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The Screening of Major Hepatotoxic Components of Tripterygium wilfordii Hook. F. Based on Hepatotoxic Injury Patterns

Meng Li
Shuguang Hospital, Shanghai University of Traditional Chinese Medicine
Qiong Luo
Shuguang Hospital, Shanghai University of Traditional Chinese Medicine
Xi Chen
Shanghai Key Laboratory of Traditional Chinese Clinical Medicine
Furong Qiu
Shuguang Hospital, Shanghai University of Traditional Chinese Medicine
Yanyan Tao
Shuguang Hospital, Shanghai University of Traditional Chinese Medicine
Xin Sun (913637769@qq.com)
Shuguang Hospital, Shanghai University of Traditional Chinese Medicine
Chenghai Liu
Shuguang Hospital, Shanghai University of Traditional Chinese Medicine

Research Article

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Abstract

Background: *Tripterygium wilfordii* Hook. F. (TwHF), a traditional Chinese medicine, is widely used in treatment of rheumatoid arthritis (RA), etc.. Due to multiorgan toxicity, particularly hepatotoxicity, the application of TwHF is restricted. In order to clarify the hepatotoxic substances, zebrafishes, hepatocytes and macrophages were used for screening based on hepatotoxic injury patterns. It provided a basis for further hepatotoxic mechanism of TwHF.

Methods: First, 12 compounds were selected according to the chemical categories of TwHF. The fluorescence area and fluorescence intensity of zebrafishes liver were observed and calculated. The viability of two hepatocyte lines were detected by CCK_8. TNF-α and IL-1β mRNA expression of BMDM was used to evaluate macrophage activation, a factor of potential indirect hepatotoxicity. Finally, hepatotoxic characteristics of 4 representative components were verified by mice *in vivo*.

Results: Parthenolide, Triptolide, Triptonide, Triptobenzene H, Celastrol, Demethylzeylasteral, Wilforlide A, Triptotriterpenic acid A and Regelidine can significantly reduce the fluorescence area and fluorescence intensity of zebrafish liver. The viability of L-02 or AML-12 was significantly inhibited by Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral, Triptotriterpenic acid A. Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral and Triptobenzene H significantly increased TNF-α and IL-1β mRNA levels of BMDM, while Triptophenolide, Hypodiolide and Wilforine significantly reduced. Triptotriterpenic acid A, Celastrol and Triptobenzene H at dose of 10 mg/kg significantly increased the levels of mice serum ALT and AST, and aggravated liver inflammation.

Conclusions: Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral, Triptotriterpenic acid A and Triptobenzene H might be main hepatotoxic components of TwFH. Among them, Triptotriterpenic acid A only presented a direct hepatotoxicity. Triptobenzene H showed indirect liver damage by activating macrophages. Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral could directly and indirectly cause liver injury.

Introduction

Tripterygium wilfordii Hook. F. (TwHF) is a member of the Celastraceae family of perennial vine-like plants. It is a well-known herbal medicine with potential anti-inflammation and immune-modulation properties, and widely used in various autoimmune-mediated inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, psoriasis, pemphigus, Behcet's disease [1–3]. However, there are increasing reports about its side effects, such as reproductive toxicity, hepatotoxicity, nephrotoxicity, cardiotoxicity and spleen toxicity [4]. Due to these adverse effects, the clinical use of TwHF-related drugs was limited [5].

Hepatotoxicity is a main part of drug safety evaluation, and also an important reason for drug withdrawal after marketing. TwHF can cause multiple organ injury, among which the incidence of hepatotoxicity is the highest, up to 40% [6]. TwHF is known to contain more than 200 chemical components, such as Triptolide, Triptonide, Triptobenzene H, Celastrol, Wilforlide A, Triptotriterpenic acid A, etc. The alkaloids, diterpenoids and triterpenoids in TwHF have varying degrees of toxicity [7, 8]. It was known that Triptolide (TP) is the hepatotoxic substance which could directly injury hepatocytes through mitochondrial depolarization [9, 10], but some herbal or composed products containing much lower TP also cause liver injury reportedly. It indicates that there are many hepatic toxics and mechanism yet to know. In Recent years, a number of experts and scholars have proposed a "new pattern" of indirect liver injury, differ from direct or idiosyncratic liver injury, which was drug induced immune cells mediated liver injury [11]. As an immunomodulator, more new indirect liver injury mechanisms of TwHF need to be further explored. The identification and elucidation of the toxic components and mechanism is helpful to the rational clinical application of TwHF preparations.

In current study, we aim to evaluate hepatotoxicity of different components in TwHF and its main mechanism. According to chemical categories and concentrations of components in TwHF, we performed zebrafishes-based 12 compounds toxicity screening. Furthermore, we explored the damage types of hepatotoxicity in two cell lines and validated the representative components in mice. This work contributes to a better understanding of hepatotoxic substances and possible targeted cytological characteristics of liver injury caused by TwHF, and lays a foundation for further research on the toxicological mechanism.

Materials And Methods

Drugs

Parthenolide, Triptolide, Triptonide, Triptophenolide, Triptobenzene H, Hypodiolide, Celastrol, Demethylzeylasteral, Wilforlide A, Triptotriterpenic acid A, Wilforine, Regelidine (ACMEC biochemical, Shanghai, Table 1.) were respectively dissolved in DMSO at the concentration of 50 mM and stored at -20 °C.

Table 1 The 12 chemical components of TwHF

Components		Chemical formula	No./CAS	Product code	Purity (%)
Diterpenes	Triptolide	$C_{20}H_{24}O_6$	38748-32-2	T54611	98
	Triptonide	$C_{20}H_{22}O_6$	38647-11-9	T42921	98
	Triptophenolide	$C_{20}H_{24}O_3$	74285-86-2	T67461	98
	Triptobenzene H	$C_{21}H_{28}O_4$	146900-55-2	T16850	97
	Hypodiolide	$C_{20}H_{30}O_3$	139122-81-9	T84750	90
Triterpenes	Celastrol	$C_{29}H_{38}O_4$	34157-83-0	C69551	98
	Demethylzeylasteral	$C_{29}H_{36}O_6$	107316-88-1	D17480	98
	Wilforlide A	$C_{30}H_{46}O_3$	84104-71-2	W52141	98
	Triptotriterpenic acid A	$C_{30}H_{48}O_4$	84108-17-8	T96110	97
Alkaloids	Wilforine	$C_{43}H_{49}NO_{18}$	11088-09-8	W36430	98
	Regelidine	$C_{35}H_{37}NO_8$	114542-54-0	R82510	95
Sesquiterpene	Parthenolide	$C_{15}H_{20}O_3$	20554-84-1	P15551	98

Drug toxicology screening in Zebrafishes

The transgenic liver fluorescent zebrafish Tg (L-FABP: EGFP) were used for drug toxicology screening [12]. When the zebrafishes developed to 72 hours post fertilization, the young zebrafishes with normal development were selected and transferred to a 24-well plate according to the number of 10 juveniles per well. According to the concentration range of the preliminary experiment, a blank control group and three concentration groups were set and with 2 replicate holes in each group. Then zebrafishes larvae were incubated at a constant temperature (28°C) in a light incubator, the drugs were administrated for 3 consecutive days. The death and deformity of zebrafishes were observed and recorded within 24, 48, and 72 hours after incubation. After 72 hours, the zebrafishes larvae were anesthetized with tricaine (0.3‰) and fixed on a glass slide with methylcellulose (4%) for taking pictures. The fluorescent area and intensity of the liver were calculated.

Cells Culture

According to the L-02 cells and AML-12 cells growth curve, the drugs incubation time was selected as 24 hours to evaluate the dose-effect relationship. L-02 cells were obtained from the ATCC company (CRL-12461) and maintained in 5% CO_2 at 37 °C in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, Gaithersburg, MD). AML-12 cells were obtained from the ATCC company (CRL-2254) and grown in DMEM/F-12 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1× Insulin-Transferrin-Selenium (ITS) mixture, and 40 ng/mL dexamethasone. The cells were maintained at 37 °C in an atmosphere of 5% CO_2. The bone marrow-derived macrophages (BMDM) were flushed from the tibia and femurs of 8- to 12-week-old C57BL/6 mice. Cells were cultured in DMEM (GIBCO) supplemented with 10% FBS, and 1% penicillin-streptomycin (GIBCO) and differentiated with 20% L929 supernatant for 7 days and were maintained in 5% CO_2 at 37 °C. BMDM were dispensed into 6-well culture plates at a concentration of 5×10^5 cells/well and stimulated with 12 components for 24h.

Cell viability assay

L-02 and AML-12 cells were seeded in 96-wells culture plates at a concentration of 1×10^4 cells /well and incubated at 37°C for 24 h in culture incubator. Then, the different concentration of 12 components (<1‰ DMSO preparation) was added and incubated for another 24 h. A total of 100 μl of cell counting kit-8 solution (CCK-8, Dojindo Biotechnology, Kumamotoi, Japan) homogenized in medium was added to each well, and the plates were incubated for 2 h at 37°C. The absorbance at 450 nm was measured using a microplate reader.

RT-PCR

Total RNA of BMDM was extracted using the Nucleospin RNA II kit (Takara, Japan). Quantitative RT-PCR was performed with SYBR Green (Applied Biosystems, Foster City, CA, USA). For thermal cycling procedure, an initial denaturation and activation of the hot start Taq polymerase at 95 °C for 30 s was followed by 40 cycles consisting of denaturation (95 °C for 5 s) and annealing and elongation (60 °C for 34 s). Finally, a dissociation curve step was added (95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s). See Table 2 for detailed primer sequences.

Table 2 Primer sequences

GENE	Sequences (5′-3′)	
	Forward primer	Reverse primer
TNF-α	TAG CCA GGA GGG AGA ACA GA	CCA GTG AGT GAA AGG GAC AGA
IL-1β	TAC ATC AGC ACC TCA CAA GC	AGA AAC AGT CCA GCC CAT ACT
IL-10	AGT GTG TAT TGA GTC TGC TGG	GAG AGA GGT ACA AAC GAG GTT
β-actin	TGGAATCCTGTGGCATCCATGAAAC	TAAAACGCAGCTCAGTAACAGTCCG

Mice Acute liver toxicity test

Five representative components were selected for further liver toxicity detection *in vivo*, which in order to reversely confirm the reliability of their hepatotoxicity. Wild-type C57BL/6 mice (male, 8 weeks old) were obtained from Shanghai Lab. Animal Research Center (Shanghai, China), they were randomly divided into 11 groups and each group had 6 mice. While the blank control group was given equal dose of 5% DMSO, the other groups were treated respectively with different doses of Triptotriterpenic acid A (1mg/kg and 10mg/kg, dissolved in 5% DMSO, i.p.), Celastrol (1mg/kg and 10mg/kg, dissolved in 5% DMSO, i.p.), Triptobenzene H (1mg/kg and 10mg/kg, dissolved in 5% DMSO, i.p.), Wilforlide A (1mg/kg and 10mg/kg, dissolved in 5% DMSO, i.p.), Wilforine (1mg/kg and 10mg/kg, dissolved in 5% DMSO, i.p.). The mice were sacrificed 24 hours after acute administration, their plasma and liver tissue samples were collected for further detected.

Determination of Serum ALT and AST

Blood was collected and serum was separated by centrifugation (4°C, 3000 rpm, 15 min). The levels of serum ALT and AST were determined according to the requirements of the instructions provided in reagent kits (Nanjing Jiancheng Institute of Biological Engineering, Cat No.C009-1 and Cat No.A059-1).

HE staining of liver tissues

The liver tissues of mice were fixed in 10% neutral phosphate-buffered formalin solution, then dehydrated and embedded in paraffin to make conventional paraffin sections. The sections were cut into 4 μm thickness and stained with hematoxylin and eosin (H&E, Nanjing Jiancheng Institute of Biological Engineering, Cat No.D006). The histopathological changes of liver tissues were observed under the optical microscope.

Statistical analysis

All values are presented as the means ± standard deviation (SD). Data were analyzed using analysis of variance (ANOVA) with a *Dunnett's* post hoc test for multiple comparisons or student *T-test* for two groups. *P*-values < 0.05 were considered to indicate statistical significance.

Results

9 components of TwHF were hepatotoxic to zebrafishes

Firstly, Celastrol (1.25μM for ≥48h, 2.5μM for ≥24h), Demethylzeylasteral (1.25μM or 2.5μM for ≥24h), Parthenolide (30μM for 72h), Wilforine (30μM for ≥48h), Wilforlide A (30μM for ≥48h), Triptophenolide (15μM or 30μM for 72h) and Hypodiolide (30μM for 72h) were observed significant impact on the mortality of zebrafishes (Figure 1). The fluorescence area and fluorescence intensity of zebrafishes liver were calculated and evaluated the hepatotoxicity after 72h of drug intervention. The results showed that Parthenolide, Triptolide, Triptonide, Triptobenzene H, Celastrol, Demethylzeylasteral, Wilforlide A, Triptotriterpenic acid A and Regelidine significantly reduced the fluorescence area and intensity of zebrafishes liver (Figure 2, Table 3). Triptophenolide, Hypodiolide and Wilforine had no significant hepatotoxic effects on zebrafishes.

Table 3 The fluorescence area and fluorescence intensity of zebrafishes liver

Groups		Fluorescence intensity (mean±SD, %)	Fluorescence Area (mean±SD, %)	Groups		Fluorescence intensity (mean±SD, %)	Fluorescence Area (mean±SD, %)
Parthenolide	Control	100.00±5.22	100.00±0.89	Celatrol	Control	100.00±0.59	100.00±4.07
	7.5μM	70.46±0.26*	98.21±1.09		0.625μM	75.61±1.43**	63.89±0.38*
	15μM	78.61±1.94	90.65±1.70*		1.25μM	(Dead)	(Dead)
	30μM	(Dead)	(Dead)		2.5μM	(Dead)	(Dead)
Triptolide	Control	100.00±0.35	100.00±3.50	Demethylzeylasteral	Control	100.00±0.59	100.00±4.07
	7.5nM	65.14±2.36**	86.63±0.46		0.625μM	89.41±3.19	86.49±0.23
	15nM	57.50±3.44**	77.97±1.50*		1.25μM	72.48±0.28**	77.04±0.15*
	30nM	41.42±3.09**	67.35±4.48*		2.5μM	(Dead)	(Dead)
Triptonide	Control	100.00±0.59	100.00±4.07	Wilforlide A	Control	100.00±3.61	10.00±1.35
	12.5nM	76.05±2.21**	92.20±4.79		7.5nM	62.68±0.19**	84.68±0.33**
	25nM	77.24±2.25*	87.90±2.22		15nM	41.51±1.95**	60.34±4.65*
	50nM	58.31±5.71*	80.95±2.89		30nM	41.28±0.93**	62.65±4.13*
Triptophenolide	Control	100.00±0.35	100.00±3.50	Triptotriterpenic acid A	Control	100.00±0.83	100.00±0.99
	7.5μM	109.71±0.99	95.82±1.81		7.5μM	84.50±1.86*	91.81±0.08*
	15μM	(Dead)	(Dead)		15μM	80.48±3.26*	84.76±1.57*
	30μM	(Dead)	(Dead)		30μM	83.87±2.47*	87.07±0.49**
Triptobenzene H	Control	100.00±3.61	100.00±1.35	Regelidine	Control	100.00±0.35	100.00±3.50
	7.5μM	63.47±2.88*	73.38±2.39*		7.5μM	67.22±1.71**	84.22±1.29
	15μM	41.57±1.42**	61.04±2.35**		15μM	56.00±0.25**	87.71±3.29
	30μM	29.57±3.75**	50.75±1.48**		30μM	51.36±1.58**	82.11±0.57*
Hypodiolide	Control	100.00±55.22	100.00±3.61	Wilforine	Control	100.00±1.35*	100.00±3.61
	7.5μM	90.68±5.78	93.62±3.95		7.5μM	78.55±2.35	81.41±5.90
	15μM	95.26±4.42	97.55±1.30		15μM	76.17±2.62*	78.45±7.02
	30μM	89.99±1.80	89.59±1.37*		30μM	79.20±1.98*	80.09±6.30

(Notes: $N \geq 10$; *$P<0.05$, **$P<0.01$ vs. Control group.)

6 components of TwHF were direct toxic to hepatocyte (L-02 and AML-12 cell lines)

In order to screen out components with direct hepatotoxicity of TwHF, two parenchymal hepatic cells lines, L-02 and AML-12, were used for evaluation. The cell viability was detected with CCK-8 kit, and the 50% inhibitive concentration value (IC_{50}) was calculated. Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral, Triptotriterpenic acid A showed significantly inhibitory effect on hepatocyte activity, while the other six components did not (Table 4).

Table 4 The IC_{50} of 12 components on L-02 and AML-12 cell lines

Components	50% inhibitive concentration (IC_{50}) value	
	L-02 cell lines	AML-12 cell lines
Parthenolide	(11.47±1.03) μM	NA
Triptolide	(28.06±1.02) nM	(27.77±1.09) nM
Triptonide	(75.79±8.88) nM	(91.49±3.10) nM
Triptophenolide	NA	NA
Triptobenzene H	NA	NA
Hypodiolide	NA	NA
Celatrol	(6.74±1.18) μM	(4.62±1.30) μM
Demethylzeylasteral	(2.02±1.03) μM	(4.03±1.78) μM
Wilforlide A	NA	NA
Triptotriterpenic acid A	NA	(16.09±1.32) μM
Regelidine	NA	NA
Wilforine	NA	NA

(Notes: *N*=6; three experimental replicates in all panels.)

6 components of TwHF activated mice macrophages (BMDM)

Immune-mediated indirect hepatotoxicity is emerging as a liver injury pattern [13]. A large number of studies have confirmed that TNF-α, IL-1β, IL-6 and other pro-inflammatory cytokines secreted by macrophages can aggravate liver injury and promote hepatocyte apoptosis [14]. Thus, mRNA expression levels of macrophages TNF-α and IL-1β were detected by RT-PCR after each component of TwHF incubation. The results showed that Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral and Triptobenzene H significantly increased mRNA expression levels of TNF-α and IL-1β. In contrast, Triptophenolide, Hypodiolide and Wilforine significantly reduced mRNA expression levels of TNF-α and IL-1β. However, Wilforlide A, Regelidine and Triptotriterpenic acid A showed no significant effect on mRNA expression of TNF-α and IL-1β (Figure 3).

Acute hepatotoxicity validation of 5 representative components from TwHF in mice

In order to verify whether these components have the acute hepatotoxicity in mammals, we selected 5 representative components for further observation according to results above (Figure 5). The C57BL/6 mice were intraperitoneally injected with Celatrol (CEL), Triptotriterpenic acid A (TA), Triptobenzene H (TH), Wilforlide A (WA) and Wilforine (WF), respectively. After 24 hours of administration, serum ALT, AST were determined and liver tissue slices were stained by HE. The results showed that CEL, TA and TH all significantly increased serum ALT and AST at 10 mg/kg, while the other groups were normal (Figure 4A and 4B). In HE staining pathological images, these 3 groups exhibited slightly disordered liver structure, mild swelling of hepatocytes with scattered inflammatory cell infiltration in varying degrees. Normal control group and other groups slices showed the intact hepatic lobules, neatly arranged hepatic cords, without obvious infiltration of inflammatory cells (Figure 4C).

Discussion

Drug induced liver injury (DILI) has gradually become an important aspect of drug safety. The list of herbal products causing liver injury is increasing in recent years [15]. It is reported that 23% of DILI cases are caused by Chinese herbal medicine, and about 20% of hospitalized patients with acute liver injury are caused by herb-induced liver injury (HILI) [16]. In China, TwHF ranks first in the liver injury of single Traditional Chinese Medicine (TCM), and its clinical application and promotion have been greatly affected. Therefore, the hepatotoxic components and mechanisms of TwHF have gradually become a research hotspot. Triptolide, one of the main active components of TwFH, is also one of the main hepatotoxicity factors which related to membrane damage, mitochondrial destruction, metabolic dysfunction, endoplasmic reticulum stress, oxidative stress, apoptosis and autophagy [17]. Previous researchers have found that triptolide, celatrol and demethylzeylasteral are the main hepatotoxicity components of TwHF based on spectrum-effect correlation analysis [18]. Our study found that in addition to these three components, other components in TwHF also have hepatotoxicity. Moreover, the exploration of toxicity mechanism found that some components have no direct hepatotoxicity. These results suggest that TwHF may have other unknown hepatotoxicity mechanism.

Typically, DILI is classified as either intrinsic or idiosyncratic. Recently, it was recognized that indirect hepatotoxicity is the third type and lack of in-depth understanding [19]. This type of DILI usually occurs in the special population with underlying diseases or susceptibility, which is manifested as the drug action changes the body state (such as immunity homeostasis), thereby inducing liver injury or aggravating the original liver disease. Indirect hepatotoxicity is much more common than idiosyncratic hepatotoxicity, it is a total response to a whole class of drugs, such as tumor necrosis factor antagonists and checkpoint inhibitors, rather than just a specific drug like nitrofurantoin. It may exist in most of the HILI, but there is a lack of research on this aspect. This may provide new ideas for clarifying the mechanism of HILI.

As a well-recognized biological model system for toxicology research, the zebrafishes have become a workhorse model in chemical toxicity screening [20]. Firstly, by comparing the mortality rate and changes of liver fluorescence area of zebrafishes, we found that 9 components have obviously hepatotoxicity (Fig. 5). This result indicated that there were indeed other substances causing liver injury in TwHF besides TP. According to the mechanisms of DILI, it was classified as direct and idiosyncratic hepatotoxicity. In order to avoid species differences as much as possible, we adopted two kinds of liver parenchymal cell lines from different species, L-02 cell line (human) and AML-12 cell line (Mice). Our results showed that Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral, Triptotriterpenic acid A had direct hepatocellular toxicity, and the toxicity tests of the two cell lines were consistent. These six components might be the basis of hepatotoxicity of TwHF. Combined with the toxicity results of zebrafish, it suggested that 3 components, Triptobenzene H, Regelidine and Wilforlide A, which did not show direct hepatocyte toxicity might had indirect hepatotoxicity.

Macrophages that are widely distributed throughout the body participate in innate and adaptive immune responses in many diseases. The physiologic function of macrophages can vary tremendously depending on the environment in which they reside in and the local stimuli to which they are exposed [21]. The activated macrophages secrete pro-inflammatory cytokines, such as TNF-α and IL-1β, which will cause the immune inflammatory response in the liver, chemokines like MCP-1 and CXCL-10 can also be secreted to recruit peripheral macrophages and immune cells into the liver and aggravate the inflammation [22]. Therefore, macrophages activation was also evaluated after drugs treatment in addition to direct hepatocyte toxicity in our study. The TNF-α and IL-1β mRNA expression level of BMDM were detected to determine whether the activation of macrophages. We found that Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral and Triptobenzene H significantly increased mRNA expression levels of TNF-α and IL-1β. This result suggested that there were components in TwHF could activate macrophages, which may be one of the mechanisms of indirect hepatotoxicity. As shown in Fig. 5, we further classified the 12 components of TwHF into 5 groups according to the screening results of zebrafishes and cell experiments, which were only hepatocyte-toxic group, only Mac-activation group, both hepatocyte-toxic and Mac-activation group, neither hepatocyte-toxic nor Mac-activation group, no zebrafish hepatotoxicity group, respectively. Finally, 5 representative components from each group were producted for validation in mice, and the results were consistent with the cells experiment above. A series of studies demonstrated that TP induced significant liver damage in mice through a variety of immune pathways, such as activating NKT cells [23], recruiting macrophages [24], neutrophil infiltration [25] and disturbing Th17/Treg balance [26]. These results suggested that indirect liver injury is common in DILI, and the culprit of liver injury caused by TwHF cannot only be attributed to its direct toxicity, but indirect toxicity is also extremely important.

Although we compared and analyzed the 12 components toxicity of TwHF at different model levels, that's not all components in TwHF. Besides, only macrophages was considered to explain indirect hepatotoxicity in our study, ignoring the role of other immune cells, which is the limitation of this study. However, indirect hepatotoxicity is a newly proposed category of DILI that has not been fully recognized yet. Whether TwHF affects immunity or the function of the liver to causes hepatotoxicity after TwHF is metabolized in the body, it needs to be explored further more. The deeper mechanism of indirect hepatotoxicity of TwHF we proposed is not comprehensive, and remains to be further explored.

Conclusions

Through the evaluation of zebrafishes liver injury, hepatocyte viability and macrophage activation, Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral, Triptotriterpenic acid A and Triptobenzene H proved to be the main hepatotoxic components of TwFH. Among them, Triptotriterpenic acid A only presented a direct hepatotoxicity. Triptobenzene H showed indirect liver damage by activating macrophages. Parthenolide, Triptolide, Triptonide, Celatrol, Demethylzeylasteral could directly and indirectly cause liver injury.

Abbreviations

BMDM	bone marrow-derived macrophages
CCK-8	cell counting kit-8 solution
CEL	celatrol
DILI	drug induced liver injury
DMSO	dimethylsulfoxide
FBS	fetal bovine serum
HILI	herb-induced liver injury
IC50	50% inhibitive concentration value
ITS	insulin-transferrin-selenium
RA	rheumatoid arthritis
TA	triptotriterpenic acid A
TCM	traditional Chinese medicine
TH	triptobenzene H
TP	triptolide
TwHF	*Tripterygium wilfordii* Hook. F.
WA	wilforlide A
WF	wilforine

Declarations

Ethics approval and consent to participate

The studies involving zebrafsh manipulations were approved by the institutional animal use and care committee of Shandong Academy of Sciences (No. SWSfw01202101). All mice experiment protocols were approved by the Animal Experiment Ethical Committee of Shanghai University of Traditional Chinese Medicine (Ethical reference no. PZSHUTCM200821016). All experimental procedures were carried out in accordance with the ethical guidelines and regulations. All animal experiments methods are reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available

from the corresponding author or from the https://pan.baidu.com/s/11DUPUroG7UtIANV0ZpfcPQ?pwd=gaja.

Competing interests

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare no conflicts of interest.

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Authors' contributions

Meng Li and Qiong Luo wrote the manuscript; Qiong Luo and Xi Chen producted the cells experiments *in vitro*; Meng Li and Xin Sun carried out the mice experiments *in vivo*; Xin Sun prepared the figures and tables; Yanyan Tao contacted zebrafish testing facility; Furong Qiu and Yanyan Tao contributed to reviewing the manuscript; Chenghai Liu designed the research scheme and provided funding for all researches. All authors approved the final version of the manuscript.

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Authors' information

[1] Institute of Liver Diseases, Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China.

[2] Shanghai Key Laboratory of Traditional Chinese Clinical Medicine, Shanghai 201203, China.

[3] Lab of Clinical pharmacokinetics, Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China.

[4] Key Laboratory of Liver and Kidney Diseases, Ministry of Education, Shanghai 201203, China.

[5] Shanghai Innovation Center of TCM Health Service, Shanghai 201203, China

Supplementary Information

None/Not applicable

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Figures

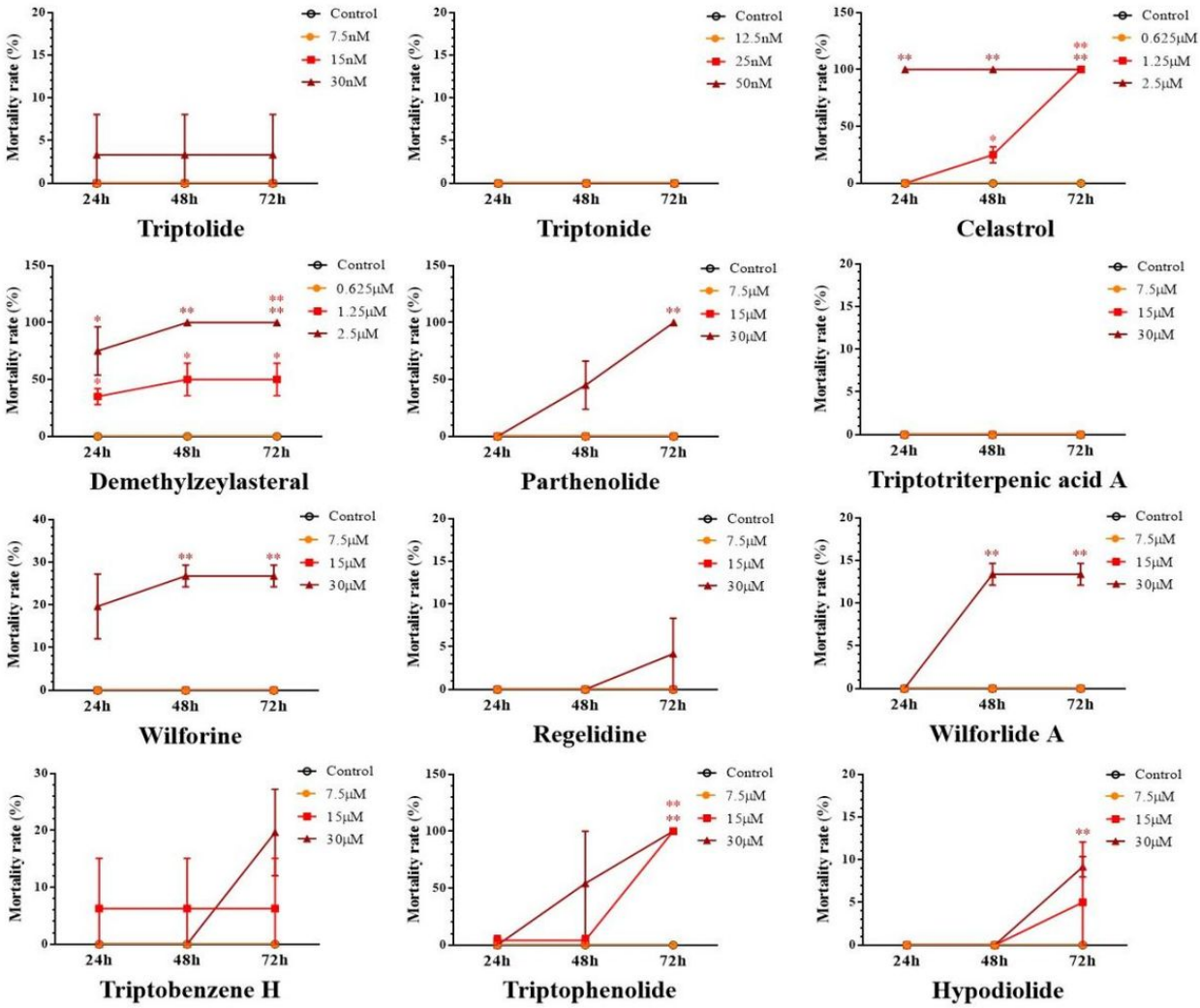


Figure 1

Effects of 12 components on mortality rate of zebrafishes

(Notes: *N*=20; **P*<0.05, ***P*<0.01 *vs.* Control group.)

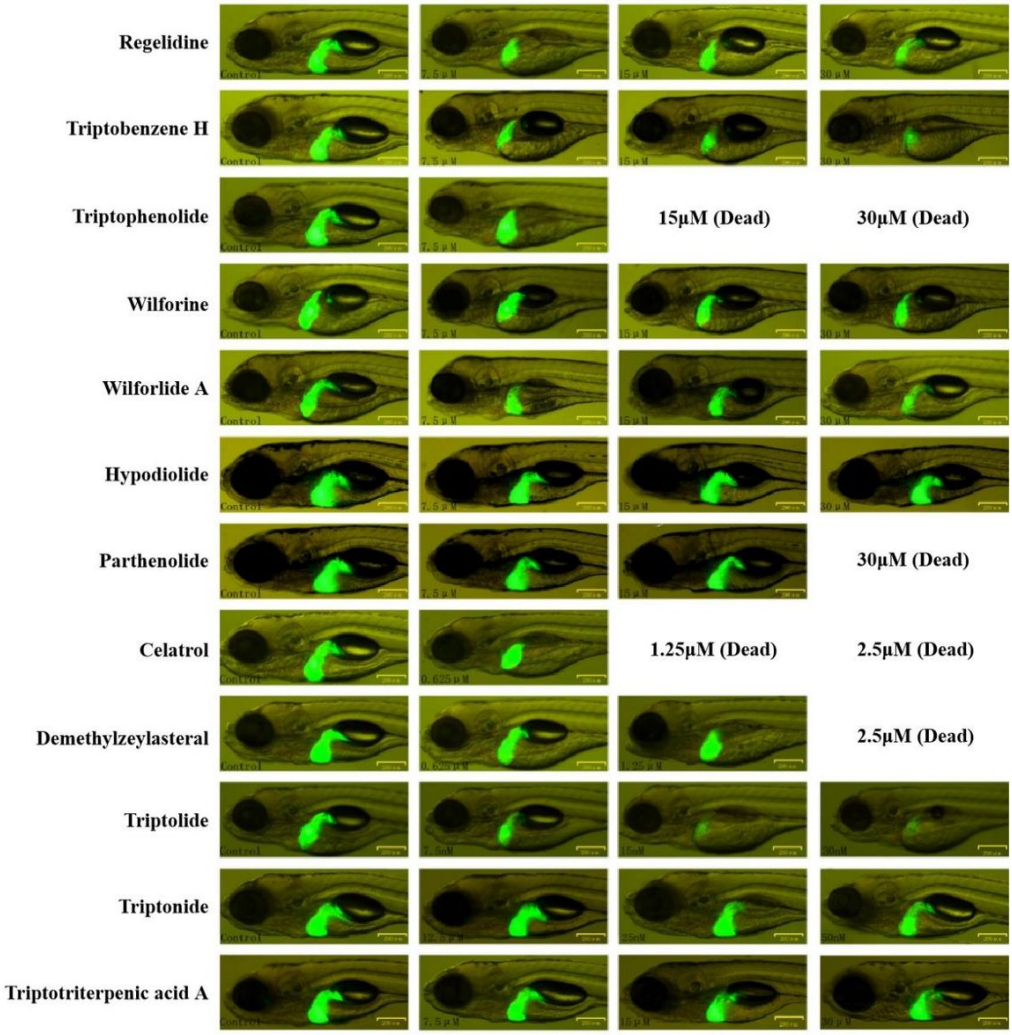


Figure 2

The fluorescent image of zebrafishes liver.

(Notes: after 72h of drug intervention; $N \geq 10$; Scale bar=200μm.)

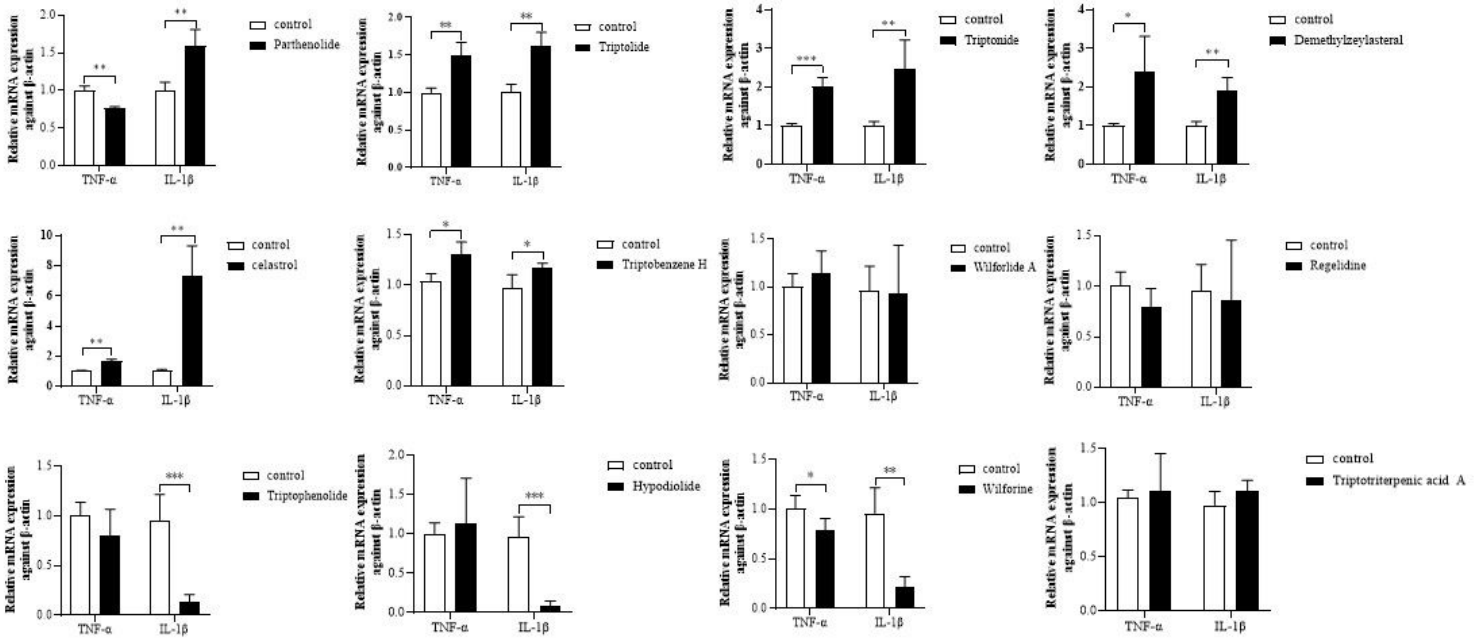


Figure 3

Effects of 12 components of TwHF on mRNA expression levels of TNF-α and IL-1β

(Notes: *N*=6; **P*<0.05, ***P*<0.01, ****P*<0.001 *vs.* Control group; three experimental replicates in all panels.)

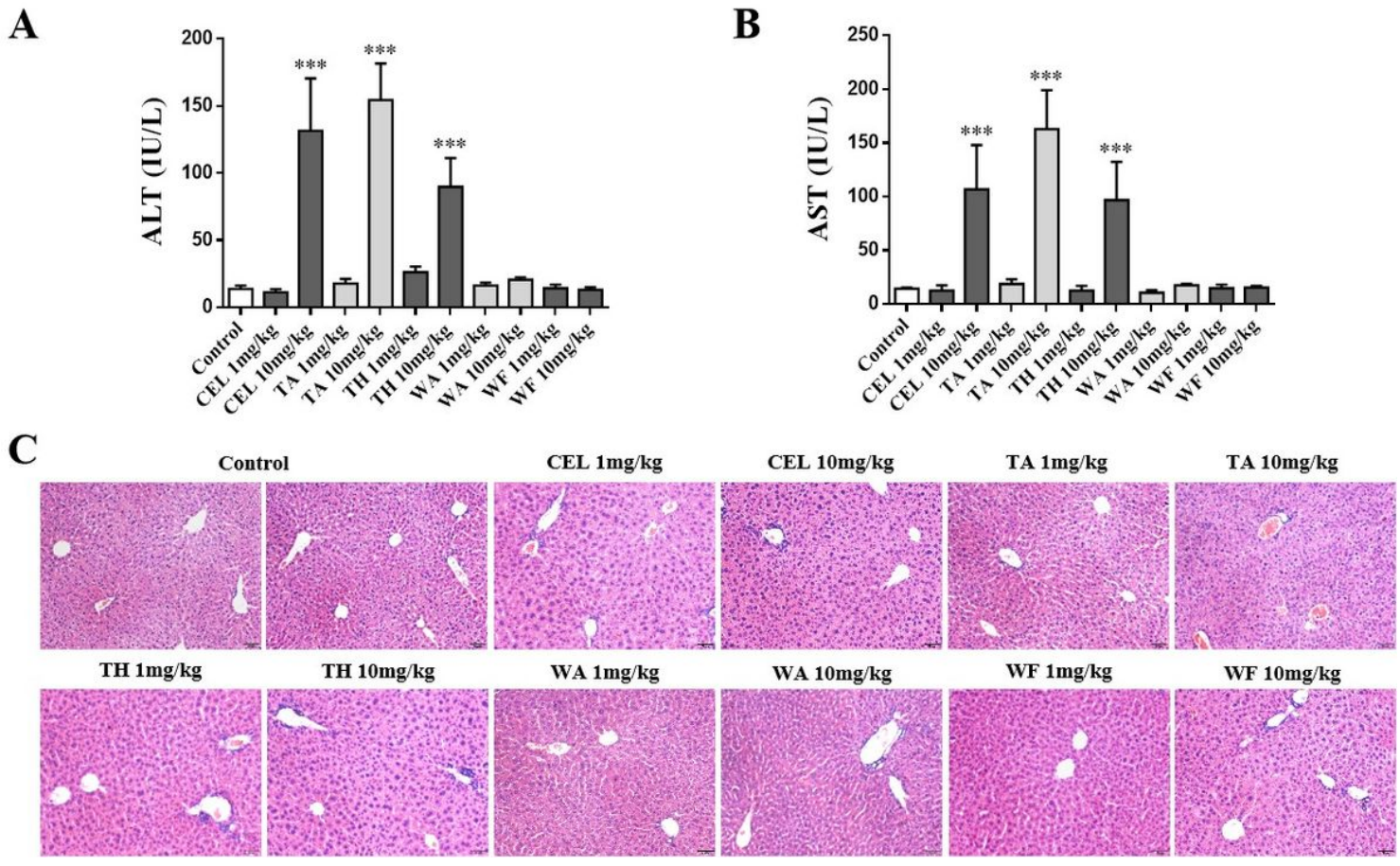


Figure 4

Acute hepatotoxicity validation of 5 representative components from TwHF in mice.

(Notes: A, serum ALT levels in mice; B, serum AST levels in mice; C, HE staining images of mice liver tissue slices, 200×; $N \geq 6$; ***$P < 0.001$ *vs.* Control group)

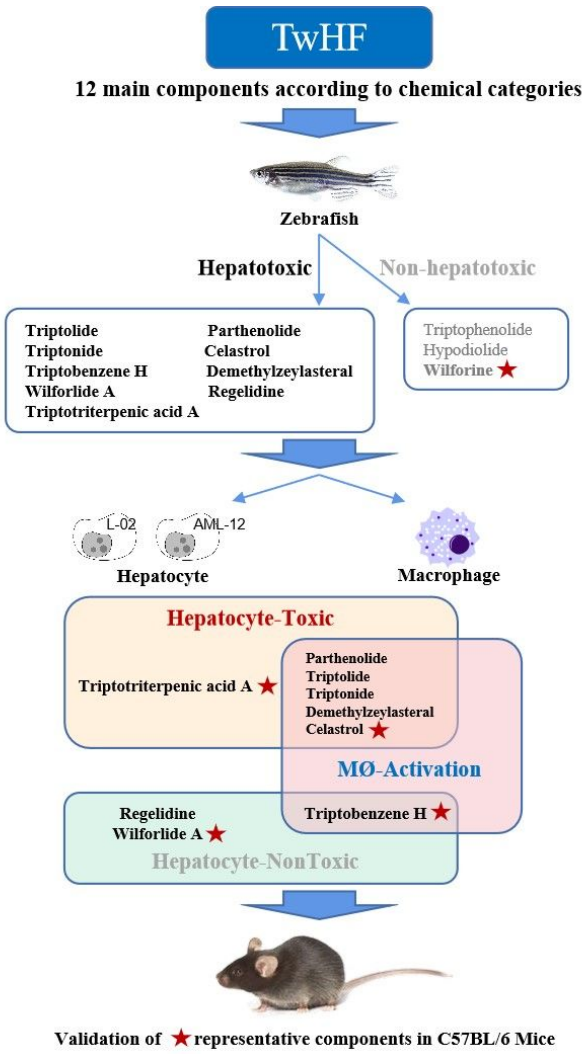


Figure 5

Graphical abstract