Acupuncture and Herbal Medicine

The correlation between chemical ingredients and acute toxicity of Psoraleae Fructus and two classic prescriptions

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Abstract

Objective: To compare the acute toxicity and chemical ingredients of Psoraleae Fructus (PF) with those of two classic prescriptions, Ershen Wan (ESW) and Sishen Wan (SSW).

Methods: Based on classical toxicological methods, body weight, food and water consumption, lethal conditions, and toxic reactions were recorded after administering single oral doses of PF, ESW, and SSW. The 50% lethal dose (LD_{50}) values of PF and ESW and the maximum tolerance dose (MTD) of SSW were determined. In addition, PF, ESW, and SSW constituents were detected using ultra-high-performance liquid chromatography/mass spectrometry (UPLC-MS), and the spectrum-toxicity correlation was analyzed.

Results: The LD_{50} of PF and ESW were 53.9 g/kg/day (46.2–63.0 g/kg/day, 95% confidence limit [CL]) and 68.3 g/kg/day (59.0–78.9 g/kg/day, 95% CL), which were respectively about 40 and 50 times the human daily dosage. The MTD of SSW was 41.0 g/kg/day, indicating the highest safety. What can be inferred from the chemical ingredients and toxicity correlation analyses is that compatibility reduced the contents of 13 potential hepatotoxin compounds in PF.

Conclusions: The classic compatibility of ESW and SSW effectively attenuated the hepatotoxicity of PF, which was related to the reduced content of potentially toxic substances, particularly coumarins. This study explored the principles of attenuating the toxicity of classic prescriptions to provide a reference for the rational clinical use of PF.

Keywords: Ershen Wan, Liver injury, Psoraleae Fructus, Sishen Wan, Toxicology

Graphical abstract: http://links.lww.com/AHM/A111

Introduction

In Chinese, the dried fruit of *Psoralea corylifolia* L. is known as Psoraleae Fructus (PF) and is a crucial component of traditional medicine. The potential therapeutic effects of PF include reinforcing the kidneys and strengthening *yang*, alleviating chronic diarrhea, and enhancing bone marrow. Owing to its historical reputation as a non-toxic remedy, PF has been included in numerous traditional Chinese medicine (TCM) prescriptions and is thus widely utilized in TCM[1]. Nevertheless, recent reports on liver injury associated with PF preparations have raised concerns[2–6]. Consequently, there is an immediate need to identify the potentially toxic constituents within PF and develop strategies to ensure the safe utilization of PF-related preparations[7,8].

Compatibility is a fundamental principle in TCM theory, encompassing synergistic effects and detoxification compatibility. Numerous PF-related treatment prescriptions demonstrate its compatibility with other documented herbs in TCM literature that are extensively utilized in clinical practice. Ershen Wan (ESW), a medicinal combination of PF and Myristicae Semen (MS) derived from the dried kernel of *Myristica fragrans* Houtt, is a well-established remedy for ailments such as diarrhea caused by spleen-kidney Yang deficiency[9–11]. Subsequently, Chinese physicians incorporated *Schisandrae chinensis Fructus* (SCF) and *Euodiae Fructus* (EF) into this formulation and named it Sishen Wan (SSW), a Chinese patent medicine on the National Essential Medicine List with a long history of clinical use and minimal reports of adverse reactions[12–14]. To address the concern of understanding the extent of ESW

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and SSW mitigation of PF toxicity, acute toxicity experiments were conducted to gather preclinical safety data. The chemical compositions of PF and the two preparations were analyzed to screen for potentially toxic substances in PF, thereby providing valuable references for safe use.

Materials and methods

Preparation of PF and two prescriptions

Professor Yue Gao identified the PF (Lot: 19072501), MS (Lot: 19070203), SCF (Lot: 21082801), and EF (Lot: 21030801), which were purchased from Beijing Lvye Pharmaceutical Co., Ltd., China. The inspection was conducted according to the specifications for medicinal materials and met all requirements. A quantity of 1 kg of PF was subjected to two rounds of decoction at a temperature of 100°C using 8 L of water, with each round lasting for 1 h. After cooling and filtration, the resultant aqueous extract was of maximum concentration.

SSW was prepared according to the medicinal ratio outlined in the China Pharmacopoeia (PF: MS: SCF: EF, 4:2:2:1), maintaining the proportions of PF and MS in ESW at 2:1. Each medicinal material was precisely weighed and subjected to two rounds of decoction using eight times the volume of water, with each round lasting for 1 h. The liquid was filtered, combined, and concentrated to the desired concentration.

Chemical analysis of three extracts

The constituents were comprehensively detected, followed by characterization of the detected compounds and quantification to rank them. Pre-analytical information obtained through literature mining was employed to identify the constituents of the candidate compounds, including their names, molecular prescriptions, and structures, using UPLC-MS accurate chromatography-mass analysis of PF. The identified constituents were characterized by comparison with the corresponding reference compounds, considering factors such as chromatographic retention time, accurate molecular mass/ionization patterns, and fragmentation profiles. Calibration was performed using the respective reference compounds (if available) or a structurally similar reference compound to quantify the characterized constituents. Alternatively, the detected constituents were compared with those from the literature-mined data.

The extract was accurately weighed, a 50% methanol solution was added twice for ultrasonic extraction, the samples were centrifuged at 4,000 rpm for 10 min, and the volume of the supernatant was adjusted to 25 mL. The 1.7 μm Waters Acquity HSS T3 column (100 mm × 2.1 mm i.d.; maintained at 45°C) was used for the chromatographic column. Mobile phase A consisted of 0.01% formic acid in water, while methanol with 0.01% formic acid comprised mobile phase B. The flow rate was set to 0.30 mL/min, the sample injection size was 5 μL, and the chromatographic gradient elution conditions were as follows: 0 to 2 min, 98% (A); 2 to 32 min, 98% to 2% (A); 32 to 37 min, 2% (A) and 37 to 42 min, 98% (A). The nontargeted primary mass spectrometric detection conditions were as follows: mass range of 50 to 1,500 *m/z*; source temperature of 120°C; cone gas flow of 50 L/h; desolvation temperature of 450°C and desolvation gas flow of 800 L/h; electrospray capillary voltage of 2.5 kV in negative ionization mode and 3.0 kV in positive ionization mode.

Experimental animals

KM mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) with the animal license number SCXK (Beijing) 2021-0011. The mice were housed in cages under a 12:12 hour light/dark cycle with commercial food pellets and water available *ad libitum*. The animals were allowed 5 days of acclimatization before the commencement of the experimental procedures.

Pre-test of acute toxicity

Half of the male and half of the female mice, weighing 18 to 22 g, were randomly divided into normal control (Con), PF, ESW, and SSW groups. After fasting for 12 h before the experiment, mice in the drug group were administered 25 mL/kg by gavage, and those in the normal Con group were administered the same volume of water by gavage. The daily dose of PF in humans is 8 g, equivalent to 1.4 g/kg in mice. The LD_0 value (0% mortality), LD_{100} value (100% mortality), and interval *r* value of the corresponding dose groups were determined according to the cause of death situation of the mice. The acute toxicity of SSW in mice indicated that the 50% lethal dose (LD_{50}) value could not be measured; therefore, the maximum tolerance dose (MTD) value was determined.

Acute toxicity

In total, 160 mice were randomly allocated to separate groups with an equal distribution of males and females. Acute toxicities were assessed in accordance with the principles outlined in the Organisation for Economic Co-operation and Development (OECD) guidelines[13], and the LD_{50} and 95% confidence limits were calculated using the bliss method. PF, ESW, and SSW were administered to the mice *via* gavage at a single dose per body weight in a volume of 25 mL/kg, following a fasting period of 12 h. The acute oral toxicity of SSW was evaluated in mice using the MTD. The crude PF content in the ESW and SSW groups was consistent with that in the PF group. The mice in the Con group were administered saline only. Any deviations in overall behavior were observed for 4 h after dosing, followed by daily monitoring for 14 consecutive days. Any significant adverse reactions, including hunched posture, weight loss, squinted eyes, ocular discharge, paralysis, dehydration, limping, sluggish movements, rough hair coating, lack of eating or drinking, poor grooming, and awkward gait, were recorded as instances of morbidity. Mice that exhibited major adverse reactions were euthanized humanely. Body weights were measured before and after administration on d 0, 1, 3, 7, and 14, while food intake and water consumption were measured on days 1, 7, and 14. This study was conducted according to the National Research Council Guide for the Care and Use

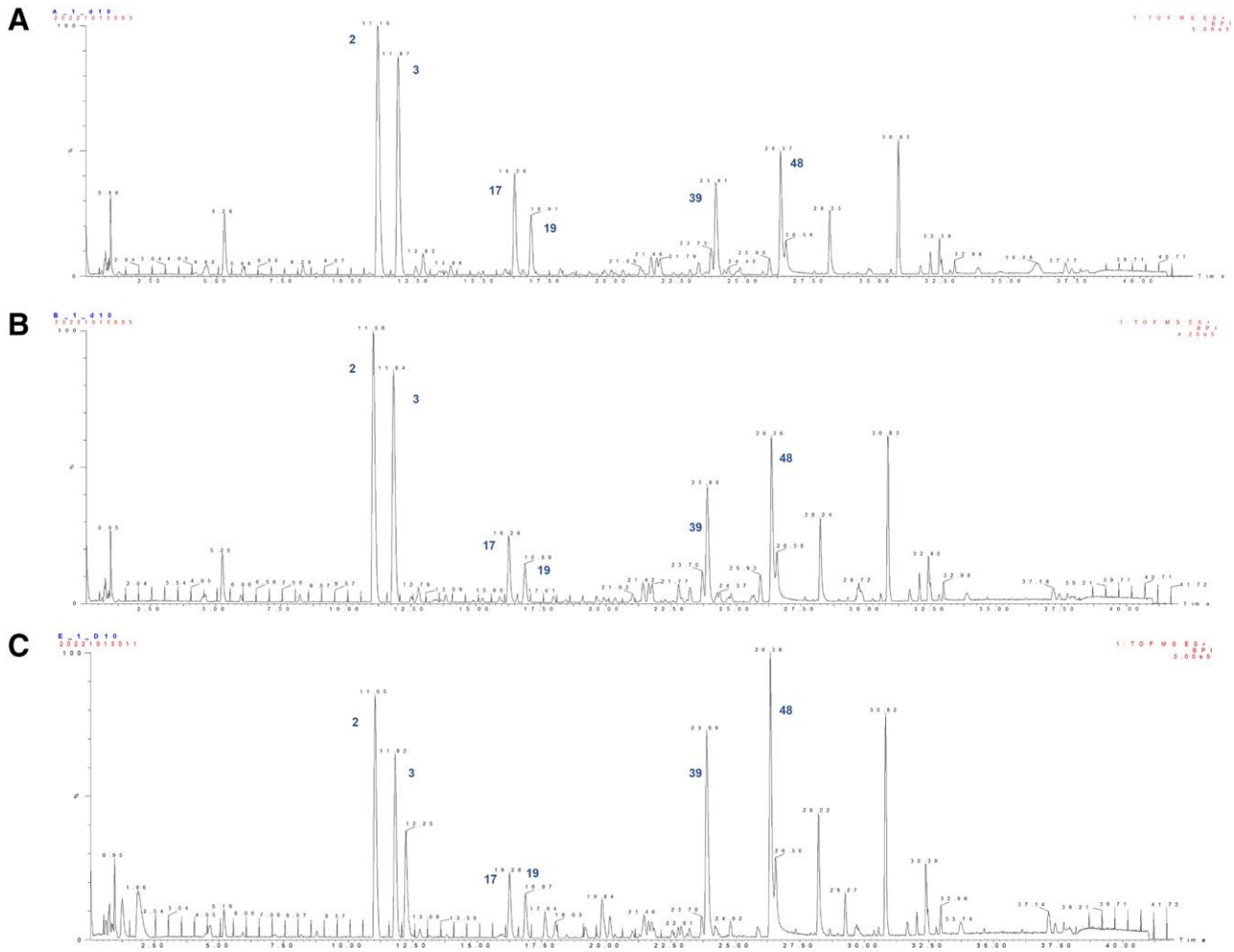

Figure 1. The liquid chromatograms of compounds in PF (A), ESW (B), and SSW (C). ESW: Ershen Wan; PF: Psoraleae Fructus; SSW: Sishen Wan.

of Laboratory Animals. The protocol was approved by the Animal Care and Use Committee of the Laboratory Animal Center of the Academy of Military Medical Sciences (Permit Number: IACUC-DWZX-2021-728).

Histopathology evaluation

At the end of the experiment, surviving mice were euthanized for a gross anatomical check to detect obvious changes in the major organs. The kidneys, liver, and spleen were excised and weighed to determine their relative weights. The excised organs were stored in 10% formalin until further histological analysis. The slides were stained with hematoxylin & eosin (H&E), scanned using an Aperio digital pathology slide scanner, and analyzed using the Image Scope software.

Statistical analysis

Pearson correlation coefficients between the chemical ingredients and acute toxicity were calculated to identify the chemical markers. The results expressed as mean ± standard deviation were statistically analyzed with the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA), and significant differences within groups were calculated using one-way analysis of variance (ANOVA) followed by Dunnett test; P value <0.05 was considered statistically significant.

Results

Composition analysis of three decoctions

As shown in Figure 1, the constituents were comprehensively detected, followed by characterization of the detected compounds and quantification for ranking and grading. Overall, 51 constituents were detected and characterized in the three decoctions, including 8 coumarins, 31 flavonoids, 2 bakuchiols, and other compounds (Table 1). The ratio of coumarins to total doses was the highest in the PF decoction, especially for isopsoralenoside and psoralenoside. In the ESW and SSW decoctions, the coumarin content was significantly reduced.

Acute toxicity

General observation and mortality

PF overdose resulted in various adverse effects in mice, including faintness, hypokinesia, and convulsions. The mortality rate of the mice began at 17 h post-administration, with all deaths occurring within 72 h. The number of deaths in the different dose groups (93.9, 71.0, 53.6, 40.6, 31.5, and 19.7 g/kg) were recorded as 10, 7, 5, 2, 0, and 0, respectively (Table 2). The LD_{100} of PF decoction was determined to be 93.9 g/kg, while the minimum lethal dose was found to be 40.6 g/kg. In accordance with the OECD guidelines[15] for acute oral toxicity testing, the LD_{50} of orally administered PF was

Table 1

Identified common compounds in PF, ESW, and SSW from the UPLC-MS analysis

No.	Identification	Molecular formula	Molecular mass (Da)	RT (min)	Q1
1	Quinic Acid	$C_7H_{12}O_6$	192.0634	4.43	215.0524 (+Na)
2	Psoralenoside	$C_{17}H_{18}O_9$	366.0951	11.10	389.0890 (+Na)
3	Isopsoralenoside	$C_{17}H_{18}O_9$	366.0951	11.83	389.0894 (+Na)
4	Schaftoside (Apigenin-6-glucoside-8-arabinoside) or isomer	$C_{26}H_{28}O_{14}$	564.1479	12.69	565.1556
5	Schaftoside (Apigenin-6-glucoside-8-arabinoside) or isomer	$C_{26}H_{28}O_{14}$	564.1479	12.87	565.1559
6	Cnidioside A or its isomer	$C_{17}H_{20}O_9$	368.1107	13.12	391.1245 (+Na)
7	Kaempferol 3-O-xylosyl-(1-2)-rhamnoside or Kaempferol 3-Rhamnoside 4'-Xyloside	$C_{26}H_{28}O_{14}$	564.1479	13.19	565.1557
8	Tetramethylchromanol glucoside or isomer	$C_{20}H_{30}O_7$	382.1992	13.70	405.1926 (+Na)
9	Apigenin-Glucoside	$C_{21}H_{20}O_{10}$	432.1056	13.85	433.1166
10	Psoralenoside isomer	$C_{17}H_{18}O_9$	366.0951	13.93	389.088
11	Chrysin 7-Rhamnosyl-Glucoside	$C_{27}H_{30}O_{13}$	562.1686	14.11	585.1568
12	Kaempferol 3-O-xylosyl-(1-2)-rhamnoside or Kaempferol 3-Rhamnoside 4'-Xyloside	$C_{26}H_{28}O_{14}$	564.1479	14.14	565.1559
13	Cnidioside A or its isomer	$C_{17}H_{20}O_9$	390.0951	14.29	391.1036
14	Isopsoralenoside isomer	$C_{17}H_{18}O_9$	366.0951	15.43	389.0896 (+Na)
15	Apigenin	$C_{15}H_{10}O_5$	270.0528	15.58	271.0668
16	Kaempferol-3-glucoside	$C_{21}H_{20}O_{11}$	448.1006	16.46	471.0935 (+Na)
17	Psoralen	$C_{11}H_6O_3$	186.0317	16.24	187.0471
18	Daidzein	$C_{15}H_{10}O_4$	254.0579	17.47	255.072
19	Isopsoralen	$C_{11}H_6O_3$	186.0317	16.89	187.047
20	3-Hydroxy-6-(4-hydroxyphenyl)-2-(2-hydroxypropan-2-yl)-2,3-dihydrofuro[3,2-g] chromen-5-one	$C_{20}H_{18}O_6$	354.1103	18.64	377.1047 (+Na)
21	Brosimacutin D/E	$C_{20}H_{20}O_5$	340.1311	19.00	341.1426
22	Brosimacutin D/E	$C_{20}H_{20}O_5$	340.1311	19.43	341.1443
23	Euchrenone a7	$C_{20}H_{20}O_5$	340.1311	20.30	341.1438
24	Psorachalcone A	$C_{20}H_{20}O_5$	340.1311	20.57	341.1444
25	Coumesterol	$C_{15}H_8O_5$	268.0368	20.60	269.0505
26	3-Hydroxypsoralenol	$C_{20}H_{18}O_6$	354.1103	20.71	377.1049 (+Na)
27	7-Hydroxy-3-(4-hydroxy-3-(2-hydroxy-3-methylbut-3-enyl) phenyl)-4H-chromen-4-one	$C_{20}H_{18}O_5$	338.1154	20.77	361.1100 (+Na)
28	Erythrinin A	$C_{20}H_{16}O_4$	320.1049	20.99	321.1183
29	Erythrinin C	$C_{20}H_{18}O_6$	354.1103	21.14	355.1229
30	Psoralenol	$C_{20}H_{18}O_5$	338.1154	21.39	339.1286
31	3-Hydroisovabachin	$C_{20}H_{20}O_5$	340.1311	21.41	341.1442
32	Corylifol C isomer	$C_{20}H_{18}O_5$	338.1154	21.67	339.1279
33	Ethyl (S)-3-Hydroxybutyrate Glucoside	$C_{20}H_{24}O_3$	312.1725	22.39	313.1774
34	Bavachromanol/bakuchalcone	$C_{20}H_{20}O_5$	340.1311	22.77	341.1441

(*Continued*)

Table 1

(Continued)

No.	Identification	Molecular formula	Molecular mass (Da)	RT (min)	Q1
35	Isobavachin	$C_{20}H_{20}O_4$	324.1362	22.92	325.1487
36	Corylifol C	$C_{20}H_{18}O_5$	338.1155	23.21	339.1280
37	3-Hydrovabachin	$C_{20}H_{20}O_5$	340.1311	23.43	341.1437
38	Corylifol B	$C_{20}H_{20}O_5$	340.1311	23.77	341.1440
39	Bavachin	$C_{20}H_{20}O_4$	324.1362	23.89	325.1487
40	6-Prenylnaringenin or its isomer	$C_{20}H_{20}O_5$	340.1311	24.07	341.1611
41	Neobavaisoflavone	$C_{20}H_{18}O_4$	322.1206	24.30	323.1337
42	8-Prenyldaizein	$C_{21}H_{22}O_4$	322.1206	24.30	323.1337
43	Corylin	$C_{20}H_{16}O_4$	320.1049	24.95	321.1118
44	13-Hydroxylbakuchiol	$C_{18}H_{24}O_2$	272.1777	24.96	295.1729
45	Isobavachrone/corylifolinin	$C_{20}H_{20}O_4$	324.1362	25.88	325.1488
46	Bavachalcone	$C_{20}H_{20}O_4$	324.1362	26.19	325.1491
47	Bavachinin A	$C_{21}H_{22}O_4$	338.1519	26.46	339.1646
48	Psoralidin	$C_{20}H_{16}O_5$	336.1000	26.35	337.1135
49	Corylifol A	$C_{25}H_{26}O_4$	390.1832	28.08	391.1945
50	8-Geranyloxypsoralen	$C_{21}H_{22}O_4$	338.1519	28.52	339.1645
51	Bakuchiol	$C_{18}H_{24}O$	256.1828	29.03	255.1744

ESW: Ershen Wan; PF: Psoraleae Fructus; RT: Retention time; SSW: Sishen Wan; UPLC-MS: Ultra-high-performance liquid chromatography/mass spectrometry.

53.9 g/kg/day, equivalent to 31 times the recommended human dosage in the clinical setting. After 14 d of recovery, no abnormal symptoms were observed in the surviving mice.

Similar symptoms were observed in the high-dose ESW group; however, the mortality rate was lower in the ESW group than in the PF group. Based on the mortality rates and dosages in mice, the maximum dose of ESW did not result in lethality in any of the mice, with a mortality rate of 90%. The LD_{50} value of orally administered ESW was determined to be 68.3 g/kg/day, which is equivalent to 39 times the human dosage (Table 3). As shown in Figure 2, the LD_{50} curve of the ESW group shifted to the right compared with that of the PF group, indicating that compatibility with ESW may alleviate the acute toxicity of PF alone.

Most mice in the SSW group exhibited no adverse effects, indicating the lower toxicity of SSW. Within the 14 d following administration, the mice displayed normal hair color, activity levels, mental state, and bowel movements. The mortality rate for the highest SSW dose was 10%, with an MTD of 41 g/kg/day.

Body weight, food intake, and water consumption

After a single oral dose of 40.6 g/kg PF, the body weights of both male and female mice significantly decreased. After 1 day of treatment, the body weight of mice in the PF group significantly decreased, and the weight remained significantly inhibited until day 14; however, it was less than that of the Con group. The weight growth trend in the PF group was correlated with the dosage. However, no differences were observed in body weight between the two compatibility groups (ESW and SSW) and the Con group.

In contrast, the administration of 40.6 g/kg PF resulted in reduced food intake and water consumption on the first and seventh day for both male and female mice (Figure 2C, D). No significant changes in food intake were observed in the ESW and SSW groups; however, a

Table 2

Survival condition of treated mice after oral PF administration

Dosage of PF raw drug (g/kg)	*n*	Dead mice in the PF group					Mortality (%)	LD_{50} and 95% confidence (g/kg)
		1 day	2 days	3 days	4–14 days	Total		
93.9	10	9	1	0	0	10	100	53.9 (46.2–63.0)
71.0	10	6	1	0	0	7	70	
53.6	10	4	0	1	0	5	50	
40.6	10	1	1	0	0	2	20	
31.5	10	0	0	0	0	0	0	
19.7	10	0	0	0	0	0	0	

LD_{50}: 50% lethal dose; PF: Psoraleae Fructus.

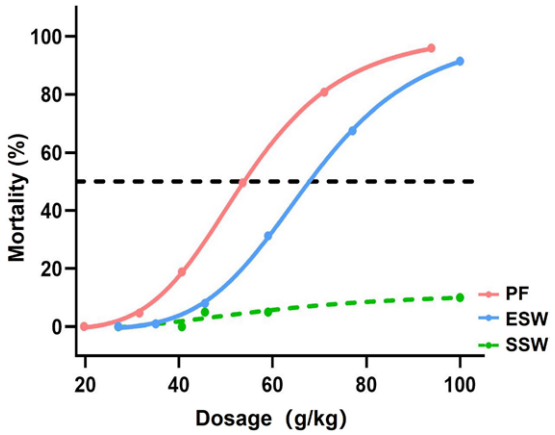

Figure 2. The LD_{50} curve of the PF and ESW and the MTD value of the SSW. ESW: Ershen Wan; LD_{50}: 50% lethal dose; MTD: Maximum tolerance dose; PF: Psoraleae Fructus; SSW: Sishen Wan.

slight decrease in water consumption on the seventh day was noted in the ESW group.

Organ weight

After a 14-day recovery period, the liver weight of female mice subjected to PF treatment remained elevated compared with that of the Con group, suggesting potential liver injury due to PF overdose. However, no significant liver or other organ weights were observed in the ESW and SSW groups (Table 4).

Histopathology analysis

Subsequent histological analysis revealed that PF caused morphological changes only in mouse liver tissue. As shown in Figure 4, PF overdose induced granular hepatocyte degeneration, swelling, necrosis in calcified foci, and inflammatory cell infiltration. Conversely, no obvious histological alterations were observed in the other organs. No obvious histological changes were observed in the ESW and SSW groups.

Correlation analysis for potential toxicity substances

The correlation coefficient was used to calculate the content of the identified compounds in PF, ESW, and SSW concerning the level of liver injury. Figure 5 shows the three clusters of the different compounds. It is noteworthy that the content of 18 compounds in the lowermost cluster decreased in the two compatibility groups compared with the PF group. Among these, 13 compounds were statistically significant ($P < 0.05$), including isopsoralen, psoralen, corylifol A, 3-hydroisovabachin, neobavaisoflavone, bavachin, psoralidin, corylifol C, psoralenoside, isopsoralenoside, isopsoralenoside isomer, daidzein, and brosimacutin D/E.

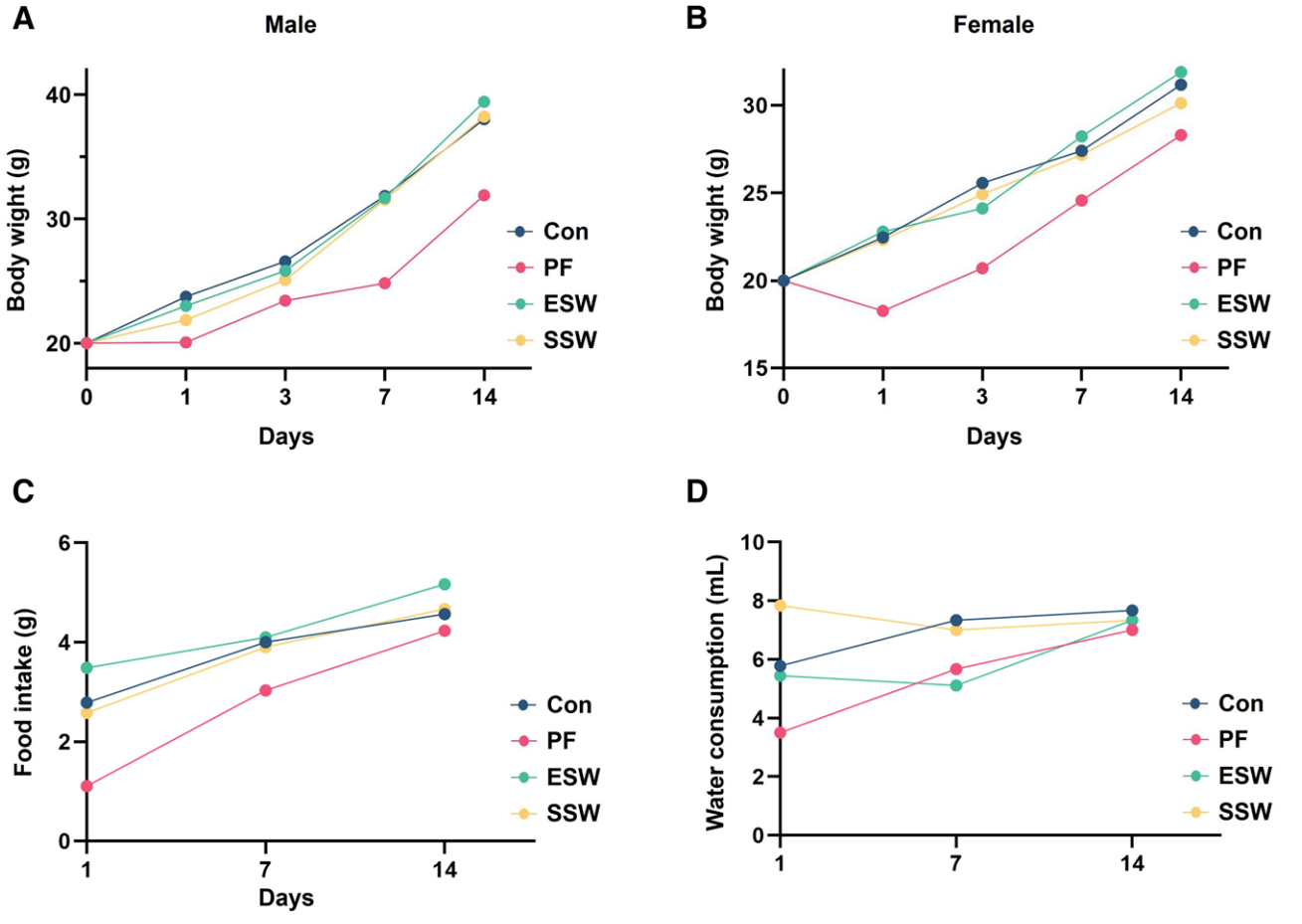

Figure 3. Effects of PF, ESW, and SSW on body weight (A, B), food intake (C), and water consumption (D) of mice during 14 days after administration. Con: Control; ESW: Ershen Wan; PF: Psoraleae Fructus; SSW: Sishen Wan.

Table 3

Survival condition of treated mice after oral ESW administration

Dosage of PF raw drug (g/kg)	*n*	Dead mice in the ESW group					Mortality (%)	LD_{50} and 95% confidence (g/kg)
		1 day	2 days	3 days	4–14 days	Total		
100.0	10	7	2	0	0	9	90	68.3 (59.0–78.9)
77.0	10	4	3	0	0	7	70	
59.0	10	2	1	0	0	3	30	
45.5	10	0	1	0	0	1	10	
35.0	10	0	0	0	0	0	0	
27.0	10	0	0	0	0	0	0	

ESW: Ershen Wan; LD_{50}: 50% lethal dose; PF: Psoraleae Fructus.

Table 4

Organ weights of mice after the 14-day recovery period in each group

Gender	Group	Heart (g)	Liver (g)	Spleen (g)	Lung (g)	Kidney (g)	Thymus gland (g)
Male	Con	0.20 ± 0.03	2.10 ± 0.23	0.13 ± 0.04	0.28 ± 0.02	0.66 ± 0.13	0.15 ± 0.01
	PF	0.20 ± 0.01	1.99 ± 0.18	0.14 ± 0.03	0.31 ± 0.04	0.61 ± 0.08	0.17 ± 0.06
	ESW	0.18 ± 0.01	1.82 ± 0.05	0.13 ± 0.03	0.33 ± 0.01	0.60 ± 0.02	0.18 ± 0.05
	SSW	0.22 ± 0.04	2.10 ± 0.15	0.14 ± 0.02	0.33 ± 0.02	0.65 ± 0.07	0.17 ± 0.02
Female	Con	0.19 ± 0.01	1.87 ± 0.23	0.16 ± 0.02	0.31 ± 0.02	0.47 ± 0.04	0.20 ± 0.03
	PF	0.20 ± 0.02	2.16 ± 0.10*	0.20 ± 0.05	0.28 ± 0.06	0.49 ± 0.04	0.19 ± 0.03
	ESW	0.23 ± 0.02	2.08 ± 0.22	0.15 ± 0.03	0.33 ± 0.03	0.50 ± 0.07	0.23 ± 0.05
	SSW	0.19 ± 0.03	1.94 ± 0.14	0.14 ± 0.02	0.32 ± 0.04	0.47 ± 0.04	0.25 ± 0.04

Con: Control; ESW: Ershen Wan; PF: Psoraleae Fructus; SSW: Sishen Wan.
*$P < 0.05$.

Discussion

PF is a non-toxic medicine with a long history of clinical applications. The results indicated that the LD_{50} of PF decoction exceeded 50 g/kg based on clinical usage, suggesting that the adverse effects of PF were minimal. However, excessive administration of PF can lead to acute liver injury in mice, which is characterized by hepatocyte swelling, necrosis, and impaired recovery. Consequently, administration of PF at an appropriate dosage with rigorous use under the medical guidance of Chinese physicians is advised.

With rare adverse events through extensive historical utilization, classic prescriptions such as ESW and SSW have demonstrated their safety in clinical practice. We considered the inherent wisdom of ancient Chinese physicians in formulating these traditional prescriptions and underscored the importance of conducting comparable research to elucidate the scientific implications of detoxification through compatibility. To our knowledge, this study presents the first evidence suggesting that the compatibility of ESW and SSW in classic prescriptions could effectively mitigate acute hepatotoxicity induced by PF using modern toxicological methods. Further mechanistic studies are required to elucidate the classical principle of detoxification by compatibility, which will provide a reference for the rational compatibility of PF.

Although some studies have explored the hepatotoxicity induced by PF, the toxic substances and mechanisms of PF remain unclear. Spectrum-toxicity correlation provides a good approach for analyzing potential toxicity-related compounds from the complex compositions of herbs[16,17]. In this study, a comprehensive chemical analysis revealed the substantial presence of coumarins in PF. Correlation analysis findings revealed that coumarins, particularly isopsoralen, psoralen, psoralenoside, and isopsoralenoside, may contribute to PF hepatotoxicity, which was significantly decreased in the two classical compatibility groups. The most pronounced toxicity attenuation in the SSW group suggests a potential association between psoralenoside and isopsoralenoside and PF hepatotoxicity, as evidenced by the substantial differences in content between the ESW and SSW groups ($P < 0.01$). Potentially toxic substances were predominantly found in the ethyl acetate and n-butanol extracts in another study and were closely associated with coumarin concentrations[18]. Additionally, psoralen and isopsoralen exhibit hepatotoxic effects in zebrafish and rats, consistent with the findings of the compatibility analysis in our study[19–22]. Nevertheless, it is imperative to note that these coumarins also possess efficacy; thus, ensuring appropriate dosage and compatibility would ensure the safe administration of PF[23]. Moreover, bavachin exhibits, stronger hepatotoxicity by aggravating oxidative damage and endoplasmic reticulum stress[24,25], suggesting that reliable spectrum-toxicity correlation analysis could provide valuable clues to reveal the toxic substance. Consequently, further investigations are warranted to delve deeper into this topic.

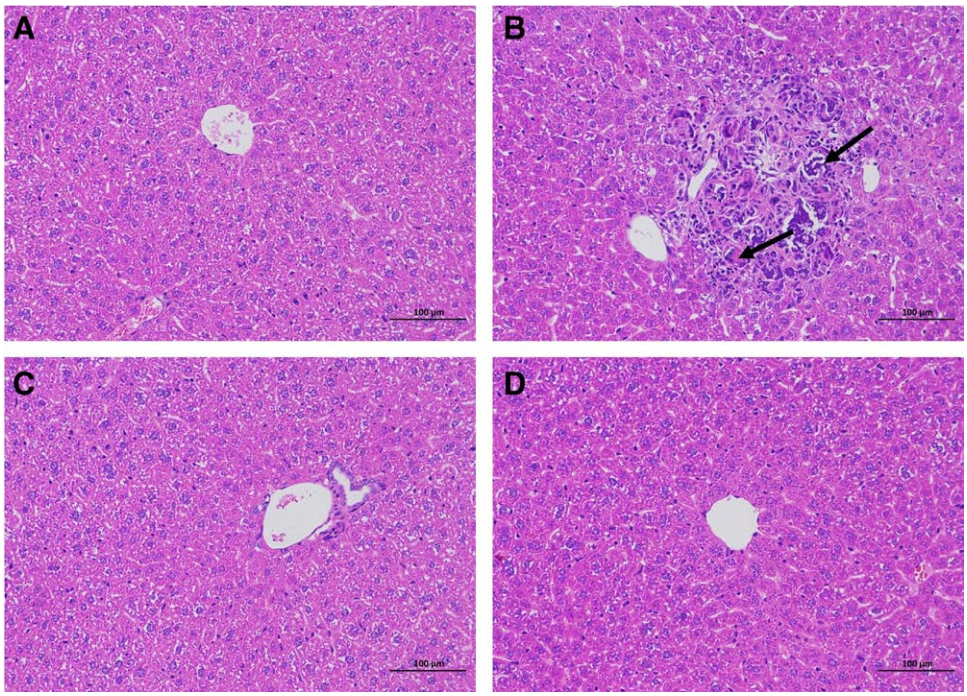

Figure 4. The H&E staining of the liver in the control (A), PF (B), ESW (C), and SSW (D) groups of mice after withdrawal for 14 d. ESW: Ershen Wan; H&E: Hematoxylin & eosin; PF: Psoraleae Fructus; SSW: Sishen Wan.

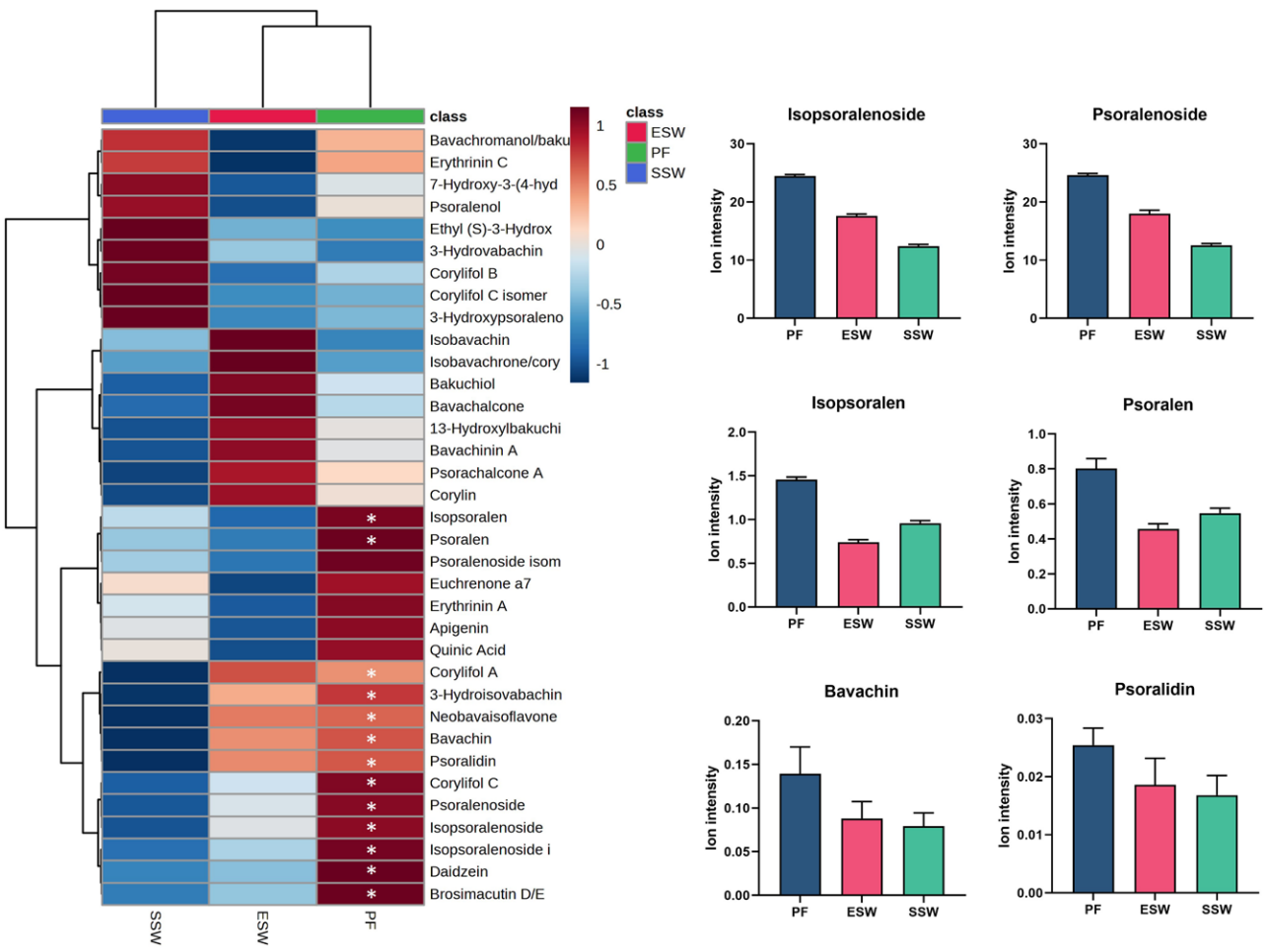

Figure 5. Correlation matrix of hepatotoxicity and main compound contents. ESW: Ershen Wan; PF: Psoraleae Fructus; SSW: Sishen Wan.

Conclusions

The classic compatibility of both ESW and SSW effectively reduced the toxicity of PF, probably due to the reduced coumarin content, which requires further verification in the future.

Conflict of interest statement

Yue Gao is editorial board member of this journal.

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Author contributions

Zhuo Shi and Jin-Chao Pan contributed equally to this study and designed and completed the study; Xiang-Jun Wu, Jia-Lu Cui, Cheng Zhang, and Fang-Yang Li analyzed the data under the guidance and supervision of Yu-Guang Wang and Yue Gao; Mao-Xing Li, Cheng-Rong Xiao, Zeng-Chun Ma, Yu-Guang Wang, and Yue Gao revised the manuscript. All the authors have read and approved the final manuscript.

Ethical approval of studies and informed consent

This study was approved by the Animal Care and Use Committee of the Laboratory Animal Center of the Academy of Military Medical Sciences (Permit Number: IACUC-DWZX-2021-728).

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None.

Data availability

All relevant data are within the manuscript.

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