terpenoids, secoiridoid, iridoids, swertiamarin, glycosides, and ursolic acid (Yamahara *et al.*, 1978; Kikuzaki *et al.*, 1996; Brahmachari *et al.*, 2004). It is used extensively in the Tibetan traditional system of medicine as a hepatoprotective agent since it is easily accessible to local communities. As rare wild plants of the Tibetan plateau, studies of the medicinal ingredients of *S. mussotii* are constantly deepening with the rapid development of national medicine. Active components, such as swertiamarin, sweroside, gentiopicroside and isoorientin, can be extracted from *S. mussotii* efficiently. The increase in the amount of pure components has allowed a better understanding of their pharmacological activity, which has shown distinct activities for some of the constituents of *S. mussotii*. For instance, sweroside inhibits the central nervous system (Zheng and Liu, 2009), while mangiferin stimulates the central nervous system (Cheng *et al.*, 1999), different doses of oleanolic acid have hepatoprotective effects or lead to cholestasis (hepatotoxicity), which are opposite effects (Liu, 2005; Lu *et al.*, 2013). The opposing activities of these compounds implies that different extraction methods may lead to the

To cite this paper: Yang, H., Y. Xiao, C. Li, T. Gao, L. Wei and Y. Du, 2019. Effects on expression of hepatic phase-1 and phase-2 metabolism and transporter genes by *Swertia mussotii* extract, a hepatoprotective herb from Tibetan traditional medicine. *Intl. J. Agric. Biol*., 21: 1036–1042

production of different efficacies and demonstrate the diversity of medication efficacies that can be obtained when the whole plant is used. Our Laboratory has found that the median lethal dose (LD_{50}) of the 75% ethanol extract of *S. mussotii* is 27.24 g/kg, whereas the maximum tolerated dose (MTD) of the water extract of *S. mussotii* is 40 g/kg (Lv *et al.*, 2010). Because of these different mechanisms underlying the activity of medicinal ingredients, the pharmacological effect of *S. mussotii* extract remains a concern.

Drugs and other xenobiotics normally experience two metabolizing phases in the body, which are called phase 1 and 2 metabolism. During the phase 1 metabolism, drugs are catalyzed or detoxified mainly by Cytochrome P450 (CYP-450). Suppressing CYP-450 enzymes can cause elevated plasma levels due to a concomitantly dispensed pharmaceutical as the enzymes play a vital role in therapeutic pharmacology. The toxicology of xenobiotics could lower or extend the pharmacological power, broaden the manifestation of drug-induced toxicity, or reduce the therapeutic effect (Jarukamjorn *et al.*, 2006; Lee *et al.*, 2013). In the phase 2 metabolism, enzymes such as glucuronyltransferase (Ugts), sulfotransferase (Sults), heme oxygenase-1 (HO-1) and quinone oxidoreductase 1 (Nqo1), and some transporters all participating in metabolism and affect the disposition of chemicals in the body (Liu, 2007; Klaassen and Aleksunes, 2010). However, almost no reports about effects of *S. mussotii* extract on metabolism enzymes and liver drug transporters. Therefore, this study was designed to evaluate the transporter-mediated hepatobiliary disposition of *S. mussotii* extract to examine the effects of *S. mussotii* extract on major phase-1 CYP and phase 2 enzyme genes. To obtain a systemic understanding of the metabolism of the *S. mussotii* extract, the efflux transporters of multidrug resistance associated protein genes (primarily Mrp1, Mrp2 and Mrp4) and 4 major hepatic organic anion-transporting polypetides (namely Oatp1a1, Oatp1b2, Oatp1a4 and Oatp2b1) were all assessed.

Materials and Methods

Reagent and Animals

Gentiopicroside (the purity of 99.9%), purchased from National Institute for Food and Drug Control (Beijing, China). All Kunming (KM) mice (Mus musculus) were eight-week-old, weighing 18–22 g, and were purchased from the Experimental Animal Center of Gansu University of Chinese Medicine (Gansu, China; Certificate No. SCXK 2015-0005), then kept in animal facilities at Northwest Institute of Plateau Biology, Chinese Academy of Sciences (NWIPB,CAS), with standard laboratory conditions of regular environment (12 h light /dark cycle, 24±1°C and 45 ± 2% humidity). All animals were acclimatized for a period of 15 days prior to the start of the experiment. All animal procedures were conducted in accordance with the WHO Guidance of Humane Care and Use of Laboratory Animals.

Plant Material and Preparation of Extract

Whole plants (include root) of *S. mussotii* were collected from Sichuan province, China (altitude, 2,729 m; latitude, 31°27.575′; longitude, 102°05.600′; slope, 10°; aspect, northeast), at the full-blooming stage in July 2015. Whole plants of *S. mussotii* were dried under shade. Air-dried *S. mussotii plants* were reduced to coarse powder, extracted with 75% ethanol four times (60 min each time) with a Soxhlet apparatus, and then concentrated in the midst of lowered temperature (45°C) and pressure in a rotary evaporator (Heidolph, Germany). The extracts were freeze-dried, then maintained in desiccators.

Standardization of Gentiopicroside through HPLC Method

The lyophilized extract of *S. mussotii* was standardized by using HPLC. Gentiopicroside was used as the standard. Agilent HPLC system equipped with G1379A degasser, G1316A column heater, G1312A pump, G1315B DAD Detector and manual injector. The column used for HPLC analysis was an Agilent Eclipse XDB-C18 column (Agilent Spherisorb, USA) with a length 250 mm × width 4.6 mm, and the column temperature set at 25°C.

Typical stock solutions of gentiopicroside and *S. mussotii* extract (1 mg/mL each) were set up in HPLC-grade methanol. The stock and extract solutions were sieved through a syringe filter. The loading volume was 10 μL. Elution was carried out with optimized mobile phase containing acetic acid (0.1%): methanol (0 min, 75:25 v/v; 17 min, 60:40 v/v; 18 min, 50:50 v/v; 30 min, 30:70 v/v; 35 min, 10:90 v/v; 40 min, 75:25 v/v) at flow rate of 1 mL/min and at wavelength of 254 nm. Prior to usage, the mobile phase was sonicated for 10 min and then degassed. The analysis was performed for a 40 min run time to take out the analyte.

The percent relative standard deviation (RSD), limit of detection (LOD) and quantification (LOQ) and the percent recovery of gentiopicroside from *S. mussotii* extract were all carried out in triplicate (n=3) and as per the recommended guideline of International Conference on Harmonization (ICH). The standard solutions were diluted to different concentration levels with methanol and were analyzed; the different concentration levels and peak area could be used to generate a calibration curve of the standard compound.

Experimental Design

All animals were randomly divided into six groups after adapted for five days, as the solvent control group (distilled water), first group (0.1 g/kg), second group (0.3 g/kg), third group (1.0 g/kg) and fourth group (3.0 g/kg) of *S. mussotii* extract, 20 mL/kg intragastric gavage (i.g.) daily, for eight days continuously. All doses were chosen according to our previous studies (Yang *et al.*, 2016). Mice were monitored

chiefly for animal activities and the body weight, during the 8 day dosing period. Blood and livers were collected at 24 h after dosing. Serum was separated and alanine aminotransferase (ALT) was analyzed with automatic blood analyzer in Northwest Institute of Plateau Biology, CAS. After recorded liver weights, part of the liver was stained with hematoxylin and eosin (H&E) after fixed in 10% neutral formalin for 48 h to examine morphology of the liver and approximately 100 mg liver was promptly put into 1.0 mL TRIzol (TakaRa Biotechnology, Dalian, China) solution for RNA isolation.

Real-time PCR Analysis

Isolated RNA was determined by the 260/280 ratios and gel-electrophoresis to analysis quality and quantity. Then the RNA was reversed transcribed with the High Capacity Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA). Primers were designed and listed in Table 1. Total RNA was extracted according to the manufacturer's instructions, followed by purification with Total RNA (Mini) Kit (Watson Biotechnology, Shanghai, China). RT-PCR analysis was performed with Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). The procedure included 5 min of denaturing at 95°C, 40 cycles of annealing and extension for 45 s at 60°C. This was followed by denaturing at 95°C for 10 s. The dissociation curve was formed after completing 40 cycles to confirm the quality of amplification and the primers. The relative expression of the genes was determined with the $2^{-\triangle\triangle Ct}$ method. Normalization was done with the housekeeping gene β-actin using an identical sample.

Statistical Analysis

Statistical analysis was done by using SPSS 21.0 software. The data were expressed as mean ± SEM. All data were analyzed by the one-way analysis of variance (ANOVA), followed by LSD multiple range test or Dunnett' s Test. *p*-value < 0.05 was considered statistically significant.

Results

Quantification of Gentiopicroside by HPLC

Standard gentiopicroside presented a good linear relation in the range of 14 to 1000 μg/mL in the calibration curve. The correlation between peak area and concentration was found to be good and had the higher correlation coefficient (r^2) value of 0.999. RSD was used to approximate the precision of instrument. The RSD values of instrumental precision and intra-assay precision of gentiopicroside were approximated as <1.8%. The LOD and LOQ of gentiopicroside were determined to be 0.064 and 0.58 μg/mL, respectively. This suggested that the HPLC procedure for gentiopicroside was exact and the sensitivity of the technique was good enough to identify the gentiopicroside in the extract. At various concentrations, the retrieval of gentiopicroside was found to be 98% with RSD values that were < 1.5%, suggesting that gentiopicroside can be fully retrieved from the *S. mussotii* extract. Gentiopicroside was determined and quantified in the *S. mussotii* extract through a comparison of the retention time (R_t = 15.19 min) of typical gentiopicroside. These outcomes were deemed satisfactory and appropriate for the quantitative analysis. Gentiopicroside present in *S. mussotii* extract was found to be 27.48% (w/w).

Animal General Health

Mice received oral administrations of distilled water and various amounts of *S. mussotii* extract (SME) (0.1, 0.3, 1.0, and 3.0 g/kg) for eight days. The body weight, movements, and overall health of the mice were noted. Body weight gain in various *S. mussotii* extract groups was obviously different compared to the controls, but all mice displayed typical movements and overall health. In comparison, with the doses increase, the animal body weight decreases significantly (23.35 ± 0.32, 22.33 ± 0.42, 21.77 ± 0.29, 21.83 ± 0.06 and 21.35 ± 0.34 g for control, SME 0.1, 0.3, 1.0 and 3.0 g/kg group, respectively), especially the SME 0.3, 1.0 and 3.0 g/kg groups, were significantly different to control group (P < 0.05). At necropsy, the ratios of liver/body weight were not different from the control (5.67 ± 0.18, 5.25 ± 0.24, 5.06 ± 0.07, 6.07 ± 0.19 and 5.50 ± 0.16% for control, SME 0.1, 0.3, 1.0, and 3.0 g/kg group, respectively). Blood biochemistry results indicated that ALT (45.01 ± 3.58, 44.98 ± 4.14, 44.61 ± 4.07, 41.48 ± 3.72 and 40.39 ± 3.56 U/L for control, SME 0.1, 0.3, 1.0 and 3.0 g/kg group, respectively) were not observably different among groups.

Effect of *S. mussotii* Extract on Cytochrome P450 Enzymes

To verify the impact of *S. mussotii* extracts on P450 enzyme gene, we determined the mRNA levels of major P450 for metabolism (Table 2). The results showed the expression of Cyp2b10 was increased with the dose increase, while Cyp2e1 and Cyp3a11 were inhibition significantly at the highest doses (SME 3.0), which showed statistical difference when compare to the control group. The expression of Cyp2e1 increased at doses of 0.1 and 0.3 g/kg. It gradually decreased at doses of 1.0 and 3.0 g/kg. *S. mussotii* extract disclosed numerous induction activities toward CYP-450.

Effect of *S. mussotii* Extract on Phase 2 Enzyme Genes

Induction of phase-2 enzymes is broadly acknowledged as an effective method to lower the risk of various diseases associated with exposure to toxins, carcinogens and mutagens (Talalay *et al.*, 1995; Wilkinson and Clapper, 1997; Talalay, 2000; Zhang and Gordon, 2004).

Table 1: Primer sequences for RT-PCR analysis

Gene	Accession	Forward	Reverse
β-actin	V01217	TGACCGAGCGTGGCTACAG	GGGCAACATAGCACAGCTTCT
Cyp1a2	NM_009993	GACATGGCCTAACGTGCAG	GGTCAGAAAGCCGTGGTTG
Cyp2b10	AF128849	AAGGAGAAGTCCAACCAGCA	CTCTGCAACATGGGGGTACT
Cyp2e1	NM_021282	TCCCTAAGTATCCTCCGTGA	GTAATCGAAGCGTTTGTTGA
Cyp3a11	NM_007818	ACAAACAAGCAGGGATGGAC	CCCATATCGGTAGAGGAGCA
Cyp4a10	NM_010011	CACACCCTGATCACCAACAG	TCCTTGATGCACATTGTGGT
Sult1a1	NM_133670	GGATGTAGCTGAGGCAGAGG	CAGCTCCCAGTGGCATTTAT
Sult1b1	NM_019878	GGTGGGAAAAGAGGGAAGAG	AAGGCCTCTTCATCCAAGGT
Ugt1a1	NM_201645	ACACCGGAACTAGACCATCG	ATACCATGGGAGCCAGAGTG
Ugt1a6	NM_145079	ATACCATGGGAGCCAGAGTG	ACCAGAACTGTGAGGGTTGG
HO-1	M33203	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
Nqo 1	BC004579	TATCCTTCCGAGTCATCTCTAGCA	TCTGCAGCTTCCAGCTTCTTG
Oatp1a1	NM_013797	ATCCAGTGTGTGGGGACAAT	GCAGCTGCAATTTTGAAACA
Oatp1a4	NM_030687	GGAAGATTGGACACGCATCT	GGCATTGTGACTGAAGCAGA
Oatp1b2	NM_020495	CAAACTCAGCATCCAAGCAA	GGCTGCCAAAAATATCCTGA
Oatp2b1	NM_020495	CTAGGCCAAATGCCAGAAAG	TTGCTTGGATGCTGAGTTTG
Mrp1	NM_008576	GCCCTCTTTGCAGTCATCTC	CAGTCTCTCCACTGCCACAA
Mrp2	NM_013806	TCCTAGACAGCGGCAAGATT	GCTAGAGCTCCGTGTGGTTC
Mrp4	BC150822	GCAAAGCCCATGTACCATCT	ACCACGGCTAACAACTCACC

Table 2: Effect of *S. mussotii* extract on mouse liver P450 gene expression

	control	SME 0.1	SME 0.3	SME 1.0	SME 3.0
Cyp1a2	47.728±1.774	49.306±4.452	46.714±0.862	48.970±4.191	45.806±7.416
Cyp2b10	4.394±0.851	4.516±0.738	5.890±1.319	7.094±1.915	8.537±0.841*
Cyp2e1	494.120±14.863	849.859±78.910*	760.111±24.777*	545.213±69.469	351.232±9.561*
Cyp3a11	363.198±46.642	260.480±14.369	231.055±42.871	237.883±27.602	161.914±25.259*
Cyp4a10	0.583±0.196	0.297±0.066	0.942±0.071	1.083±0.034	1.244±0.339

*$P < 0.05$ vs control

Therefore, the effect of *S. mussotii* extracts on Sults, Ugts, HO-1and Nqo1 mRNA levels in this study were examined. The expression of HO-1 and Nqo1 were increased 2-fold at highest doses of *S. mussotii* extract and Sult1a1 was also increased, while it had no obvious effects on Sult1b1, Ugt1a1and Ugt1a6 (Fig. 1).

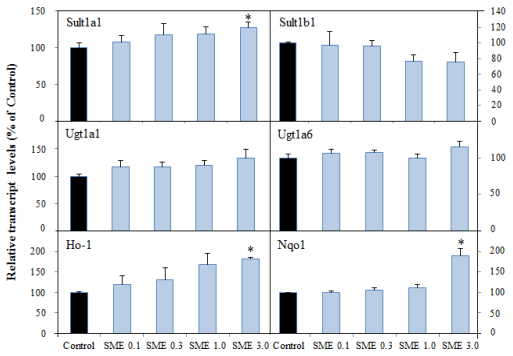

Fig. 1: Outcome of *S. mussotii* extract on the expression of phase 2 enzyme genes in adult KM mouse livers. RT-PCR analysis the total RNA, which from the mice that were given *S. mussotii* extract orally at doses of 0.1 g/kg, 0.3 g/kg, 1.0 g/kg and 3.0 g/kg for 8 days. Data represents the mean ± SEM. ($P < 0.05$ vs control)

Effect of *S. mussotii* Extract on Hepatic Uptake Transporters

The initial step of xenobiotic metabolism elevates their uptake and every one of the similar treatments that can raise hepatic uptake could have a major effect on drug metabolism. As noted in the literature, in the liver, organic anion transporting peptides (Oatps) are the primary transporters (Cheng *et al.*, 2005). In this study, we choose Oatp1a1, Oatp1a4, Oatp1b2 and Oatp2b1 to observe. But Fig. 2 clearly demonstrates that *S. mussotii* extract decreased the mRNA levels of Oatp1a1 and Oatp1b2. The change of expression of Oatp2b1 and Oatp1a4 was not evident.

Effect of *S. mussotii* Extract on Hepatic Efflux Transporters

As the prime hepatic efflux transporters (Klaassen and Aleksunes, 2010), multidrug resistance proteins (Mrps) play a crucial role in the transfer of drug metabolites and drugs from the liver. *S. mussotii* extract had remarkable effect on Mrp1, Mrp2 and Mrp4 mRNA levels, especially at the dose of 3.0 g/kg for Mrp1 and Mrp4 (Fig. 3).

Histopathological Alterations

Characteristics H & E microphotos are given in Fig. 4. The livers of the mice that were orally given *S. mussotii* extract (SME 0.1, 0.3, 1.0 and 3.0 g/kg) for eight days were comparable to the controls. This indicates that no obvious liver pathology lesions were visible with the dosage

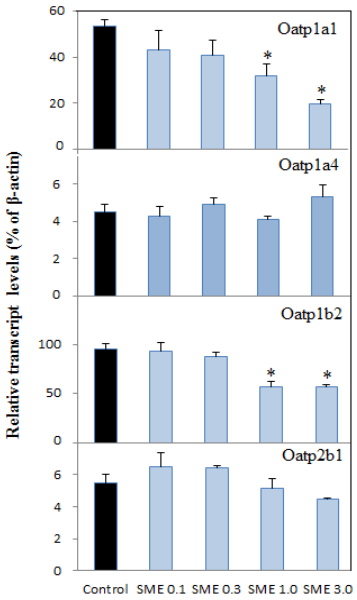

Fig. 2: Outcome of *S. mussotii* extract on the expression of uptake transporters in adult KM mouse livers. RT-PCR analysis the total RNA, which from the mice that were given *S. mussotii* extract orally at doses of 0.1 g/kg, 0.3 g/kg, 1.0 g/kg and 3.0 g/kg for 8 days. The data represents the mean ± SEM. ($P < 0.05$ vs control)

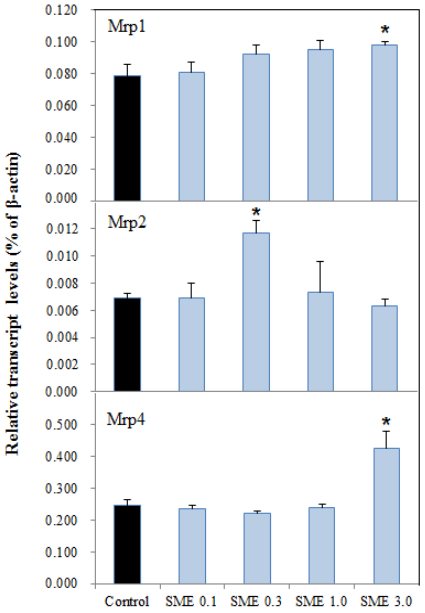

Fig. 3: Outcome of *S. mussotii* extract on the expression of efflux transporters in adult KM mouse livers. RT-PCR analysis the total RNA, which from the mice that were given *S. mussotii* extract orally at doses of 0.1 g/kg, 0.3 g/kg, 1.0 g/kg and 3.0 g/kg for 8 days. The data represents the mean ± SEM. ($P < 0.05$ vs control)

of the *S. mussotii* extract utilized in the current evaluation.

Discussion

Using herbs from natural sources as therapeutics has grown quickly (Malviya *et al.*, 2010). The current evaluation clearly shows that *S. mussotii* extract had a great impact on hepatic drug processing enzyme gene expression. It initiated CYP enzyme genes, had an impact on Sults and Ugts expression and elevated hepatic uptake and efflux transporter expression. Every one of these effects could have an integral part, and the outcomes from this systemic evaluation of the impact of *S. mussotii* extract on liver drug processing genes is pivotal when quantifying the specific bioactive molecule in the complex herbal extract.

S. mussotii extract has many substantial impacts on hepatic CYP450s. The hepatic CYP450 1 to 4 family gene expression levels we selected in the evaluation changed after the mice received *S. mussotii* extract for eight successive days. Cyp2b10 expression was elevated 2-fold at the largest dosage and Cyp3a11 expression was suppressed at all of the doses. This reinforced that the activation of Cyp genes is essential herb–drug interactions mechanization (Guengerich, 1997; Su *et al.*, 2007; Choi *et al.*, 2011). Besides the effects of *S. mussotii* extract on cytochrome P450s have been observed, the effects of extract on Phase 2 metabolism also have been studied for their importation for herb–drug interactions (Zamek-Gliszczynski *et al.*, 2006). The outcomes of this evaluation obviously showed that *S. mussotii* extract has a specific impact on the mRNA levels of the primary enzyme genes for sulfation conjugation-Sult1a1 at the largest dosage of 3 g/kg. In contrast, effect of *S. mussotii* extract on Ugt1a1 and Ugt1a6 was not obvious, and there was no relevant study to support our results.

The Nrf2/ARE antioxidant pathways are primary defense mechanisms against oxidative stress (Liu *et al.*, 2013), which causes cells to restore toxic stimuli. Nrf2 separates from its partner, Keap1 and then it initiates a group of cell defense genes, including Nqo1, one of the primary Nrf2 target genes (Itoh *et al.*, 1997; Ishii *et al.*, 2002). In this study, highest dose of *S. mussotii* extract increased the expression of Nqo1 2-fold, which imply the potential liver injury sides effects, meanwhile, the expression of Ho-1 was changed in livers of mice that were given orally *S. mussotii* extract (0.3 g/kg) indicative of the oxidative stress to the liver. As a sensitive biomarker for oxidative liver injury (Lu *et al.*, 2011), the level change of Nqo1 and Ho-1 all indicated the further studies are necessary to expound the effects.

Organic anion-transporting polypeptides are related to drug-drug interactions (Koenen *et al.*, 2011; Badolo *et al.*, 2013), for they are responsible for cellular influx of some xenobiotics and endogenous substances (Obaidat *et al.*, 2012; Gong and Kim, 2013). As a kind of membrane uptake transporters, they are known to play a crucial part in transporting substrates from the blood in to the liver (Evers and Chu, 2008). Oatp1a1 is important in liver bile acid

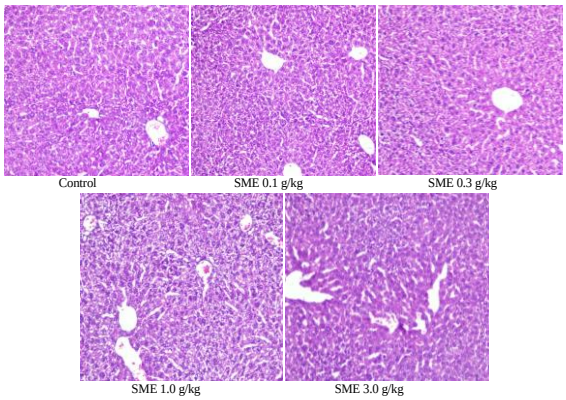

Fig. 4: Histopathological examination with representative photos from livers of mice orally administrated with distilled water (Control, 20 mL/kg) and *S. mussotii* extracts (SME 0.1, 0.3, 1.0 and 3.0 g/kg) for 8 days. Magnification: 200X

homeostasis and mainly expressed in liver and kidney (Cheng *et al.*, 2005; Fu *et al.*, 2012; Zhang *et al.*, 2012). The primary hepatic uptake transporter Oatp1b2 (Lu *et al.*, 2008) plays crucial roles in the hepatic uptake of therapeutics, environmental chemicals and toxicants (Meier-Abt *et al.*, 2004), as well as internal materials, such as bile acids (Csanaky *et al.*, 2011). In the present study, Oatp1a1 declined with an increase in dose, and similar decrease of Oatp1b2 was also evident, with over 40% decrease in SME 3 g/kg group compared to control group and the expression of Oatp1b2 was decreased at all doses of *S. mussotii* extract groups. These results distinctly proved the effect of *S. mussotii* extract on Oatp1a1, Oatp1a4, Oatp1b2 and Oatp2b1 and the effect on the hepatic uptake or ganicanion transporting peptides could be regarded as a basis of *S. mussotii* extracts – drug interactions.

ATP-dependent plasma membrane transporters named Mrps, are in charge of the removal of xenobiotics (Klaassen and Aleksunes, 2010). In the present study, we have examined the hepatic efflux transporters, Mrp1, Mrp2 and Mrp4, they are multidrug resistance-associated protein genes. As showed in Fig. 3, *S. mussotii* extract had prominent effects on the Mrp1 and Mrp4 mRNA levels, especially at dose of 3.0 g/kg. MRP1 has been demonstrated to extrude natural product drugs, *S. mussotii* extract as a natural product drugs, maybe Mrp1 extrude it in a cotransport for the result of its high expression level (Borst *et al.*, 1999; Kool *et al.*, 1999). Mrp4 has been considered as a detoxification protein though the normal expression of Mrp4 in the liver is low (Chen and Klaassen, 2004; Russel *et al.*, 2008), it is also called a versatile efflux transporter for drugs and toxicants (Borst *et al.*, 2007) by transport of structurally diverse endogenous and xenobiotic compounds (Russel *et al.*, 2008). The results of our study provided a clue that high dose of *S. mussotii* extract may have a side effect. Mrp2 is in charge of biliary removal, the flow of compounds into bile, and detoxification of internal and xenobiotic organic anions (Anne and Dietrich, 2007; Klaassen and Aleksunes, 2010). Mrp2 is part of the multidrug resistance protein subfamily restricted solely to the apical membrane domain of hepatocytes, and it is a crucial participant in herb-drug interactions. In this study, effect of *S. mussotii* extract on Mrp2 had no dose-effect relationship and it is contrary to the results of Mrp1 and Mrp4.

Conclusion

This study systemically substantiated that multidrug resistance-associated protein gene expressions and hepatic organic anion-transporting polypeptides may be stimulated by *S. mussotii* extract. The metabolism of *S. mussotii* extract requires activation of hepatic transporters, cytochrome P450 enzyme genes, and phase-2 enzyme genes. The impact of *S. mussotii* extract on drug processing genes may have an integral part in generating herb-drug interactions. Besides these, the results implied that traditional herb medicine, especially those based on experience and custom, may have potential side effects, which require our more attention and in-depth study.

Acknowledgments

This work was supported by the Key Laboratory Special Development Program of Qinghai Province (2017-ZJ-Y08) Qinghai Key Laboratory of Tibetan Medicine Pharmacology and Safety Evaluation.

References

Anne, T.N. and K. Dietrich, 2007. The apical conjugate efflux pump ABCC2 (MRP2). *Pflugers Arch. Eur. J. Physiol.*, 453: 643–659

Badolo, L., C. Bundgaard, M. Garmer and B. Jensen, 2013. The role of hepatic transport and metabolism in the interactions between pravastatin or repaglinide and two rOatp inhibitors in rats. *Eur. J. Pharm. Sci.*, 49: 767–772

Balasundari, P., S.K. Singh and S. Kavimani, 2005. Free radical scavenging of xanthones from *Swertia chirata* Buchham and tumor cell growth inhibition. *Main Group Chem.*, 4: 177–185

Borst, P., C. de Wolf and K. van de Wetering, 2007. Multidrug resistance-associated proteins 3, 4 and 5. *Pflugers Arch. Eur. J. Physiol.*, 453: 661–673

Borst, P., R. Evers, M. Kool and J. Wijnholds, 1999. The multidrug resistance protein family. *BBA-Biomembranes*, 1461: 347–357

Brahmachari, G., S. Mondal, A. Gangopadhyay, D. Gorai, B. Mukhopadhyay, S. Saha and A.K. Brahmachari, 2004. Swertia (Gentianaceae): chemical and pharmacological aspects. *Chem. Biodivers*, 1: 1627–1651

Chen, C. and C.D. Klaassen, 2004. Rat multidrug resistance protein 4 (Mrp4, Abcc4): molecular cloning, organ distribution, postnatal renal expression, and chemical inducibility. *Biochem. Biophys. Res. Commun.*, 317: 46–53

Cheng, H.L., Y.H. Li and Q.Y. Bian, 1999. Effects of Enzyme and morphological change of mangiferin on experimental liver damage in rats. *Chin. J. Lab. Anim. Sci.*, 9: 24–27

Cheng, X., J. Maher, C. Chen and C.D. Klaassen, 2005. Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps). *Drug Metab. Dispos.*, 33: 1062–1073

Choi, Y.H., Y.W. Chin and Y.G. Kim, 2011. Herb-drug interactions: focus on metabolic enzymes and transporters. *Arch. Pharm. Res.*, 34: 1843–1863

Csanaky, I.L., H. Lu, Y. Zhang, K. Ogura, S. Choudhuri and C.D. Klaassen, 2011. Organic anion–transporting polypeptide 1b2 (Oatp1b2) is important for the hepatic uptake of unconjugated bile acids: Studies in Oatp1b2-null mice. *Hepatology*, 53: 272–281

Evers, R. and X.Y. Chu, 2008. Role of the murine organic anion-transporting polypeptide 1b2 (Oatp1b2) in drug disposition and hepatotoxicity. *Mol. Pharmacol*., 74: 309–311

Fu, Z.D., I.L. Csanaky and C.D. Klaassen, 2012. Gender-divergent profile of bile acid homeostasis during aging of mice. *PLoS One*, 7: e32551

Gong, I.Y. and R.B. Kim, 2013. Impact of genetic variation in OATP transporters to drug disposition and response. *Drug Metab. Pharmacokinet*, 28: 4–18

Guengerich, F.P., 1997. Role of cytochrome P450 enzymes in drug–drug interactions. *Adv. Pharmacol*., 43: 7–35

Hase, K., Q. Xiong, P. Basnet, T. Namba and S. Kadota, 1999. Inhibitory effect of tetrahydroswertianolin on tumor necrosis factor-α-dependent hepatic apoptosis in mice. *Biochem. Pharmacol.*, 57: 1431–1437

Ishii, T., K. Itoh and M. Yamamoto, 2002. Roles of Nrf2 in activation of antioxidant enzyme genes via antioxidant responsive elements. *Method. Enzymol*., 348: 182–190

Itoh, K., T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh and M. Yamamoto, 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.*, 236: 313–322

Jarukamjorn, K., K. Don-in, C. Makejaruskul, T. Laha, S. Daodee, P. Pearaksa and B.O. Sripanidkulchai, 2006. Impact of Andrographis paniculata crude extract on mouse hepatic cytochrome P450 enzymes. *J. Ethnopharmacol.*, 105: 464–467

Karan, M., K. Vasisht and S.S. Handa, 1999. Antihepatotoxic activity of Swertia chirata on paracetamol and galactosamine induced hepatotoxicity in rats. *Phytother. Res.*, 13: 95–101

Kikuzaki, H., Y. Kawasaki, S. Kitamura and N. Nakatani, 1996. Secoiridoid glucosides from *Swertia mileensis*. *Planta Med.*, 62: 35–38

Klaassen, C.D. and L.M. Aleksunes, 2010. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol. Reveg.*, 62: 1–96

Koenen, A., H.K. Kroemer, M. Grube and H.E. Meyer zu Schwabedissen, 2011. Current understanding of hepatic and intestinal OATP-mediated drug–drug interactions. *Expert Rev. Clin. Phar.*, 4: 729–742

Kool, M., M. Van Der Linden, M. de Haas, G.L. Scheffer, J.M.L. De Vree, A.J. Smith, G. Jansen, G.J. Peters, N. Ponne, R.J. Scheper, R.P. Elferink, F. Baas and P. Borst, 1999. MRP3, an organic anion transporter able to transport anti-cancer drugs. *Proc. Natl. Acad. Sci. USA*, 96: 6914–6919

Kotoky, J. and P.N. Das, 2008. Medicinal plants used for liver diseases in some parts of Kamrup district of Assam, a North Eastern State of India. *Fitoterapia*, 79: 384–387

Lee, S.Y., J.Y. Lee, W. Kang, K.I. Kwon, S.K. Park, S.J. Oh, J.Y. Ma and S. K. Kim, 2013. Cytochrome P450-mediated herb–drug interaction potential of Galgeun-tang. *Food Chem. Toxicol.*, 51: 343–349

Li, J.C., L. Feng, B.H. Sun, T. Ikeda and T. Nohara, 2005. Hepatoprotective activity of the constituents in Swertia pseudochinensis. *Biol. Pharm. Bull.*, 28: 534–537

Liu, J., K.C. Wu, Y.F. Lu, E. Ekuase and C.D. Klaassen, 2013. Nrf2 protection against liver injury produced by various hepatotoxicants. *Oxid. Med. Cell. Longev*., Article ID: 305861

Liu, J., 2005. Oleanolic acid and ursolic acid: Research perspectives. *J. Ethnopharmacol.*, 100: 92–94

Liu, X.P., 2007. *Extract of Ginkgo Biloba Induces the Phase 2 Enzymes through Keap1-Nrf2-ARE Signaling Pathway*. Anhui Medical University, China

Lu, H., S. Choudhuri, K. Ogura, I.L. Csanaky, X. Lei, X. Cheng, P.Z. Song and C.D. Klaassen, 2008. Characterization of organic anion transporting polypeptide 1b2-null mice: essential role in hepatic uptake/toxicity of phalloidin and microcystin-LR. *Toxicol. Sci.*, 103: 35–45

Lu, Y.F., X.L. Wan, Y. Xu and J. Liu, 2013. Repeated oral administration of oleanolic acid produces cholestatic liver injury in mice. *Molecules*, 18: 3060–3071

Lu, Y.F., Q. Wu, S.X. Liang, J.W. Miao, J.S. Shi and J. Liu, 2011. Evaluation of hepatotoxicity potential of cinnabar-containing An-Gong-Niu-Huang Wan, a patent traditional Chinese medicine. *Regul. Toxicol. Pharm.*, 60: 206–211

Lv, P., L.X. Wei, Y.Z. Du, H.X. Yang and M. Peng, 2010. Hepatoprotective and toxic characteristics of the whole herb of traditional Tibetan folk medicine Swertia mussotii Franch. *J. Med. Plants Res.*, 4: 706–709

Malviya, N., S. Jain and S. Malviya, 2010. Antidiabetic potential of medicinal plants. *Acta. Pol. Pharm.*, 67: 113–118

Meier-Abt, F., H. Faulstich and B. Hagenbuch, 2004. Identification of phalloidin uptake systems of rat and human liver. *BBA-Biomembr*., 1664: 64–69

Obaidat, A., M. Roth and B. Hagenbuch, 2012. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. *Annu. Reveg. Pharmacol.*, 52: 135–151

Ramesh, N., M.B. Viswanathan, A. Saraswathy, K. Balakrishna, P. Brindha and P. Lakshmanaperumalsamy, 2002. Antimicrobial and phytochemical studies of *Swertia corymbosa*. *Fitoterapia*, 73: 160–164

Russel, F.G., J.B. Koenderink and R. Masereeuw, 2008. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. *Trends Pharmacol. Sci.*, 29: 200–207

Sadeghi, Z., S. Elmi, A. Elmi, M. Ghazi-Khansari, H. Hajimehdipoor, Y. Amanzadeh, S.E. and Sadat-Ebrahimi, 2006. Protective effects of Swertia longifolia Boiss. and its active compound, swerchirin, on paracetamol-induced hepatotoxicity in mice. *J. Pharm. Pharmacol.*, 58: 277–280

Saha, P., S. Mandal, A. Das, P.C. Das and S. Das, 2004. Evaluation of the anticarcinogenic activity of Swertia chirata Buch. Ham, an Indian medicinal plant, on DMBA-induced mouse skin carcinogenesis model. *Phytother. Res.*, 18: 373–378

Scartezzini, P. and E. Speroni, 2000. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.*, 71: 23–43

Sieniawska, E., T. Baj, J. Dudka, R. Gieroba, L. Swiatek, B. Rajtar and M. Polz-Dacewicz, 2013. Cytotoxicity, antioxidant activity and an effect on CYP3A4 and CYP2D6 of *Mutellina purpurea* L. extracts. *Food Chem. Toxicol*., 52: 188–192

Su, C.R., Y.F. Ueng, N.X. Dung, M. Vijaya Bhaskar Reddy and T.S. Wu, 2007. Cytochrome P3A4 inhibitors and other constituents of *Fibraurea tinctoria*. *J. Nat. Prod.*, 70: 1930–1933

Talalay, P., J.W. Fahey, W.D. Holtzclaw, T. Prestera and Y. Zhang, 1995. Chemoprotection against cancer by phase 2 enzyme induction. *Toxicol. Lett.*, 82: 173–179

Talalay, P., 2000. Chemoprotection against cancer by induction of phase 2 enzymes. *Biofactors*, 12: 5–11

Tripathi, R., H. Mohan and J.P. Kamat, 2007. Modulation of oxidative damage by natural products. *Food Chem.*, 100: 81–90

Wilkinson, J.T. and M.L. Clapper, 1997. Detoxication enzymes and chemoprevention. *P. Soc. Exp. Biol. Med.*, 216: 192–200

Yamahara, J., T. Konshima, T. Sawada and H. Fujimura, 1978. Biologically active principles of crude drugs: pharmacological actions of *Swertia japonica* extracts, swertiamarin and gentianine. *Yakugaku Zasshi*, 98: 1446–1451

Yang, H.X., C. Li, T.T. Gao, L.J. Geng, L.X. Wei and Y.Z. Du, 2016. Infrared spectroscopy analysis and pharmacological activity of ethanol extracts of wild and cultivated *Swertia mussotii* Franch. *Nat. Prod. Res. Dev*., 28: 112–119

Zamek-Gliszczynski, M.J., K.A. Hoffmaster, K.I. Nezasa, M.N. Tallman and K.L. Brouwer, 2006. Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. *Eur. J. Pharm. Sci.*, 27: 447–486

Zhang, Y., I.L. Csanaky, X. Cheng, L.D. Lehman-McKeeman and C.D. Klaassen, 2012. Organic anion transporting polypeptide 1a1 null mice are sensitive to cholestatic liver injury. *Toxicol. Sci.*, 127: 451–462

Zhang, Y. and B. Gordon, 2004. A strategy for cancer prevention: stimulation of the Nrf2–ARE signaling pathway. *Mol. Cancer Ther*., 3: 885–893

Zheng, L.S. and X.Q. Liu, 2009. Advances in the Research of Iridoids. *Natu. Pro. Res. Dev.*, 21: 702–711

(Received 13 November 2018; Accepted 23 November 2018)