**Research article**

**Tripterygium wilfordii multiglycoside-induced hepatotoxicity via inflammation and apoptosis in zebrafish**

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**[ABSTRACT]** *Tripterygium wilfordii* multiglycoside (GTW) is a commonly used compound for the treatment of rheumatoid arthritis (RA) and immune diseases in clinical practice. However, it can induce liver injury and the mechanism of hepatotoxicity is still not clear. This study was designed to investigate GTW-induced hepatotoxicity in zebrafish larvae and explore the mechanism involved. The 72 hpf (hours post fertilization) zebrafish larvae were administered with different concentrations of GTW for three days and their mortality, malformation rate, morphological changes in the liver, transaminase levels, and histopathological changes in the liver of zebrafish larvae were detected. The reverse transcription-polymerase chain reaction (RT-PCR) was used to examine the levels of miRNA-122 (miR-122) and genes related to inflammation, apoptosis, cell proliferation and liver function. The results showed that GTW increased the mortality of zebrafish larvae, while significant malformations and liver damage occurred. The main manifestations were elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), significant liver atrophy, vacuoles in liver tissue, sparse cytoplasm, and unclear hepatocyte contours. RT-PCR results showed that the expression of miR-122 significantly decreased by GTW; the mRNA levels of inflammation-related genes *il1b*, *il6*, *tnfα*, *il10*, *cyc2* and *ptges* significantly increased; the mRNA level of *tgfl* significantly decreased; the mRNA levels of apoptosis-related genes, *caspase-8* and *caspase-9*, significantly increased; the mRNA level of *hcl2* significantly decreased; the mRNA levels of cell proliferation-related genes, *top2a* and *uhrf1*, significantly reduced; the mRNA levels of liver function-related genes, *alr* and *cyp3c1*, significantly increased; and the mRNA level of *cyp3a65* significantly decreased. In zebrafish, GTW can cause increased inflammation, enhanced apoptosis, decreased cell proliferation, and abnormal expression of liver function-related genes, leading to abnormal liver structure and function and resulting in hepatotoxicity.

**[KEY WORDS]** Tripterygium wilfordii multiglycoside; Zebrafish; Hepatotoxicity; Inflammation; Apoptosis


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**Introduction**

*Tripterygium wilfordii* multiglycoside (GTW), a fat-soluble extract derived from the root of the plant *Tripterygium wilfordii* in the Celastraceae family, exerts anti-inflammatory, immunosuppressive, immunomodulatory and anti-tumor effects [1]. Currently, GTW is a commonly used immunomodulatory drug for the treatment of rheumatoid arthritis, primary glomerulonephritis, nephrotic syndrome, anaphylactoid purpura nephritis and lupus nephritis [1]. However, GTW frequently induces adverse reactions, especially abnormal liver function [2]. FU [3] reported that GTW caused acute liver injury, and patients often presented gastrointestinal reactions (nausea, vomiting, and loss of appetite), yellowish staining of the sclera, skin and urine, liver tenderness and other symptoms. Moreover, there were a few patients with slow onset and severe cholestatic liver injury. GTW consists of diterpenoids, triterpenes, sesquiterpenes, alkaloids, glycosides, and other compounds [1]. Triptolide (TP) is a diterpenoid and the main active ingredient of GTW. In recent years, there have been reports concerning the mechanism of TP-induced hepatotoxicity. WANG [4] and FU [3] demonstrated that TP in-
duced oxidative stress, resulting in hepatotoxicity in rats. WANG et al reported that TP induced the secretion of interleukin (IL)-17 and aggravated liver damage. According to recent reports, TP-induced hepatotoxicity was associated with inhibition of the Sirt1/FXR signaling pathway. However, the mechanism of GTW-induced hepatotoxicity is not completely understood. To ensure drug safety, it is necessary to investigate GTW-induced hepatotoxicity.

Zebrafish is an ideal vertebrate model organism with 70%–80% genomic homology to the human genome. Compared with conventional experimental animals, zebrafish has the advantages of a short reproductive cycle, in vitro embryo development, transparent vertebrate embryos, stable species, small individual differences, and easy feeding. Zebrafish exhibits high similarity with mammals in liver structure and functions, with high application value in drug-induced hepatotoxicity studies. The use of zebrafish in evaluating hepatotoxicity induced by traditional Chinese herbal medicine requires less dosage of test drugs, shortens research cycle and reduces research expense.

In the current study, a transgenic Tg (L-FABP : EGFP) zebrafish line and a wild-type zebrafish AB strain were used as models to evaluate the hepatotoxicity of GTW and explore related mechanism involved. There findings will provide theoretical evidence for the subsequent clinical use of and research on GTW.

Material and Methods

Reagents

GTW was purchased from Zhejiang DND Pharmaceutical Co., Ltd. (batch No. 0802702, Zhejiang Xinchang, China). According to the quality standards test, the content of wilforlide A in GTW was 4.3%, and chemical analysis of its constituents has been published. The stock solution was prepared by dimethyl sulfoxide (DMSO) and stored at 4 °C. The GTW solution was diluted with zebrafish water to obtain the indicated concentration used in the experiments. The compositions of zebrafish water were 5 mmol·L⁻¹ NaCl, 0.17 mmol·L⁻¹ KCl, 0.4 mmol·L⁻¹ CaCl₂, and 0.16 mmol·L⁻¹ MgSO₄.

Zebrafish

In the current study, transgenic zebrafish with liver-specific fluorescence Tg (L-FABP : EGFP) and wild-type AB strain zebrafish were used, which were provided by the Key Laboratory for Drug Screening Technology of Shandong Academy of Sciences (Jinan, China). The male and female zebrafish were separately housed in standard light/dark conditions (14 h/10 h) at 28 °C and regularly fed on granular bait and Artemia. Before egg harvesting, healthy and mature male and female zebrafish were separately placed into a mating tank at a ratio of 1 : 1 or 1 : 2. On the next day, the wall was removed for mating, and the fertilized eggs were obtained after 1–2 hours. These fertilized eggs were then disinfected and washed before transferred into zebrafish embryo culture medium at 28 °C with light control. All experimental protocols were approved by and performed under the supervision of Biology Institute, Qilu University of Technology Animal Ethics Committee.

Drug treatment

After egg fertilization at 72 hours post fertilization (hpf), normal zebrafish larvae were selected and placed in 6-well plates (30 larvae/well). A control group (with zebrafish water as embryo culture medium, containing 0.1% DMSO) and treatment groups containing different concentrations of GTW (1 μg·mL⁻¹, 2.5 μg·mL⁻¹, 4 μg·mL⁻¹, 5 μg·mL⁻¹, 10 μg·mL⁻¹, 12 μg·mL⁻¹, and 15 μg·mL⁻¹) were prepared, with three replicates per well for each treatment group. The zebrafish larvae were incubated in an incubator at 28 °C with light control, and GTW was administered for three consecutive days. The medium was changed every day, and the development of zebrafish embryos were observed.

The mortality of zebrafish

The death of zebrafish larvae in each group was recorded at 24, 48, and 72 hpe (hours post exposure). The mortality was calculated, and lack of heartbeat was used as the indicator.

Morphological changes and malformation score of zebrafish

The zebrafish larvae were anesthetized with tricaine (category No. A5040, Sigma Aldrich) at a mass concentration of 0.3%, and methyl cellulose (Sigma Aldrich) solution (10 mg·mL⁻¹) was used to fix zebrafish larva (the fish body was laterally positioned with binocular overlap) on a slide. Each group was observed under a stereo fluorescence microscope (Olympus SZX16, Tokyo, Japan), and the morphology of the zebrafish was photographed. The malformation was scored as previously described.

Morphological changes in the liver of zebrafish

When determining the two-dimensional (2D) liver morphology, the zebrafish larvae were anesthetized and fixed on a slide. The 2D liver morphology of zebrafish in each group was photographed under a fluorescence microscope (Olympus FSX100, Tokyo, Japan), and the 2D liver fluorescence area was calculated. Meanwhile, the overall morphology of zebrafish was photographed, and the yolk sac area was calculated.

Determination of the transaminase activity in zebrafish

A total of 150 zebrafish larvae (72 hpe) were collected from each group for tissue homogenization, and the supernatant was collected. A Nanodrop One Microvolume Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine protein concentration. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were examined according to the manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, China).

Histopathological examination of the liver in zebrafish

Five randomly selected zebrafish larvae (72 hpe) in each group were fixed with 4% paraformaldehyde (Sigma Aldrich), dehydrated in an ethanol gradient, cleared with xylene for transparency, embedded in paraffin, sectioned, stained with hematoxylin-eosin (HE) (Beyotime Biotechnology), and mounted. Tissue sections were photographed under a microscope (OlympusFSX100, Tokyo, Japan).

Determination of the expression of miR-122

The 72 hpe zebrafish larvae were fully ground using a
homogenizer. miRNAs were extracted using the column centrifugation method, the purity and content of miRNAs were determined by the Nanodrop One Microvolume Spectrophotometer, and the integrity of miRNAs was detected using agarose gel electrophoresis. The complementary DNA (cDNA) was obtained by reverse transcription (RT) of miRNA samples of each group with miR-122 and U6-specific RT primers, and then determined with specific forward primers and universal reverse primers using a LightCycler 96 instrument (Roche), a real-time fluorescence polymerase chain reaction (PCR) system. The results were analyzed with U6 as the internal reference and the expression of each target gene in the control group normalized as 1. The relative quantitative analysis was performed using LightCycler® 96 software. The primer sequences of miR-122 are shown in Table 1.

**Table 1. The primer sequences of miR-122 for quantitative real-time PCR**

<table>
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<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>miR-122</td>
<td>miR122(RT)</td>
<td>GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGGATACGACCAAACA</td>
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<tr>
<td></td>
<td>miR122</td>
<td>CCGTTGGAGGGTGACAATGGAGTGCAGGGTCCGAGGTATT</td>
</tr>
<tr>
<td>U6</td>
<td>U6(RT)</td>
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<tr>
<td></td>
<td>U6</td>
<td>CCGTTGGAGGGTGACAATGGAGTGCAGGGTCCGAGGTATT</td>
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</table>

**Results**

**Effects of GTW on the mortality of zebrafish**

To determine the toxicity of GTW on zebrafish, the mortality of zebrafish exposed to different concentrations of GTW was examined. No death occurred in the zebrafish larvae at a low concentration of GTW. With the GTW concentration increased and treatment time extended, the death of zebrafish larvae began to increase. At 24 hpe, although high-concentration GTW caused a certain amount of death, the mortality was maintained at a relatively low level. At the dose of 15 μg mL⁻¹ for 48 h, the mortality of zebrafish larvae reached 100%, and at 72 hpe, the mortality of larvae was 100% for the 10 μg mL⁻¹ GTW treatment group (Fig. 1A).

**Effects of GTW on the morphology of zebrafish**

GTW exerted a certain impact on the morphological development of zebrafish larvae. The effects of different concentrations of GTW and different treatment times on the morphology of zebrafish larvae were observed under a stereo fluorescence microscope, and the teratogenicity of GTW on zebrafish larvae was scored (Figs. 1B and C). The scores were evaluated according to several aspects, including the liver, notochord, brain, jaw, swim bladder, and yolk sac. The scoring system is listed as follows: 5, normal structure; 4, normal structure with minor malformation, suggesting that there are some developmental delays or changes, which however can be recovered; 3, minor structural malformation; 2: moderate structural malformation; and 1: severe structural malformation [12].

The results showed that GTW caused malformation in zebrafish larvae, which was manifested as abnormalities in the jaws (indicated by green arrowheads), diminished or absent swim bladders (indicated by red solid arrows), spinal curvature (indicated by yellow solid arrows), and delay of yolk sac absorption (indicated by blue dotted arrows). These findings indicated that with the increase in GTW concentrations and treatment time, the morphology scores of zebrafish larvae decreased, which further suggested the toxic effect of GTW on the morphological development of zebrafish larvae.

**Effects of GTW on liver morphology in zebrafish**

The 2D morphology of the liver in zebrafish larvae was observed under a fluorescence microscope (Figs. 2A, B). The results showed that at 24 hpe, the liver area of zebrafish larvae was changed, which however was not significant. At 48 hpe and 72 hpe, 1 μg·mL⁻¹ GTW increased the liver area of zebrafish larvae to some extent. With the increase of GTW concentrations, the fluorescent area in the liver of zebrafish larvae significantly decreased, indicating that GTW caused liver damage in zebrafish larvae.

The size of the yolk sac is one of the most important liver function indicators. Analysis of the yolk sac area (Fig. 2C) showed that low-concentration GTW exerted little impact on yolk sac absorption in zebrafish larvae. With the increases of GTW concentrations and treatment time, delayed yolk sac absorption became evident.

**Effects of GTW on the transaminase activity in zebrafish**

The levels of ALT and AST are liver function indicators, which reflect the health of the liver. As shown in Fig. 3, after...
GTW treatment for 72 h, the levels of ALT and AST significantly increased in the 5 μg·mL\(^{-1}\) treatment group compared with the control group, indicating that GTW treatment caused liver damage in zebrafish larvae.

**Effects of GTW on histopathological changes in the liver of zebrafish**

To further observe the liver injury in zebrafish, sections of the livers of zebrafish larvae were observed (Fig. 4). The results showed that the liver cells of normal zebrafish larvae were neatly arranged, the cytoplasm of liver cells was uniform, the nucleus in the center of the cells was regular and round, and the connections between the cells were tight. After treatment with GTW for 72 h, a small amount of vacuoles appeared in the liver tissues of the 1 μg·mL\(^{-1}\) treatment group; for the 2.5 μg·mL\(^{-1}\) treatment group, the cytoplasm of hepatocytes was sparse, the nucleus was atrophic, and vacuolization occurred; and for the 5 μg·mL\(^{-1}\) treatment group, the cytoplasm of hepatocytes was sparse, nuclear atrophy occurred, and vacuolization became more serious, which further elucidated that GTW-induced liver damage in zebrafish larvae might be exacerbated with the increase in GTW concentrations.

**Effects of GTW on the expression of liver injury-related genes**

To explore the mechanism of GTW-induced hepatotoxicity in zebrafish larvae, miR-122 expression (Fig. 5), inflammation (Fig. 6A), apoptosis (Fig. 6B), cell proliferation (Fig. 6C) and the expression of liver function-related genes (Fig. 6D) were examined at 72 hpe.

The results showed that, compared with the control group, the RNA level of miR-122 decreased after GTW treatment, while the decrease in the RNA level of miR-122 of the 5 μg·mL\(^{-1}\) group was significantly. GTW treatment significantly increased the mRNA levels of pro-inflammatory cytokines, interleukin 1β (il1β), interleukin 6 (il6) and tumor necrosis factor-a (tnfa), significantly increased the mRNA level of interleukin 10 (il10), the negative regulator of inflammation, and significantly increased the mRNA levels of prostaglandin-related genes, cyclooxygenase 2 (cox2) and Prostaglandin E2 Synthase (ptges). The mRNA level of transforming growth factor β (tgfb) remarkably decreased; the mRNA level of apoptosis inhibitory gene B cell lymphoma 2 (bcl2) remarkably decreased; the mRNA levels of apoptosis related genes, caspase-8 and caspase-9, remarkably increased; the mRNA levels of cell proliferation related genes, topoisomerase IIα (top2α) and ubiquitin-like with PHD and RING finger domains 1 (ubhf1), remarkably decreased; the mRNA level of the augmenter of liver regeneration (alr) remarkably increased; the mRNA levels of cytochrome enzyme cyp3a65 remarkably decreased and the mRNA level of cyp3c1 remarkably increased.

**Discussion**

The current study used zebrafish to investigate GTW-induced hepatotoxicity. The effects of GTW on the mortality and hepatotoxicity in zebrafish were dose- and time-dependent. Long-term treatment with medium- and high-dose GTW resulted in significant liver injury in zebrafish, which was manifested as elevated levels of ALT and AST, significant liver atrophy, vacuoles in liver tissues, sparse cytoplasm, and unclear hepatocyte contours. Meanwhile, the zebrafish treated with GTW presented significant malformation, such as abnormalities in the jaws, diminished or absent swim bladders, spinal curvature, and delayed yolk sac absorption.

Transaminase is mainly present in hepatocytes and acts as a classic indicator of hepatotoxicity. In the case of inflammation, necrosis, and poisoning, hepatocytes may be damaged, resulting in remarkable increases in the levels of transaminases [13]. In this study, the levels of ALT and AST in zebrafish significantly increased after GTW treatment, suggesting that GTW causes liver damage in zebrafish.

About 70% of yolk sac components in zebrafish are neutral lipids, which are mainly metabolized in the liver. Therefore, the size of the yolk sac can be used as one of the indicators to reflect liver function [19]. In this study, the delayed absorption of yolk sac in the zebrafish in the GTW treatment groups was also a manifestation of GTW-induced liver damage in zebrafish.

Increasing evidence suggests that miRNAs are involved in liver development, regeneration and metabolism. Disruption of miRNA networks in the liver has been associated with

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Table 2  The primer sequences for quantitative real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>β-actin</td>
<td>Cell</td>
<td>il1β</td>
<td>TGGCGAAGACTCATCCAAAG</td>
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<tr>
<td></td>
<td>Proliferation</td>
<td>il6</td>
<td>GGAGACCTGGGGCAGCATA</td>
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<td></td>
<td></td>
<td>tnfa</td>
<td>TCAGAGACGGGAGGTTTGGAAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cox2</td>
<td>GCCTTCCTTTGGCAGTGTGCT</td>
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<td></td>
<td></td>
<td>PtgEs</td>
<td>TCACCATATCCCGCTTGGATTCC</td>
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<td></td>
<td></td>
<td>Cyp3c1</td>
<td>AATCGTGTGCAAGGTTCCCA</td>
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<td></td>
<td>Cyp3a65</td>
<td>CAAGGGTGCGGGGTATAT</td>
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<td></td>
<td>Alr</td>
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<td>Tgfb1</td>
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<td>Reference</td>
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<tr>
<td></td>
<td></td>
<td>Caspase-8</td>
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<td></td>
<td>Caspase-9</td>
<td>AAGACCTATGCTGGACTTACG</td>
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<td></td>
<td>Top2a</td>
<td>CGGAGGAGTAGGAGAAGATTTG</td>
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<td>Gg13</td>
<td>CTGCTAGGACATGGAAATAG</td>
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<td>Cyp3a65</td>
<td>AAGGCTACCGGGAGGAGACA</td>
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<td>Cyp3c1</td>
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<td>AAGGGTCAAGGAGGATTTGTCG</td>
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<td>AGAGCTATGAGCCTGGACG</td>
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<tr>
<td></td>
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<td>CGCGAAGATCCCATACCCA</td>
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many liver diseases, such as hepatitis, cirrhosis, and hepato-cellular carcinoma. miR-122, specifically expressed in hepatocytes, is the most frequent miRNA in adult liver. It serves a regulatory role in a variety of biological processes, including hepatocyte growth, lipid metabolism and stress response. miR-122 is a biomarker of liver injury in chronic hepatitis B or C, non-alcoholic fatty-liver disease (NAFLD) and drug-induced liver disease. It has been reported that drug treatment reduced miR-122 level and caused hepatotoxicity in previous studies. In this study, we used the expression of miR-122 to evaluate liver injury. The RNA levels of miR-122 in the GTW treatment groups significantly
IL-1β, IL-6, and TNF-α are pro-inflammatory cytokines and act as important inflammatory mediators. In this study, the mRNA levels of il1β, il6, and mfa significantly increased after GTW treatment, suggesting that GTW induces inflammation. IL-10 is a negative regulator of inflammation. In this study, the mRNA levels of il10 in the GTW treatment groups significantly increased, which may represent feedback regulation: that is, the inflammation stimulates the expression of il10, which then inhibits inflammation. Prostaglandin E2 (PGE2) is also an inflammatory mediator. Macrophages and other cells are stimulated by inflammation to produce an important enzyme that regulates the synthesis of prostaglandins, COX. One of the isoenzymes, COX-2, acts as an inducible enzyme and can participate in inflammation regulation. PTGES can be activated and regulated by COX-2 to produce PGE2 during the inflammatory response. In this study, the mRNA levels of cox2 and ptges in the GTW treatment groups significantly increased, indicating that GTW treatment results in increased inflammation in zebrafish. TGF-β serves an important regulatory role in inflammation, tissue repair, embryonic development, cell growth and differentiation, and immune function, and can inhibit the production of cytokines. In this study, GTW treatment caused a significant decrease in the expression of tgfβ, which is consistent with the above results that the expression of cytokines increased. These results indicated that GTW induces inflammatory response.

Inflammatory response is a defensive response of the body to internal and external stimuli. The inflammatory response triggers the activation of immune cells and then releases a series of inflammatory cytokines to remove foreign stimuli to promote tissue healing. However, excessive inflammatory responses can cause tissue damage.

Pathological results also showed that a large number of vacuoles appeared in the liver tissues after GTW treatment. To detect the apoptosis in zebrafish after GTW treatment, the expression of apoptosis-related genes were examined. Bcl2 is an anti-apoptotic protein. The results showed that the mRNA level of bcl2 in the GTW treatment groups significantly decreased, indicating that its inhibitory effects on apoptosis are weakened, and that apoptosis is enhanced. Caspase-8 and Caspase-9 are proteases that serve a key role in apoptosis, and their mRNA levels also significantly increased after GTW treatment, further indicating that GTW treatment can induce apoptosis in zebrafish.

decreased, which is consistent with the above results and further indicates that GTW can induce liver damage in zebrafish.

How does GTW induce liver injury in zebrafish? According to previous reports, miR-122 inhibition led to significant alteration in the expression pattern of key genes in inflammation (IL-6 and TNF-α) and even liver cell death. Nakamura et al. reported that miR-122 inhibited the production of inflammatory cytokines, such as IL-6 and IL-1β, by targeting the PKR activator PACT. So the mRNA expression of inflammation-related genes were examined.
Fig. 6  Effects of GTW on the expression of liver injury-related genes at 72 hpe. (A) Gene expression of inflammatory cytokines. (B) Gene expression of apoptosis signaling pathway. (C) Gene expression of cell proliferation signaling pathway. (D) Gene expression of liver function-related genes. *P < 0.05, **P < 0.01, ***P < 0.001 vs control

Subsequently, the expression of the genes involved in cell proliferation were examined. TOP2α is mainly associated with cell proliferation and cell pluripotency [30]. UHRF1 also serves an important role in maintaining the methylation status of daughter cells, cell growth, and genomic stability [31]. In this study, the mRNA levels of top2α and uhrf1 significantly decreased after GTW treatment, indicating that GTW causes abnormal cell proliferation.

The expression of liver-related genes were also examined. Acute phase proteins (APPs) are synthesized and secreted by the liver in response to external stimuli, such as inflammation, infection, trauma, and shock [32]. Increased APPs serve as a marker of acute inflammation [23]. ALR is an acute phase protein secreted by liver cells with a strong role in promoting liver regeneration and the proliferation of damaged liver cells. In addition, ALR can also participate in the regulation of inflammatory responses by stimulating Kupffer cells to secrete TNF-α, IL-6 and nitric oxide (NO) [34, 35]. ALR can be used as a marker for early stage liver injury [35]. In this study, the mRNA levels of alr significantly increased after GTW treatment, indicating that GTW causes liver injury. With respect to the above results that the mRNA levels of pro-inflammatory cytokines significantly increased, there may be a mutual regulatory effect between ALR and pro-inflammatory cytokines. Cytochrome P450 (CYP) is a group of isozymes encoded by superfamily genes with related structure and functions: they are mainly distributed in the liver and represent the major molecules in the liver that exert detoxification effects. Most drugs are metabolized by CYP enzymes in the liver microsomes [36]. Mammalian CYP3A proteins are the most abundant CYPs in the liver and responsible for the oxidative metabolism of numerous medications. There are five orthologues of human CYP3A, cyp3a65 and cyp3c1–4, in zebrafish [37, 38]. Here we detected the expression of cyp3a65 and cyp3c1 in zebrafish. The results showed that GTW caused a significant decrease in the mRNA level of cyp3a65, a significant increase in the mRNA level of cyp3c1, and abnormalities in the metabolic enzymes of the liver, leading to...
abnormal liver function.

In summary, GTW treatment results in increased inflammation, enhanced apoptosis, decreased cell proliferation, and abnormal expression of liver function-related genes, which causes abnormal liver structure and function and liver injury, resulting in hepatotoxicity in zebrafish.

References