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Hepatotoxicity induced by psoralen and isopsoralen from Fructus Psoraleae: Wistar rats are more vulnerable than ICR mice

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ABSTRACT

Fructus Psoraleae (FP) causes cholestatic liver injury; however, its main toxic constituents that are responsible for causing hepatotoxicity remained undetermined in previous studies. In the present study, psoralen and isopsoralen, the two main constituents of FP, were administered orally to rats (80 and 40 mg/kg, respectively) and mice (320 and 160 mg/kg, respectively) for 28 days, followed by biochemical and histopathological examinations to evaluate their hepatotoxicity. The results showed that psoralen and isopsoralen could induce the toxic reactions of liver and other organs in rats, while mice were not sensitive to these two compounds. Furthermore, the corresponding results indicated that administration of psoralen and isopsoralen repressed the expression of CYP7A1, BSEP, MRP2 and SULT2A1 and increased the expression of FXR and MRP3 in the rat liver. In summary, the toxic reactions of psoralen and isopsoralen are different in different species. In this study, multiple organ toxicity, such as cholestatic liver injury, occurs in rats, but not in mice. Psoralen and isopsoralen are the two main toxic constituents of FP. In addition, psoralen and isopsoralen cause liver injury, possibly through inhibiting bile acid excretion in the liver, leading to the accumulation of toxin in hepatocytes.

1. Introduction

In past decades, an increasing number of reports has been published concerning liver injury caused by traditional Chinese medicines and natural medicines. Fifty-seven different traditional Chinese medicine herbs and mixtures could induce liver injury (Teschke et al., 2015). The risk of liver injury caused by traditional Chinese medicines has attracted extensive attention at home and abroad (Bunchorntavakul and Reddy, 2013; Li et al., 2016). Fructus Psoraleae (FP) is a commonly used herbal medicine in Asian countries for the treatment of osteoporosis, vitiligo and other diseases (Chopra et al., 2013). However, there is a certain clinical risk during the application of FP, which could lead to cholestatic liver injury as supported by growing research evidence. For example, a Korean woman who drinks black tea containing FP was found to develop liver injury with symptoms such as elevated transaminase and liver damage (Nam et al., 2005). Some patients who had elevated levels of aminotransferases were reported to have acute hepatitis associated with the use of FP-related proprietary medicines in Hong Kong (Cheung et al., 2009). The Chinese Food and Drug Administration has issued that Chinese patent medicines, such as ZhuangGu GuanJie Pills and BaiShi Pills, which are produced with FP as the main ingredient, may induce side effects, such as liver injury (Cheng and Cai, 2000). Additionally, animal studies have shown that FP extract could cause damage to the liver and reproductive system (Tamotsu et al., 2002). Furthermore, FP could inhibit the expression of 7α-hydroxoylase (CYP7A1), bile-salt export pump (BSEP) and other bile

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSEP, bile-salt export pump; CAP, College of American Pathologists; CFDA, China's food and drug administration; CYP7A1, 7α-hydroxoylase; FP, Fructus Psoraleae; FXR, farnesoid X receptor; MRP2, multidrug resistance-associated protein 2; MRP3, multidrug resistance-associated protein 3; RT-PCR, quantitative real-time PCR; SULT2A1, sulfotransferase 2A1; TBA, total bile acid; TBIL, total bilirubin; γGT, γ-glutamyl transpeptidase

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transport-related proteins, as well as the expression of CYP450, resulting in cholestatic liver injury in the rats (Wang et al., 2012a).

Presently, more than 117 compounds have been isolated and identified from FP (Wei et al., 2018). Among them, psoralen and isopsoralen are the major bioactive components of FP, and their contents were 0.16–0.94% and 0.12–0.88%, respectively (Qiao et al., 2007). The total content of psoralen and isopsoralen from FP should be above 0.70% according to the China Pharmacopeia (2015 edition) (Commission, 2015). A previous research showed that both psoralen and isopsoralen are CYP3A4 inhibitors in *in vitro* study (Liu and Flynn, 2015). In addition, an *in vivo* study showed that psoralen and isopsoralen could induce liver damage by inhibiting cytochrome CYP450 in the mice and might be the two main toxic constituents (Wang et al., 2012b). However, the toxic degree of liver injury induced by psoralen and isopsoralen in mice did not match that induced by FP extract in rats (Wang et al., 2012a). Additionally, Diawara found that 8-methoxypsoralen and 5-methoxypsoralen could not significantly induce dose-dependent toxicities in male and female mice, suggesting that mice may be not suitable for the toxicity study of furocoumarins (Diawara et al., 2000). Thus, the toxicity experiments using these two compounds in mice were not entirely representative of cases. Therefore, in this study, psoralen and isopsoralen were administered orally to rats and mice to perform toxicological tests, including serum biochemistry and histopathology, to find a suitable animal system to reevaluate the toxicity of psoralen and isopsoralen.

In the light of abovementioned studies, biochemistry and histopathological analyses were carried out after 28 days of administration of psoralen and isopsoralen in rats and mice. It was found that psoralen and isopsoralen were prone to cause cholestatic liver injury in rats, accompanied by adrenal and male reproductive system injury. Our study confirmed that psoralen and isopsoralen could induce the toxic reactions of liver and other organs in rats, while mice were not sensitive to these two compounds. Additionally, the mRNA levels and protein contents of CYP7A1, BSEP, multidrug resistance-associated protein 2 (MRP2), sulfotransferase 2A1 (SULT2A1), farnesoid X receptor (FXR), and multidrug resistance-associated protein 3 (MRP3), which are related to the transportation, metabolism and excretion of bile acid in rat livers, were detected to investigate the toxicity mechanism of psoralen- and isopsoralen-induced cholestatic liver injury.

2. Materials and methods

2.1. Preparation of test substance

Psoralen and isopsoralen were extracted and isolated from FP, and their structures were confirmed by HRMS, ^{1}H and ^{13}C NMR. The purities of psoralen and isopsoralen were more than 99% by HPLC analysis (Fig. S1). The detailed procedure for the purification of these two compounds is described in the supplementary data. Psoralen, isopsoralen and Tween 80 were fully ground in a mortar. The sample was fully dissolved and was diluted to the certain concentration using 0.5% CMC-Na. Fresh samples were prepared once every day, and Tween 80 accounted for 10% of the total volume.

2.2. Experimental animals

This study was conducted at the Shanghai Institute for Food and Drug Control (SIFDC, Shanghai, China). All protocols were approved by the Institutional Animal Care and Use Committee of SIFDC (Approval No. 20180620). The Wistar rats and ICR mice were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. (License No. 20170005, Shanghai, China). Wistar rats and ICR mice were kept in a room maintained at 23 ± 2 °C, relative humidity of 40%–70%, under a 12 h light/dark cycle.

2.3. Experimental design

Wistar rats and ICR mice were fed a standard diet for 10 days to adapt to the environment before the experiments and then were divided into five groups randomly with a random distribution of body weight. Rats were orally administered with 0 (vehicle control), 40 or 80 mg of the test substance/kg bw/day. Mice were orally administered with 0 (vehicle control), 160 or 320 mg of the test substance/kg bw/day. Each group comprised 50% male and 50% female animals. The body weights of all animals were measured once a week, and the volume of the test substance administered to each animal was adjusted according to their body weight.

On the 28th experimental day, the animals were fasted for 18 h but with free access to water. The animals' blood was drawn via the abdominal aorta of each animal and was collected for serum biochemistry and histopathological examination.

2.4. Serum biochemistry

The serum samples of all animals were accumulated by centrifugation at 804 × *g* for 10 min at 4 °C (Hettich Rotanta 460R Centrifuge, Tuttlingen, Germany). The biochemistry of the serum samples was tested using a Hitachi 7060 Automatic Biochemical Analyzer (Naka, Japan).

2.5. Necropsy and histopathology analysis

Livers, adrenal glands and male reproductive organs were weighed. A complete standard set of tissues was preserved in neutral buffered formalin, and the male reproductive organs were preserved in Davison's fixative. The organ coefficients (organ weight × 100/body weight) were calculated. The samples of all organs were stored under −80 °C without repeated freeze-and-thaw steps. The organs and tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H & E) and Hall's stain (liver). The cryo-sectioned livers were washed with running tap water, rinsed with isopropanol and stained with Oil Red O mixed with isopropanol. Histological diagnosis (H & E staining) was performed according to the related protocol of the College of American Pathologists (CAP) (Creasy et al., 2010; Thoolen et al., 2010). Lipid droplets in cytoplasm were observed by oil red O staining. The contents of intracellular bile pigments were detected by Hall staining.

2.6. Immunohistochemistry

Immunohistochemistry staining for BSEP, MRP2, MRP3, SULT2A1, FXR and CYP7A1 was performed using the rat livers. The slide sections were treated with antibodies using commercial kits (XinYu Bio Tech Co., Ltd., Shanghai, China).

2.7. Quantitative real-time PCR

Total RNA was extracted from the rat livers of rats using Trizol (Takara, Shiga, Japan) according to the manufacturer's directions. Next, RNA was reverse transcribed using the PrimeScript RT reagent kit. Quantitative PCR was conducted using forward and reverse primers containing SYBER Green (Takara, Shiga, Japan). Real-time PCR was then performed using the StepOnePlus real-time PCR system. The primers used in the present study are listed in Table 1.

3. Results

3.1. Twenty-eight days toxicity study in rats and mice

3.1.1. Body weight

Compared with the control group, the body weight of rats in the experimental group decreased significantly ($P < 0.05$ or $P < 0.01$).

Table 1
Primer sequences of genes for RT-PCR.

Gene name	Primer sequence (5′-3′)
GADPH	F: ACAGCAACAGGGTGGTGGAC
	R: TTTGAGGGTGCAGCGACCTT
BSEP	F: CGTGCTTGTGGAAGAAGTTG
	R: GGGAGTAGATGGGTGTGACTG
MRP2	F: CCAATGTTTTGAATGCGGAG
	R: AGGATCGATGAGGTCACCATG
MRP3	F: CACCATCATCGTCATTCCTCT
	R: TAACTGGCAAACCTGATACGG
SULT2A1	F: ATCCGTGCCTGGCTGTCTAT
	R: GAGGACCAAATCCAGCTCATCT
FXR	F: AGGCCATGTTCCTTCGTTA
	R: TTCAGCTCCCCGACACTTTT
CYP7A1	F: TGGATCAAGTGCAACTGAATGAC
	R: GCACTGGAAAGCCTCAGAGC

Among them, the rats' body weight in the isopsoralen group were lower than those in the psoralen group ($P < 0.05$; Tables 3 and 3). Abnormal changes in the body weight of mice were not found in the dose-exposed group compared with that in the control group (Fig. S2).

3.1.2. Serum biochemistry

The levels of ALP, ALT, AST, γGT, TBA and TBIL were obviously elevated in male and female rats of all dose groups ($P < 0.05$ or $P < 0.01$). The rats treated with isopsoralen showed higher ALT, AST, γGT, TBA and TBIL levels than those treated with psoralen ($P < 0.05$ or $P < 0.01$; Fig. 1). During the experimental period, the variations of all biochemical indices of mice treated with psoralen and isopsoralen were not significant compared to those in the control group (Fig. S3).

3.1.3. Organ coefficients

A significant decrease was found in the organ coefficients of the prostate and seminal vesicle in male rats of all dose groups ($P < 0.05$ or $P < 0.01$; Fig. S5). The organ coefficients of liver and adrenal gland were remarkably elevated in male and female rats of all dose groups ($P < 0.01$ or $P < 0.05$; Fig. S5), especially, female rats treated with 80 mg/kg isopsoralen showed higher liver coefficients than those treated with 80 mg/kg psoralen ($P < 0.01$ or $P < 0.05$; Fig. 2). No obvious changes were found in the livers of male and female mice of all dose groups (Fig. S4).

3.1.4. Histopathology

Histopathological examination showed that hepatocytes were hypertrophic with hepatic focal necrosis in the male and female rats of all dose groups (Fig. 2). Moreover, the adrenal cortex became wider in all male and female rats of each dose group. The prostate and seminal vesicle were atrophied in all male rats of each dose group, and the intraluminal tissue fluid was also decreased (Fig. S5). In the dose-treated rats, increased lipid droplets and bile pigments were noted in the hepatocytes ($P < 0.01$ or $P < 0.005$). Among them, the isopsoralen group showed higher bile pigments than the psoralen group ($P < 0.05$ or $P < 0.01$; Fig. 3). Additionally, no abnormal alteration was found in mice in any of the dose groups on pathological examination (Fig. S4).

3.2. mRNA expression

To further investigate the toxic effects of psoralen and isopsoralen on livers of rats, we used quantitative real-time PCR (RT-PCR) assay to measure MRP3, FXR, BSEP, MRP2, SULT2A1 and CYP7A1 mRNA expression levels in livers. The RT-PCR results suggested that the expression levels of MRP3 and FXR mRNA were increased in the livers of all dose groups ($P < 0.05$ or $P < 0.01$), and the expression levels of BSEP, MRP2, SULT2A1 and CYP7A1 were decreased to some extent ($P < 0.05$ or $P < 0.01$; Fig. 4).

3.3. Immunohistochemical staining and quantitative data

To further confirm the psoralen- and isopsoralen-induced hepatotoxicity and cholestasis in rats, MRP3, FXR, BSEP, MRP2, SULT2A1 and CYP7A1 immunohistochemical staining in livers was performed. The immunohistochemical results suggested that significantly increases were found in the levels of MRP3 and FXR in the liver of dose groups ($P < 0.05$ or $P < 0.01$), and the levels of BSEP, MRP2, SULT2A1 and CYP7A1 in the liver were decreased to some extent ($P < 0.05$ or $P < 0.01$; Figs. 5 and 6).

4. Discussion

Liver is an important organ participating in drug metabolism, and DILI may be induced due to the direct toxicity or heterogeneous reactions of drugs or their metabolites to the liver (Stephens et al., 2014). Previous epidemiological studies have documented that nearly half of DILI was cholestatic liver injury caused by drug induced biliary dysfunction (Tajiri and Shimizu, 2008). It was highly complex with respect to the chemical constituents and pharmacological activities of Chinese medicines. Long-term and large-dose medication of Chinese medicines could induce liver injury and even hepatic carcinoma, such as *GuanMuTong*, *GuangFangJi* and *MaDouLing* (Ma et al., 2014).

FP has various pharmacological effects; however, the long-term use of FP and its related preparations could cause liver injury manifested as cholestasis (Cheung et al., 2009). Psoralen and isopsoralen, two furocoumarins, are the main active constituents of FP, both of which are possibly correlated with FP-induced cholestatic liver injury (Wang et al., 2012b). Furocoumarins produced obvious pathological changes, suggesting that some early events leading to hepatic injury after furocoumarins are administered to rats (Uehara et al., 2008). In this study, psoralen and isopsoralen were administered to rats at the dosages of 80 and 40 mg/kg, respectively. After administration, the weight of rats in the administration group was decreased significantly (Tables 2 and 3), and the liver coefficients was increased remarkably (Fig. 2). The weight loss in the male rats was less obvious than that in female rats when

Table 2
Summary of body weight (g) in male rats ($n = 6$).

Days	Control	Psoralen 80 mg/kg	Psoralen 40 mg/kg	Isopsoralen 80 mg/kg	Isopsoralen 40 mg/kg
1	210.42 ± 11.58	220.35 ± 2.11	210.23 ± 6.41	213.70 ± 9.84	214.99 ± 9.00
3	225.67 ± 12.47	227.45 ± 3.89	230.98 ± 21.23	209.97 ± 7.59$^{*\#}$	217.78 ± 11.25$^{\Delta}$
7	230.67 ± 12.47	232.45 ± 3.89	235.98 ± 21.23	214.97 ± 7.59$^{*\#}$	222.78 ± 11.25
14	262.73 ± 13.16	257.02 ± 9.87*	258.72 ± 6.67*	234.45 ± 8.31$^{**\#}$	244.95 ± 17.58
21	283.2 ± 11.90	257.33 ± 6.95*	270.93 ± 4.96	236.82 ± 7.29**	243.77 ± 14.33$^{*\Delta}$
28	300.20 ± 11.9	258.37 ± 5.84**	279.20 ± 6.48	236.57 ± 11.01$^{**\#}$	253.77 ± 14.33$^{**\Delta}$

Data were expressed as mean ± SD. * A significant difference at $P < 0.05$ level compared with the control. ** A significant difference at $P < 0.01$ level compared with the control. $^{\#}$ A significant difference at $P < 0.05$ level compared with the 80 mg/kg psoralen group. $^{\Delta}$A significant difference at $P < 0.05$ level compared with the 40 mg/kg psoralen group.

Table 3
Summary of body weight (g) in female rats ($n = 6$).

Days	Control	Psoralen 80 mg/kg	Psoralen 40 mg/kg	Isopsoralen 80 mg/kg	Isopsoralen 40 mg/kg
1	201.82 ± 4.87	198.62 ± 7.31	199.35 ± 4.19	200.78 ± 10.18	197.47 ± 9.21
3	199.82 ± 5.31	191.25 ± 11.79*	195.37 ± 3.05	190.97 ± 10.59*	191.62 ± 8.62**
7	204.82 ± 5.31	196.25 ± 11.70*	200.37 ± 2.04	195.97 ± 10.09**	196.62 ± 8.02*
14	204.93 ± 4.54	193.67 ± 8.59*	197.97 ± 5.36	192.77 ± 12.22*	191.35 ± 7.93**
21	209.47 ± 5.12	196.27 ± 10.10*	204.20 ± 3.23	190.17 ± 9.83**	200.03 ± 4.01*
28	216.47 ± 5.12	199.12 ± 206.78*	204.20 ± 3.23*	190.17 ± 9.83**#	200.03 ± 4.01*

Data were expressed as mean ± SD. * A significant difference at $P < 0.05$ level compared with the control. ** A significant difference at $P < 0.01$ level compared with the control. # A significant difference at $P < 0.05$ level compared with the 80 mg/kg psoralen group.

treated with the same dose. Additionally, there were obviously increased trends in the levels of ALT, AST, ALP, γGT, TBA and TBIL (Fig. 1), as well as in the number of hypertrophic hepatocytes (Fig. 2). Point necrosis of hepatocytes occurred occasionally in the 80 mg/kg group (Fig. 2). Additionally, the cortex of the adrenal gland was widened in all rats of each dose group, accompanied by seminal vesicle and prostate atrophy in all male rats of each dose group (Fig. S5). Nevertheless, these toxic reactions were not observed in mice administered with psoralen and isopsoralen at the dosages of 320 and 160 mg/kg (Figs. S2–4). These results suggest that, compared with mice, rats were more sensitive to psoralen- and isopsoralen-induced hepatotoxicity. The kinetics of o-hydroxyphenylacetaldehyde (o-HPA) detoxification was metabolic activation as the major determinant of species differences in coumarin-induced hepatotoxicity (Vassallo et al., 2004). However, the rat appears to be the most susceptible to this toxicity; mice are generally resistant.

Furthermore, isopsoralen was found to be more toxic than psoralen. It was previously reported that isopsoralen showed more cytotoxic than psoralen in *in vitro* experiments (Wang et al., 2015; Zhou et al., 2015), but its mechanism needed to be further investigated. Additionally, the female rats had more serious liver damage than the male rats. Previous studies showed sexually dimorphic responses of drug metabolizing enzymes in the liver, which could be linked to the increased sensitivity of females to drugs and xenobiotics (Campesi et al., 2013). Besides, FP-induced clinical adverse response mainly happened in women (Cheung et al., 2009; Nam et al., 2005). The sexual dimorphism of psoralen- and isopsoralen-induced toxicity, however, remains to be further explored.

Both biochemical and histopathological examinations indicated that psoralen and isopsoralen could cause cholestatic liver injury (Figs. 1–3). Clinical reports and experimental studies confirmed that hepatoxicity could lead to hepatocellular damage and reduced bile flow, especially, cholestasis would be reflective of the primary event leading to hepatoxicity (Humbert et al., 2012; Ozer et al., 2008). Moreover, the direct impact of chemicals or medicines on bile acids homeostasis, by whatever mechanisms, could lead to an increase in the intracellular concentrations of toxic bile acids (Vaz and Ferdinandusse, 2017). Bile acids are conjugated with taurine or glycine in the liver, and the sodium and potassium salts of these conjugated bile acids are bile pigments (Chiang, 2009). In this study, HE staining, oil red staining and Hall staining showed that hepatocytes were enlarged in the administration groups of psoralen and isopsoralen, and the enlarged hepatocytes contained a large amount of bile pigments and lipids, and even hepatic focal necrosis (Figs. 2 and 3). Therefore, additional studies are required to elucidate the transportation of bile acids in psoralen- and isopsoralen-induced cholestatic liver injury of rats.

BSEP is the driving force of bile pigment enterohepatic circulation,

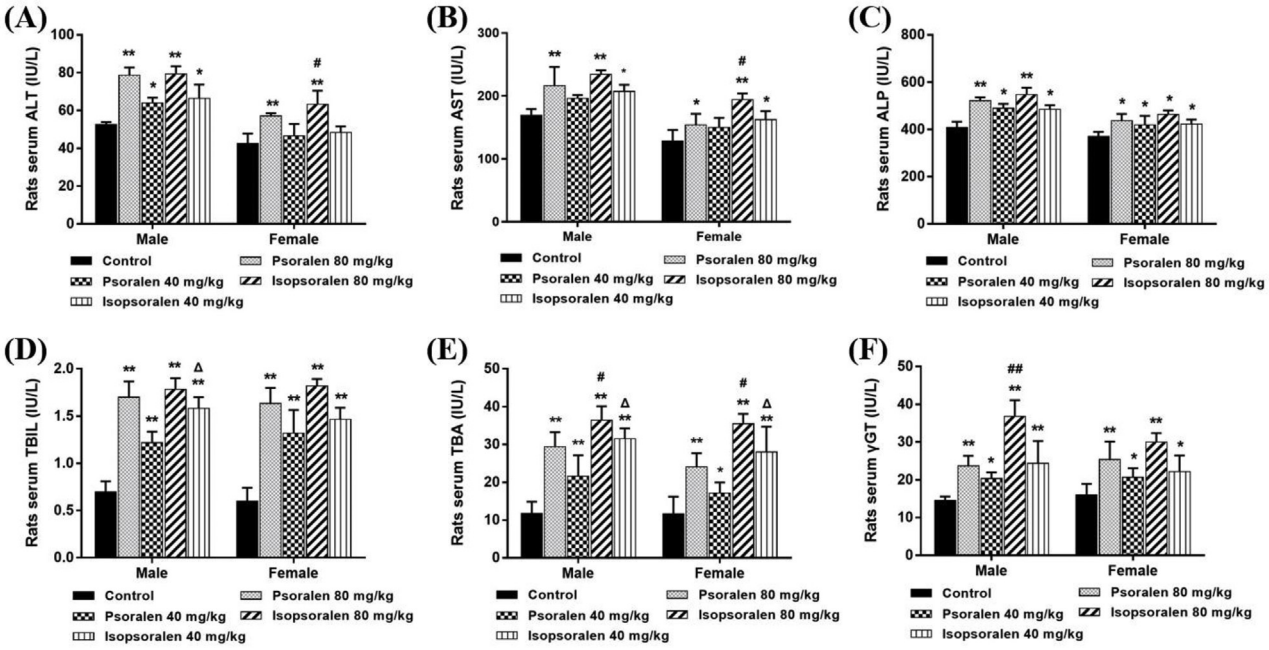

Fig. 1. Effects of psoralen and isopsoralen on the serum biochemistry in rats ($n = 6$). (A) ALT, (B) AST, (C) ALP, (D) TBIL, (E) TBA and (F) γGT. Symbols and bars represent the means ± SEM ($n = 6$). * A significant difference at $P < 0.05$ compared to the control. ** A significant difference at $P < 0.01$ compared with the control. # A significant difference at $P < 0.05$ compared with the 80 mg/kg psoralen group. ## A significant difference at $P < 0.01$ compared with the control. Δ A significant difference at $P < 0.05$ compared with the 40 mg/kg psoralen group.

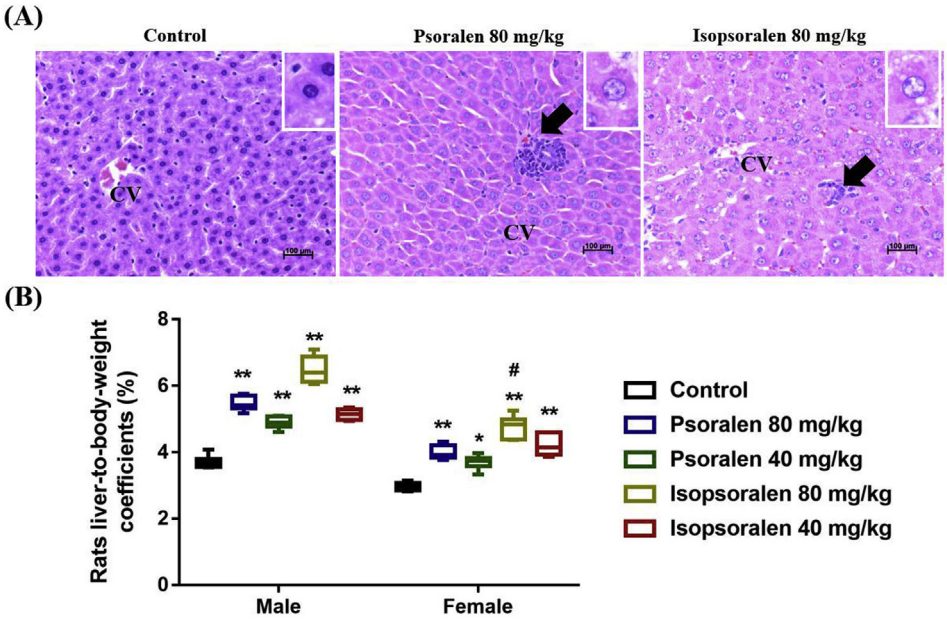

Fig. 2. Effects of psoralen and isopsoralen on the livers for 28 days. (A) Histopathological changes in the livers of rats stained with H & E. CV, central veins; Arrows, hepatic focal necrosis. (B) Organ coefficients liver in rats of all dose groups. Symbols and bars represent the means ± SEM (n = 6). * A significant difference at $P < 0.05$ compared to the control. ** A significant difference at $P < 0.01$ compared with the control. # A significant difference at $P < 0.05$ compared with the 80 mg/kg psoralen group.

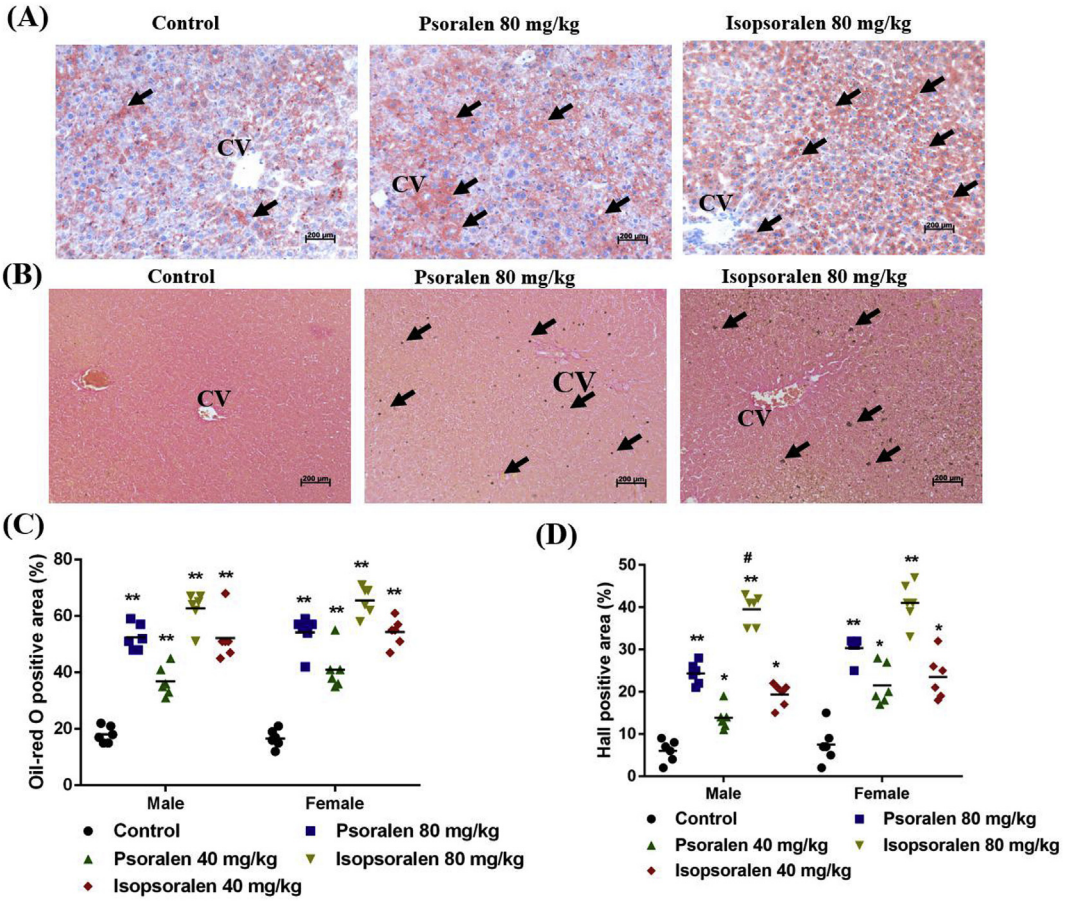

Fig. 3. Representative images of liver samples of rats stained with Oil Red O and Hall. Histopathological changes in the liver stained with (A) Oil Red O and (B) Hall, and lipid droplets and bile pigments were quantified in the (C) and (D), respectively. CV, central veins; Arrows, positive positions. Symbols and bars represent the means ± SEM (n = 6). * A significant difference at $P < 0.05$ compared with the control. ** A significant difference at $P < 0.01$ compared with the control. # A significant difference at $P < 0.05$ level compared with the 80 mg/kg psoralen group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

which could mediate the secretion of monovalent bile acid into the bile duct in hepatocytes and stimulates the formation of bile pigment-dependent bile flow (Stieger, 2010). MRP2 is the only transporter on the bile capillary surface of hepatocytes, which transports conjugated bilirubin from hepatocytes to the bile ducts (Soroka, 1997). MRP3 is a discharge pump on the hepatocyte membrane, which is mainly

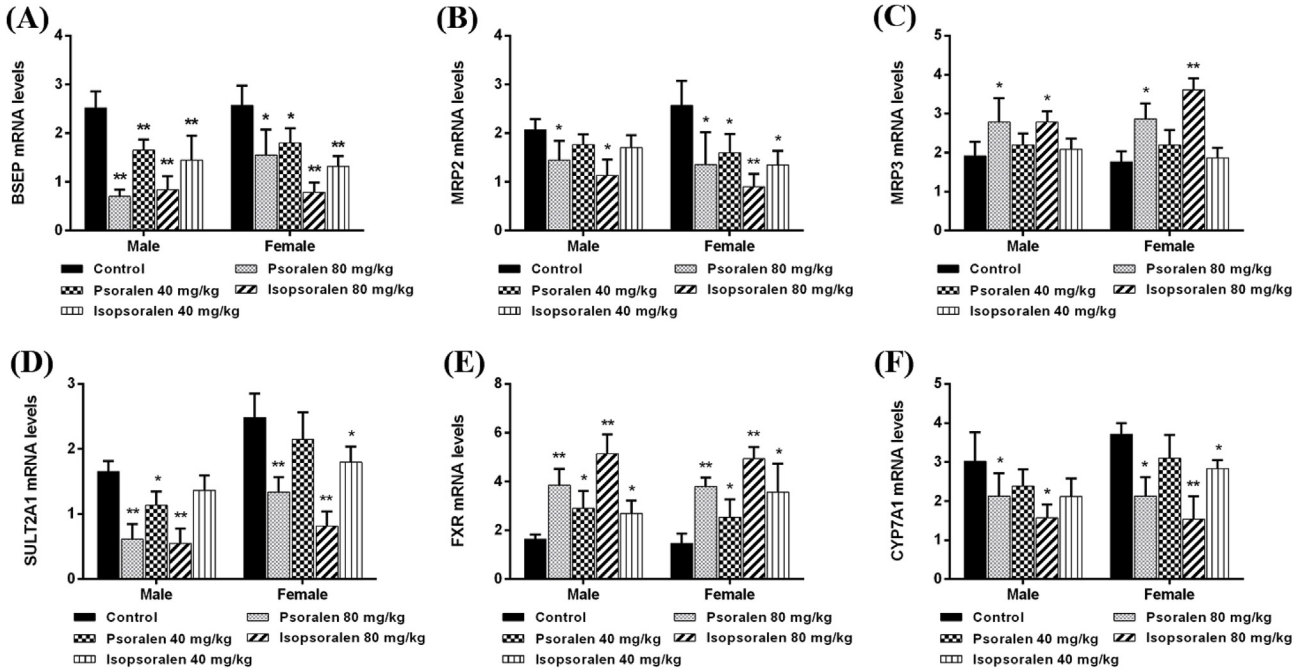

Fig. 4. Effects of repeated psoralen and isopsoralen administration on the mRNA expression levels of (A) BSEP, (B) MRP2, (C) MRP3, (D) SULT2A1, (E) FXR, and (F) CYP7A1 in the livers of rats. Symbols and bars represent the means ± SEM ($n = 6$). * A significant difference at $P < 0.05$ compared with the control. ** A significant difference at $P < 0.01$ compared with the control.

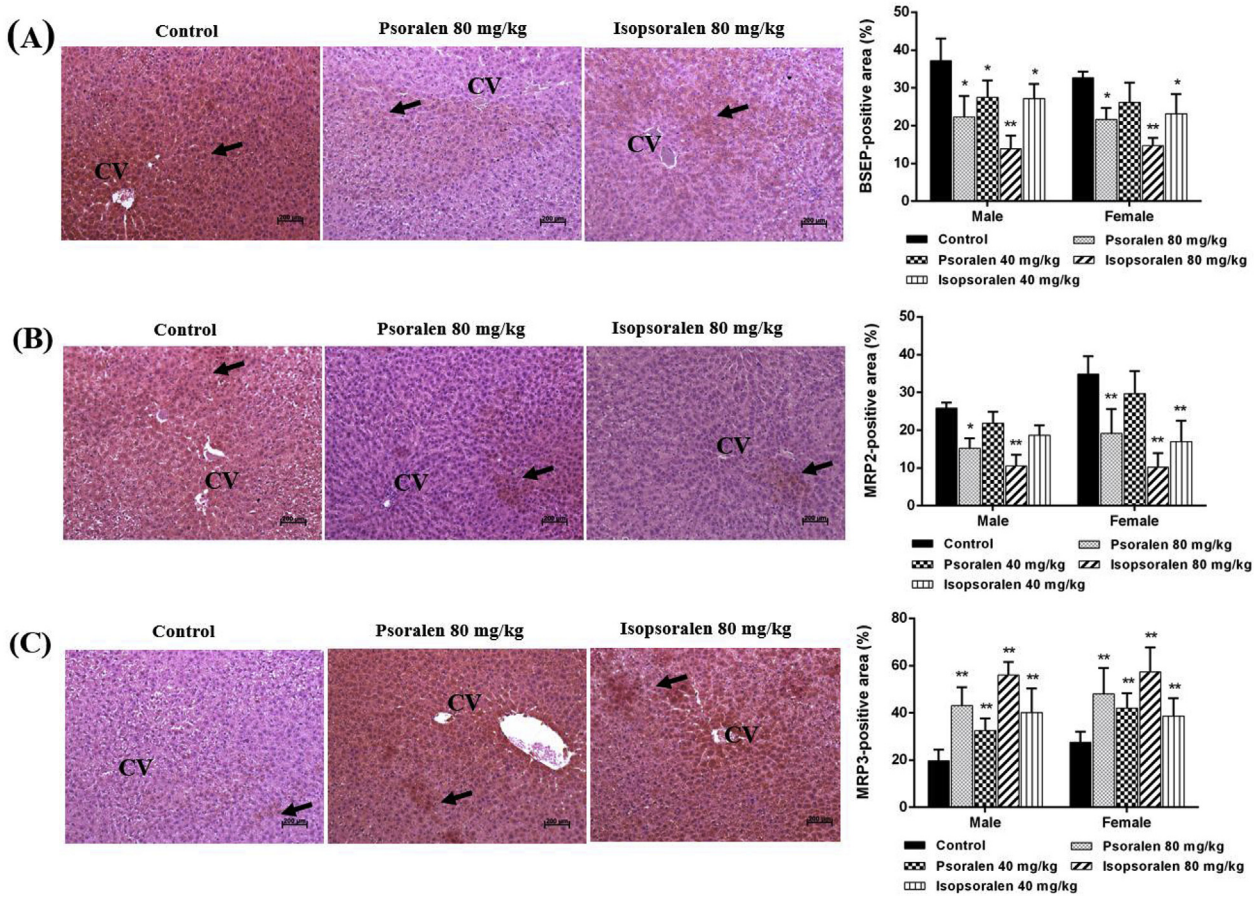

Fig. 5. Immunohistochemical staining and quantitative data for (A) BSEP, (B) MRP2, and (C) MRP3 of livers in rats. CV, central veins; Arrows, positive positions. Symbols and bars represent the means ± SEM ($n = 6$). * A significant difference at $P < 0.05$ compared with the control. ** A significant difference at $P < 0.01$ compared with the control.

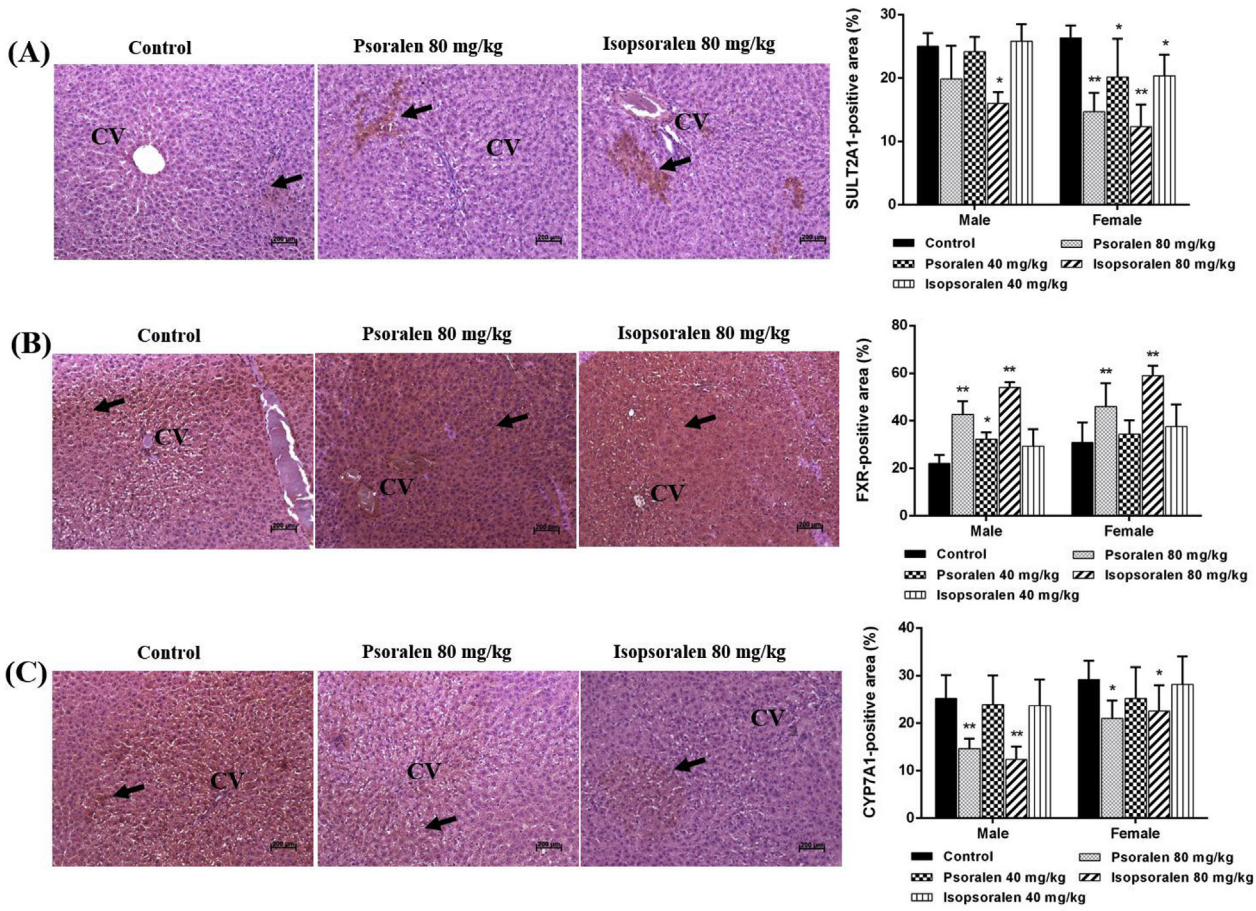

Fig. 6. Immunohistochemical staining and quantitative data of the livers for (A) SULT2A1, (B) FXR, and (C) CYP7A1 in rats. CV, central veins; Arrows, positive positions. Symbols and bars represent the means ± SEM ($n = 6$). * A significant difference at $P < 0.05$ compared with the control. ** A significant difference at $P < 0.01$ compared with the control.

responsible for the transportation of bile pigments, conjugated organic anions and direct bilirubin from hepatocytes to sinusoids (Lee et al., 2004). In this study, the expression levels of BSEP and MRP2 were decreased by psoralen and isopsoralen, suggesting the inhibition of bile acid excretion. Meanwhile, the expression level of MRP3 was up-regulated (Figs. 4 and 5), which could absorb bile pigments from bile back into the portal vein to reduce its deposition in the liver. It was also one of the reasons to cause the increase in the serum TBIL of rats (Fig. 1).

Bile acids are converted from cholesterol and could cause the entry of excessive cholesterol from the liver to the bile. It was generally believed that the main pathway of bile acid synthesis was cholesterol CYP7A1-mediated cholesterol-to-bile acid conversion in the liver, in which CYP7A1 was the rate-limiting enzyme for bile acid synthesis (Liu et al., 2014). This process was the main approach of cholesterol metabolism in the liver, which was negatively regulated by bile acids. Moreover, the expression and activity of CYP7A1 in the liver are inhibited by the feedback of serum and intestinal bile acids. When bile acid excretion was blocked and serum bile acid concentration was increased, FXR in hepatocytes could be activated to inhibit the transcription of the rate-limiting enzyme CYP7A1 in bile acid synthesis, resulting in a decrease in the bile acid synthesis rate (Kong et al., 2012). Significantly, the negative feedback regulation of bile acids by CYP7A1 could ensure the control of overall bile acid production strictly. Therefore, the inhibition of CYP7A1 expression and the up-regulation of FXR in the psoralen- and isopsoralen-treated groups (Figs. 4 and 6) were deduced to be a compensatory reaction.

Prior studies have demonstrated that furocoumarins could exert an inhibitory role in the activity of bile metabolic enzymes (Fukuda et al., 1997). SULT2A1 is the main metabolic enzyme that detoxifies bile acids in the body (Duanmu et al., 2002). Our study revealed that psoralen and isopsoralen could inhibit the expression levels of SULT2A1 (Figs. 4 and 6), further suppressing the glucosidation and sulfation of hydroxylated bile acids (Huang et al., 2010). Thus, bile acids could not be converted into glycol- or tauro-conjugated bile acids in time which may cause hepatotoxicity owing to the accumulation of intracellular bile acids (Yang et al., 2015).

The excessive cholestasis in the liver could lead to the accumulation of intracellular cytotoxic factors, such as bile acids, thereby activating the JNK pathway (Krajarng et al., 2015). Subsequently, highly activated JNK could induce the mitochondrial release of pro-apoptotic cytochrome C and activation of caspase-9 and caspase-8 to induce cell death (Saberi et al., 2014). Intrahepatic cholestasis is generally caused by the accumulation of bile acids at a high concentration in hepatocytes and blood under the circumstance of dysfunctional bile acid synthesis, absorption and excretion (Tanaka et al., 2009). Intrahepatic cholestasis is characterized by bile regurgitation into the blood or accumulation in extrahepatic tissues, resulting in systemic injury symptoms, subsequent liver fibrosis with the continuous development of the disease and liver failure.

5. Conclusion

In summary, the present study reported, for the first time, that psoralen and isopsoralen, the main constituents of FP, could induce the toxic reactions of liver and other organs in rats, while mice were not

sensitive to these two compounds. Simultaneously, for the first time, our study discovered that psoralen and isopsoralen could also cause adrenal gland, seminal vesicle and prostate damage. The primary cause of liver injury may lie in that psoralen and isopsoralen inhibits bile acid excretion in the liver, leading to the accumulation of toxins in hepatocytes. However, it remains to be further explored concerning the mechanism of psoralen- and isopsoralen-induced toxicities of the liver and other organs.

Conflicts of interest

The authors have declared that there is no conflict of interest.

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Appendix A. Supplementary data

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Transparency document

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