Triptolide increases resistance to bile duct ligation-induced liver injury and fibrosis in mice by inhibiting RELB

Zihang Yuan¹, Jie Wang¹, Haoran Zhang¹, Yingying Miao¹, Qianhui Tang¹, Ziqiao Yuan¹, Cheng Nong¹, Zhicheng Duan¹, Luyong Zhang¹,², Zhenzhou Jiang¹,³* and Qinwei Yu¹*

¹New Drug Screening Center, Jiangsu Center for Pharmacodynamics Research and Evaluation, State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China, ²Center for Drug Research and Development, Guangdong Pharmaceutical University, Guangzhou, China, ³Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, Nanjing, China

Cholestasis is a common, chronic liver disease that may cause fibrosis and cirrhosis. Tripterygium wilfordii Hook.f (TWHF) is a species in the Euonymus family that is commonly used as a source of medicine and food in Eastern and Southern China. Triptolide (TP) is an epoxy diterpene lactone of TWHF, as well as the main active ingredient in TWHF. Here, we used a mouse model of common bile duct ligation (BDL) cholestasis, along with cultured human intrahepatic biliary epithelial cells, to explore whether TP can relieve cholestasis. Compared with the control treatment, TP at a dose of 70 or 140 µg/kg reduced the serum levels of the liver enzymes alanine transaminase, aspartate aminotransferase, and alkaline phosphatase in mice; hematoxylin and eosin staining also showed that TP reduced necrosis in tissues. Both in vitro and in vivo analyses revealed that TP inhibited cholangiocyte proliferation by reducing the expression of RelB. Immunohistochemical staining of CK19 and Ki67, as well as measurement of Ck19 mRNA levels in hepatic tissue, revealed that TP inhibited the BDL-induced ductular reaction. Masson 3 and Sirius Red staining for hepatic hydroxyproline showed that TP alleviated BDL-induced hepatic fibrosis. Additionally, TP substantially inhibited BDL-induced hepatic inflammation. In summary, TP inhibited the BDL-induced ductular reaction by reducing the expression of RelB in cholangiocytes, thereby alleviating liver injury, fibrosis, and inflammation.

KEYWORDS
RelB, bile duct ligation (BDL), triptolide, TNFSF14, cholangiocyte
Introduction

Cholestasis can reflect either a functional defect in bile formation at the hepatocyte level or impairment in bile secretion/flow at the bile duct level (1). Cholestasis manifests as the excessive accumulation of biliary components (e.g., bile acid, cholesterol, and bilirubin) in the liver and systemic circulation. The clinical symptoms include liver injury, severe pruritus, jaundice, and fatigue; severe cases can cause acute liver failure (2). Chronic cholestasis may eventually lead to liver fibrosis and cirrhosis (3).

Ursodeoxycholic acid and chenodeoxycholic acid are the preferred drugs for treatment of cholestasis (4). However, tolerance may develop (5). Recently, obeticholic acid (a 6-ethyl derivative of chenodeoxycholic acid) was approved for the treatment of primary biliary cirrhosis—in conjunction with ursodeoxycholic acid—in patients with inadequate responses to ursodeoxycholic acid; it was approved as monotherapy for patients who are unable to tolerate ursodeoxycholic acid (6–8). However, hepatic decompensation, liver failure, and death have been reported in patients with Child-Pugh B or C cirrhosis who receive doses of obeticholic acid above the recommended level. Thus, the Food and Drug Administration placed a black box warning on the obeticholic acid label for patients with decompensated liver disease. New, inexpensive therapeutic agents are needed for effective relief of cholestasis symptoms.

Traditional Chinese medicine dietary supplements alleviate various forms of liver injury including cholestasis (9–12). *Tripterygium wilfordii* Hook.f (TWHF) is a species of Tripterygium in the Euonymus family (13); the dried root (“thunder god vine”) serves as a “bitter and cold” traditional Chinese medicine (14) in Eastern and Southern China. The root is also cooked in southern China. Triptolide (TP) is an epoxy diterpene lactone of TWHF (15), as well as the principal active ingredient in TWHF (13). TP exhibits potent immunosuppressive and antiproliferative activities (16); it effectively treats rheumatoid arthritis (17), diabetic kidney disease (18), and prostate cancer (19, 20). A TP dietary supplement reportedly alleviates senile osteoporosis (21); it reduces stress, and increases longevity (22). We previously showed that TP was active against colon cancer (23, 24). The NF-κB protein complex regulates cell survival (25), aging (26), cytokine production (27), and obesity (28); NF-κB is the principal target of TP. NF-κB transcriptional inhibition by TP can suppress inflammation (29) and tumor growth (30). Thus far, the effects of TP on cholestasis remain unknown. In this study, we used a mouse model of common bile duct ligation (BDL) to explore whether TP can effectively treat cholestasis. Our findings provide a rationale for TP as complementary medicine of the preferred drugs or alternative medicine for cholestasis.

Materials and methods

Materials

Triptolide (CAS number 38748-32-2, purity > 98%) was purchased from Sanleng Biotech (Guilin, China). TNFSF14 Elisa Kit (CSB-EL023991MO) was purchased from Cusabio (China).

Primary antibodies against RelB (10544), α-SMA (19245) and F4/80 (70076) was purchased from Cell Signaling Technology (USA). Primary antibody against CK19 (TROMA-III) was purchased from DSHB (USA). Primary antibody against GAPDH (60004-1-lg) was purchased from Proteintech (China). Primary antibody against Ki67 (ab16667) was purchased form Abcam (USA). Primary antibody against Ly6g (4-5931-82) was purchased from Thermofisher (USA). Rabbit and Mouse secondary antibody (31460, 31430) were purchased from Santa Cruz (USA). Primary antibody against Ly6g (4-5931-82) was purchased from Abcam (USA). Primary antibody against Ki67 (ab16667) was purchased from Thermofisher (USA). Rat secondary antibody (GB23302) were purchased from Servicio (China).

Animal surgery procedure

Male C57BL/6J mice (6–8 weeks) were supplied by Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China). The animal study was reviewed and approved by the China Pharmaceutical University Experimental Animal Ethics Committee. Mice were housed in conditions with controlled light (12 h light/dark cycle), temperature (24 ± 2°C), and humidity (50–60%) and had adequate food and tap water. Cholestasis was induced by common bile duct ligation (BDL). Mice were anesthetized using 2–3% isoflurane during the entire infection procedure, where the abdominal cavity was opened from the abdominal midline. All experiments on mice were performed under the guidelines of Ethical Committee of China Pharmaceutical University. Triptolide in powder was suspended in 0.5% CMC-Na and administered to mice by gavage. The doses selected for TP in animal experiments were 70 µg/kg and 140 µg/kg (31). The common bile duct was ligated twice with a 7-0 nylon suture. The sham operation group involved the same operation, but the common bile duct was not ligated. After one-week acclimatization, the mice were then randomly separated into four groups (*n* = 6 per group): (1) Control mice (sham operated); (2) BDL mice; (3) BDL with TP at 70 µg/kg administration; (4) BDL with TP at 140 µg/kg administration. BDL performed at three days after TP treatment. Mice in sham and BDL group were given corresponding vehicle. After BDL the mice still were treated with TP once a day. Seven days after BDL surgery, mice were sacrificed (Figure 1A).

Serum alanine aminotransferase (ALT) levels were measured using kits from Whitman Biotech (Nanjing, China). Hepatic hydroxyproline was measured using kits from Nanjing
Triptolide alleviates liver injury induced by bile duct ligation. Male C57BL/6 mice were sacrificed at seven days after BDL or sham surgery. (A) The diagrammatic experimental procedures. (B) Body weight of mice (each group n = 6). (C) Serum levels of ALT, AST and ALP in mice sacrificed at 7 days after BDL or sham surgery. (D) Representative images of H&E (The black dotted line indicates the necrotic area) from liver tissues. Necrosis area statistics of H&E. Scale Bar: 100 µm. Data are shown as the mean ± SD. Data represent at least 6 independent experiments with triplicate measurements. Analysis of variance (one-way ANOVA) was used. p values represent significance different from BDL group.
FIGURE 2
Triptolide inhibits proliferation and RelB expression in HiBEC. (A) The growth curve of HiBEC after transfection of siRNA-Relb. (B) The protein level of RelB in HiBEC after TP treatment. (C) The growth curve of HiBEC after TP treatment. (D) Double immunofluorescence staining for CK19 (green) and Ki67 (red) from HiBEC after TP treatment. Nuclei were counter-stained with DAPI (blue). (E) Relb, Tnfsf14 and Ltβ mRNA was measured in HiBEC after TP treatment. Data are shown as the mean ± SD. Data represent at least 3 independent experiments with triplicate measurements. Analysis of variance (one-way ANOVA) was used. p values represent significance different from control group.
Cell culture

Human intrahepatic biliary epithelial cells (HiBEC) were purchased from ScienCell and cultured in a EpiCAM (ScienCell) (Zhongqiaoxinzhou) containing 2% fetal bovine serum (FBS), EpiCGs (ScienCell), 5 µg/mL insulin and 0.5 µM hydrocortisone.

Small interference RNAs (siRNA) were purchased from GenePharma (Shanghai). The siRNA sequences used in this study are as follows: RelB-1 siRNA sense: 5′-GCCGUGUAGCCAGAAATT-3′; antisense: 5′-UUUCUGUAGACGCGCTT-3′. RelB-2 siRNA sense: 5′-GCACAGUUAGCGAGTA-3′; antisense: 5′-AUCUCAACUGUAGCCGCTT-3′. Negative control siRNA: 5′-UUCUCGAACUGUAGCCGCTT-3′. HiBECs were seeded in six-well plate one day before transfection. HiBECs were transfected using Lipofectamine 3000 transfection kit (thermoshifer, USA) according to the manufacturer’s instructions. Transfected cells were used for the subsequent experiments 48 h after transfection.

The growth cure of HiBECs was measured with the cck-8 kit (vazyme, China) assay. HiBEC were seeded in 96-well plate (5000 cells per well). The plates were incubated in full EpiCAM. Cck-8 working fluid were added in the plate 100 µL per well at 24h, 48h, 72h, 96h. After 1 h incubation with cck8, OD450 was detected using a spectrophotometer (Multiskan MK3, Thermofisher, USA).

Quantitative real-time polymerase chain reaction

RNA from tissues and cells was extracted with TRIzol (vazyme, China). The RNA concentration was determined using Nanodrop2000 Spectrophotometers (Thermo Scientific, USA). cDNA was generated using BIO-RAD MyCyclerThermal Cycler (BIO-RAD, USA) and the HighCapacity cDNA Reverse Kit. qPCR was performed using StepOnePlus (Applied Biosystems, USA) with specific primers (Table 1). Primers were purchased from Genescript (China). Results were normalized using GAPDH as an internal control.

Immunoblot analysis

Protein content was analyzed by lysing tissues and cells with RIPA buffer containing protease inhibitors and the Bradford Protein Assay Kit. Western blot analysis was performed following a previously described method (32). Protein bands were detected with a Tanon 5200Muti (Tanon, China) using ECL reagents. The gray density of the protein bands was determined using ImageJ. All quantitative comparisons between samples were on the same gels/blots.
FIGURE 4
Triptolide relieves BDL-induced bile duct hyperplasia. (A) Representative images of IHC for CK19 and Ki67 (The black arrows indicate the regenerated cholangiocytes) from liver tissues. Scale Bar: 100 µm. (B) Statistical analysis of immunohistochemically positive regions of (A). (C) The mRNA level of Ck19 in liver tissues. Data are shown as the mean ± SD. Data represent at least 6 independent experiments with triplicate measurements. Analysis of variance (one-way ANOVA) was used. *p values represents significance different from BDL group.

TABLE 1 Primer sequences used for RT-PCR analysis.

| Gene     | Forward primer (5′-3′)                          | Reverse primer (5′-3′)                          |
|----------|-----------------------------------------------|-----------------------------------------------|
| mouse Ccn2 | GGGCCCTCTTCCTTCGGATTC                              | ATCACGCGCAAGTGCAATGGTA                         |
| mouse Gapdh | CTTG GCCATTGGAGAGGCC                            | CAGGGATGATGGCTGGGCA                           |
| mouse Acta2 | TGCTGAGAGACGGACACTGGAA                         | CAGTTGACCTGCGAGGGCATAG                         |
| mouse Colla1 | CTTCAGGAGATCTGAGCACAC                         | CAGAGGAGCTGGTTGAGGA                           |
| mouse Tgf-β1 | GCCATGCCCCATCTCTCTT                             | CACCAGGGAGACACTGTCATAC                        |
| mouse F4/80 | CTGGTTGTGTTGGTGAGCAGA                         | TGGACCTCCTCAGAGGTCGAGCA                       |
| mouse Il-1β | TGACCTCCTCAGAGGTCGAGCA                         | CACCACATGTTGACAGGAGAAC                       |
| mouse Krt19 | ATGGCGAGCTGGAGTGGAA                             | CTGGAGGTCACCTCAGGAGCA                        |
| mouse Tnf-a | GGTACCCTAGGTCGCTTCTCTT                         | GCCATGAGCTGAGGAGAC                           |
| mouse Relb | GTTCTTTGGACCTTCCTCTCTCTT                      | TAGGGCAAGGCACATGCGCAGGCA                       |
| mouse Tgif14 | GGAGACATAGGTCATCATTCC                         | CACCAATACATCACAGGCGC                         |
| mouse Ltbp | CCGTTGTGTGGATGCGCTATC                         | GAGGCTTTGTTGCGCTATC                          |
| Human Relb | TGTTGAGAGATCTGAGGTCAG                          | TGGGCAATCCCGAGGCTGAT                          |
| Human Tgif14 | GGTCTCTGCTGTGCTGCTGCTAG                       | TTGACCTGAGGACCTGCTGCTAG                      |
| Human Ltbp | GTTCTGAGAGCTGCGAGAGAGA                        | GCTGAGAAAGGCCTGCGCTGCT                        |
| Human Gapdh | GTCTCTGACTCAGACAGGCG                         | ACCACCTGTTGCTGAGGAGCA                        |
FIGURE 5
Triptolide relieves BDL-induced liver fibrosis. (A) Representative images of Masson 3, Sirius Red, and IHC for α-SMA from liver tissues. Scale Bar: 100 μm. (B) Collagen positive area statistics of Masson3 and Sirius Red. (C) Hydroxyproline assay of liver tissues. (D) The mRNA levels of Acta2, Col1a1, Ccn2, and Tgf-β1 from liver tissues. Data represent at least 6 independent experiments with triplicate measurements. Analysis of variance (one-way ANOVA) was used. p values represents significance different from BDL group.
Immunofluorescence

Cells on coverslips were fixed in 4% paraformaldehyde for 15 min, washed with PBS, and permeabilized in PBS with 1% Triton for 10 min. Cells were then incubated with 5% goat serum in PBS for 1 h at room temperature before being incubated with antibodies overnight at 4°C. The next day, cells on a round coverslip were washed three times with PBS and incubated for 1 h at room temperature with secondary Alexa antibodies and DAPI. Fluorescence images were scanned using a FV3000 (Olympus, Japan).

Statistical analysis

All data were shown as mean ± SD and at least three replicate experiments were performed in vitro and in vivo. The necrotic, Masson3 positive and Sirius Red positive area were analyzed using Image J software. Statistical significance was determined using one-way analysis of variance as appropriate (GraphPad Prism 9, GraphPad Software Inc., CA).

Results

Triptolide alleviates bile duct ligation-induced liver injury

To explore the effects of TP on cholestasis-induced liver injury, we established a mouse model of BDL and administered two TP doses by oral gavage; such doses were previously reported to attenuate chronic kidney disease (31). A schematic of the mouse model is depicted in Figure 1A. Figure 1B shows that TP at a dose of 70 or 140 µg/kg attenuated the BDL-induced weight loss. BDL increased the serum levels of the liver enzymes alanine transaminase, aspartate aminotransferase, and alkaline phosphatase. Either dose of TP substantially reduced the levels of these enzymes (Figure 1C). Histopathological staining revealed less necrosis around the portal tract when BDL mice were treated with TP (Figure 1D). Thus, TP effectively treated BDL-induced liver injury.

Triptolide inhibits proliferation and RelB expression in human intrahepatic biliary epithelial cells

Bile duct hyperplasia is common in patients with cholestasis; cholangiocyte proliferation and a ductular reaction contribute to the onset and progression of liver disease (32–34). Members of the NF-κB family of transcription factors act through a canonical pathway and a non-canonical pathway. Non-canonical NF-κB signaling activates predominantly p100-sequestered NF-κB proteins, the most important of which is RelB (35). This protein is involved in the ductular reaction; the bile ducts of patients with primary sclerosing cholangitis and primary biliary cirrhosis exhibit increased levels of RelB. RelB and its downstream target lymphotxin β (LTβ) affect the proliferation of bile duct epithelial cells (36). TP inhibits the expression of NF-κB proteins (37). Here, we analyzed HiBECs in vitro. We hypothesized that TP would reduce cholangiocyte proliferation by inhibiting the expression of RelB.

Triptolide inhibits bile duct ligation-induced expression of RelB and downstream genes

Western blotting revealed that the protein level of RelB increased after BDL. Both TP doses substantially reduced the level of RelB (Figure 2A). Enzyme-linked immunosorbent assay analysis showed that BDL increased the expression of serum tumor necrosis factor superfamily member 14 (TNFSF14), whereas TP inhibited this increase (Figure 3B). qPCR analysis of hepatic tissue showed that BDL upregulated the mRNA expression levels of Relb and the downstream genes Tnfsf14 and Ltβ (Figure 2E).

Triptolide relieves bile duct ligation-induced bile duct hyperplasia

The above results indicated that TP inhibited the BDL-induced upregulation of Relb and downstream genes (Tnfsf14 and Ltβ) in hepatic tissue. Increased levels of RelB lead to a ductular reaction. Cytokeratin-19 (CK19) is solely expressed by cholangiocytes. Immunohistochemical analysis of CK19 revealed that BDL induced prominent bile duct hyperplasia; TP inhibited this process (Figures 4A,B). Immunohistochemical analysis of Ki67 revealed many positive cells (black arrows) in bile ducts after BDL; TP significantly reduced the numbers of these cells (Figures 4A,B), indicating that TP alleviated bile duct hyperplasia.
hyperplasia. qPCR analysis showed that TP significantly reduced the BDL-induced upregulation of Ck19 (Figure 4C).

**Triptolide relieves bile duct ligation-induced liver fibrosis**

TNFSF14, which acts downstream of RelB, promotes hepatic stellate cell activation and exacerbates liver fibrosis (38). Staining with Masson 3 and Sirius Red confirmed that TP decreased collagen deposition around the portal fields in BDL mice (Figures 5A,B). α-Smooth muscle actin [also known as actin alpha 2 (ACTA2)] is a marker of hepatic stellate cell activation; immunohistochemical staining of α-smooth muscle actin decreased around the portal area (Figure 5A). Hydroxyproline is a characteristic component of collagen; in BDL mice, the hepatic levels of hydroxyproline were substantially lower after treatment with TP at a dose of 70 or 140 μg/kg, compared with those levels in the control group (Figure 5C). Next, we examined the expression of liver fibrosis-related genes. Col1a1 is an important collagen component, and its expression significantly increases in fibrotic tissues. Connective tissue growth factor [also known as cellular communication network factor 2 (CTGF/CCN2)] and transforming growth factor beta-1 (TGF-β1) are markers of liver fibrosis, and they both directly activate hepatic stellate cells and promote collagen deposition; BDL elevates the levels of both proteins (39, 40). The mRNA expression levels of Acta2, Col1a1, Ccn2, and Tgf-β1 were
downregulated when BDL mice were treated with TP at a dose of 70 or 140 µg/kg (Figure 5D).

**Triptolide relieves bile duct ligation-induced hepatic inflammation**

Ductular reactions are often accompanied by inflammatory infiltrates (33, 41). Therefore, we examined the effect of TP on hepatic inflammation. For this purpose, we conducted immunohistochemical staining of F4/80 (also known as mouse EGF-like module-containing mucin-like hormone receptor-like 1), which is expressed by various mature macrophages including Kupffer cells; we also performed immunohistochemical staining of the lymphocyte antigen 6 complex locus G6D (LY6G), a neutrophil-specific marker. BDL-induced enhancement of F4/80 and LY6G staining was decreased by TP at a dose of 70 or 140 µg/kg; thus, TP reduced hepatic inflammatory infiltration (Figure 6A). The mRNA expression levels of genes encoding the inflammatory factors F4/80, interleukin-1β, and tumor necrosis factor-α were significantly reduced when BDL mice received TP at a dose of 70 or 140 µg/kg (Figure 6B). These findings indicated that TP attenuated hepatic inflammatory infiltration in BDL mice.

**Discussion**

We explored whether TP protected against liver injury progression in a mouse model of common BDL. TP at a dose of 70 or 140 µg/kg effectively treated BDL-induced liver injury. Liver enzyme measurement and H&E staining revealed that TP at a dose of 70 or 140 µg/kg significantly alleviated liver damage. Analysis of the liver hydroxyproline content, along with Masson 3 and Sirius Red staining, revealed that TP inhibited BDL-induced liver fibrosis. qPCR analysis of Ck19 transcripts, as well as immunohistochemical staining of CK19 and Ki67, showed that TP significantly inhibited the BDL-induced ductular reaction. TP substantially reduced hepatic inflammatory infiltration after BDL, as revealed by immunohistochemical staining of F4/80 and Ly6G, as well as the mRNA expression levels of F4/80, Il-1β, and Tnf-α in hepatic tissue. In vitro analysis demonstrated that TP dramatically downregulated the protein and mRNA expression levels of these inflammatory factors.
expression levels of RelB, as well as the downstream genes Tnfsf14 and Ltβ, thereby slowing the growth of HiBECs. Assessment of protein and mRNA expression levels in hepatic tissue revealed that TP attenuated the BDL-induced upregulation of RelB and downstream genes. The serum TNFSF14 assay confirmed that TP alleviated the BDL-induced upregulation of RelB. Graphic abstract was shown in Figure 7.

**Tripterygium wilfordii** Hook.f (TWHF) exhibits anti-inflammatory (41), anti-fertility (42), anti-colitis (43), and anti-cancer activities (44). At present, the clinical medication of TWHF is mainly used for rheumatoid arthritis, lupus and purpuric nephritis, psoriasis, erythroderma and allergic diseases. There are no clinical trials linking TWHF with cholestatic disease. However, the therapeutic window for TP is narrow; clinical applications are compromised by severe toxicities, including hepatotoxicity (37). The doses in this study were chosen because TP at a dose of 70 µg/kg substantially alleviated chronic kidney disease (31); we also used a higher dose for comparison. In our previous study, it was found that there was no obvious liver toxicity and cholestasis symptoms when TP 250 µg/kg was administered for 7 days (45). Therefore, we believe that the TP dose used in this study is a safe dose. TP-induced hepatotoxicity cannot be ignored. We previously found that TP was hepatotoxic at a dose of 500 or 600 µg/kg (46, 47). Evidently, TP at a dose of 70 or 140 µg/kg significantly alleviated BDL-induced liver injury, liver fibrosis, the ductular reaction, and hepatic inflammatory infiltration. Therefore, the role of TP in cholestasis is dose-dependent. The specific mechanism may be related to the complex immune homeostasis in the liver, and the specific mechanism will be carried out in future studies. The above content shows that TP needs more research before its clinical use.

**Conclusion**

**Tripterygium wilfordii** Hook.f (TWHF) exhibits anti-inflammatory (41), anti-fertility (42), anti-colitis (43), and anti-cancer activities (44). At present, the clinical medication of TWHF is mainly used for rheumatoid arthritis, lupus and purpuric nephritis, psoriasis, erythroderma and allergic diseases. There are no clinical trials linking TWHF with cholestatic disease. However, the therapeutic window for TP is narrow; clinical applications are compromised by severe toxicities, including hepatotoxicity (37). The doses in this study were chosen because TP at a dose of 70 µg/kg substantially alleviated chronic kidney disease (31); we also used a higher dose for comparison. In our previous study, it was found that there was no obvious liver toxicity and cholestasis symptoms when TP 250 µg/kg was administered for 7 days (45). Therefore, we believe that the TP dose used in this study is a safe dose. TP-induced hepatotoxicity cannot be ignored. We previously found that TP was hepatotoxic at a dose of 500 or 600 µg/kg (46, 47). Evidently, TP at a dose of 70 or 140 µg/kg significantly alleviated BDL-induced liver injury, liver fibrosis, the ductular reaction, and hepatic inflammatory infiltration. Therefore, the role of TP in cholestasis is dose-dependent. The specific mechanism may be related to the complex immune homeostasis in the liver, and the specific mechanism will be carried out in future studies. The above content shows that TP needs more research before its clinical use.

**Data availability statement**

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.
Ethics statement

The animal study was reviewed and approved by China Pharmaceutical University Experimental Animal Ethics Committee.

Author contributions

ZJ and ZhY designed the overall research experiments. ZhY, JW, HZ, YM, QT, ZqY, and CN performed the experiments. ZhY and JW analyzed the data. ZhY wrote the manuscript. ZJ and QY reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Postgraduate Research Practice Innovation Program of Jiangsu Province (KYCX19_0674 to ZhY), the Natural Science Foundation of Jiangsu Province (BK202221526 to ZJ), National Natural Science Foundation of China (81773827 and 82074114 to ZJ), “Double First-Class” University project (CPU2018 GY33 to ZJ), and China Postdoctoral Science Foundation (2020M681786 to QY).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1032722/full#supplementary-material

References

1. Zollner G, Trauner M. Mechanisms of cholestasis. Clin Liver Dis. (2008) 12:1–26. doi: 10.1016/j.cld.2007.11.010
2. Cai SY, Boyer JL. Bile infarcts: new insights into the pathogenesis of obstructive cholestasis. Hepatology. (2019) 69:473–5. doi: 10.1002/hep.30291
3. European Association for the Study of the Liver. EASL clinical practice guidelines: management of cholestatic liver diseases. J Hepatol. (2009) 51:237–67. doi: 10.1016/j.jhep.2009.04.009
4. Beuers U. Drug insight: mechanisms and sites of action of ursodeoxycholic acid in cholestasis. Nat Clin Pract Gastroenterol Hepatol. (2009) 5:318–28. doi: 10.1038/ncpgastro0521
5. Samur S, Klebanoff M, Banken R, Pratt DS, Chapman R, Ollendorf DA, et al. Long-term clinical impact and cost-effectiveness of obeticholic acid for the treatment of primary biliary cholangitis. Hepatology. (2017) 65:920–8. doi: 10.1002/hep.28932
6. Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, et al. Agonist endowed with anticholestatic.
7. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, et al. Agonist endowed with anticholestatic.
8. Nevens F, Andreoni P, Mazzella G, Strasser SI, Bowls C, Invernizzi P, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med. (2016) 375:631–43. doi: 10.1056/nejmoa1509940
9. Neves F, Andreone P, Mazzella G, Strasser SI, Bowls C, Invernizzi P, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med. (2016) 375:631–43. doi: 10.1056/nejmoa1509940
10. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. (2015) 385:956–65. doi: 10.1016/S0140-6736(14)61933-4
11. Crocenni F, Roma M. Silymarin as a new hepatoprotective agent in experimental cholestasis: new possibilities for an ancient medication. Curr Med Chem. (2006) 13:1055–74. doi: 10.2174/092986706776369950
12. Wang X, Han L, Bi Y, Li C, Gao X, Fan G, et al. Paradoxical effects of emodin on anti-induced intrahepatic cholestasis and herb-induced hepatotoxicity in mice. Toxicol Sci. (2019) 168:264–78. doi: 10.1093/toxsci/kfy295
13. Li XI, Jiang ZZ, Zhang YI. Triptolide: progress on research in pharmacodynamics and toxicology. J Ethnopharmacol. (2014) 155:67–79. doi: 10.1016/j.jep.2014.06.006
14. Xia M, Liu D, Liu H, Zhao J, Tang C, Chen G, et al. Based on network pharmacology tools to investigate the mechanism of tripterygium willow arsenic cholestasis. Front Med. (2021) 8:794962. doi: 10.3389/fmed.2021.794962
15. Zhang X, Zhang X, Wang X, Wang T, Bai B, Zhang N, et al. Efficient delivery of triptolide plus a milk-30-5p inhibitor through the use of near infrared laser responsive or CADY modified MSNs for efficacy in rheumatoid arthritis. Int J Mol Sci. (2020) 23:101536. doi: 10.3390/ijms20202387
16. Datan E, Minn I, Xu P, He QL, Ahn HH, Yu B, et al. Glucose-triptolide conjugate selectively targets cancer cells under hypoxia. iScience. (2020) 23:101536. doi: 10.3390/ijms20202387
17. Fan D, Guo Q, Shen J, Zheng K, Lu C, Zhang G, et al. The effect of triptolide in rheumatoid arthritis: from basic research towards clinical translation. Int J Mol Sci. (2018) 19:376. doi: 10.3390/ijms19120376
18. Li XJ, Jiang ZZ, Zhang LY. Triptolide: progress on research in pharmacodynamics and toxicology. J Ethnopharmacol. (2014) 155:67–79. doi: 10.1016/j.jep.2014.06.006
19. Huang W, He T, Chai C, Yang Y, Zheng Y, Zhou P, et al. Triptolide inhibits the proliferation of prostate cancer cells and down-regulates SUMO-specific proteasome 1 expression. PLoS One. (2012) 7:e37693. doi: 10.1371/journal.pone.0037693
20. Yan P, Sun X. Triptolide: a new star for treating human malignancies. J Cancer Res Ther. (2018) 14(Suppl.):5271–5. doi: 10.4103/0973-1482.235340
Yuan et al. 10.3389/fnut.2022.1032722

21. Luo D, Ren H, Zhang H, Zhang P, Huang Z, Xian H, et al. The protective effects of triptolide on age-related bone loss in old male rats. *Biomed Pharmacother.* (2018) 98:280-5. doi: 10.1016/j.biopha.2017.12.072

22. Kim SL, Beak SM, Park SK. Supplementation with triptolide increases resistance to environmental stressors and lifespan in C. elegans. *J Food Sci.* (2017) 82:E1490-7. doi: 10.1111/1750-3841.13720

23. Li H, Li I, Mei H, Pan G, Wang X, Huang X, et al. Antitumor properties of triptolide: phenotype regulation of macrophage differentiation. *Cancer Biol Ther.* (2020) 21:178-88. doi: 10.1089/cbr.2019.1679555

24. Li H, Xing X, Zhang X, Li L, Jiang Z, Wang T, et al. Effects of triptolide on the sphingosine kinase - Sphingosine-1-phosphate signaling pathway in colitis-associated colon cancer. *Int Immunopharmacol.* (2020) 88:106892. doi: 10.1016/j.intimp.2020.106892

25. Luo J, Kamata H, Karin M. IKK/NF-κB signaling: balancing life and death - a new approach to cancer therapy. *J Clin Invest.* (2005) 115:2625-32. doi: 10.1172/JCI26322

26. Garcia-García VA, Alameda JP, Page A, Casanova ML. Role of nf-kb in ageing and age-related diseases: lessons from genetically modified mouse models. *Cell.* (2021) 10:1906. doi: 10.3390/cells10081906

27. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther.* (2017) 2:17023. doi: 10.1038/sigtrans.2017.23

28. Sahir JS, El-Omri A, Shaik NA, Banaganapalli B, Al-Shaeri MA, Alkenna NA, et al. Identification of key regulatory genes connected to NF-κB family of proteins in visceral adipose tissues using gene expression and weighted protein interaction network. *PLoS One.* (2019) 14:e0214337. doi: 10.1371/journal.pone.0214337

29. Yang J, Tang X, Ke X, Dai Y, Shi J. Triptolide suppresses NF-κB-mediated inflammatory responses and activates expression of Nrf2-mediated antioxidant genes to alleviate caerulein-induced acute pancreatitis. *Int J Mol Sci.* (2022) 23:1252. doi: 10.3390/ijms23031252

30. Yinjun L, Ji J, Yinguwi W. Triptolide inhibits transcription factor NF-kappab and induces apoptosis of multiple myeloma cells. *Leuk Res.* (2005) 29:99–105. doi: 10.1016/j.leukres.2004.05.014

31. Yoshida T, Yamashita M, Horimi C, Hayashi M. Smooth muscle-selective nuclear factor-κB inhibition reduces phosphate-induced arterial medial calcification in mice with chronic kidney disease. *J Am Heart Assoc.* (2017) 6:e007248. doi: 10.1161/JAHA.117.007248

32. Sato K, Marzioni M, Meng F, Francis H, Glaser S, Alpini G. Ductular reaction in liver diseases: pathological mechanisms and translational significances. *Hepatology.* (2019) 69:420–30. doi: 10.1002/hep.30150

33. Wang Y, Aoki H, Yang J, Peng K, Liu R, Li X, et al. The role of sphingosine 1-phosphate receptor 2 in bile-acid-induced cholangiocyte proliferation and phosphatidylcholine and cholesterol metabolism in mouse liver. *J Lipid Res.* (2021) 62:635–43. doi: 10.1002/hep.27744

34. Ghonem NS, Assis DN, Boyer JL. Fibrotic development in acute lung injury in a murine model. *Acta Pharm Sin B.* (2021) 11:1902. doi: 10.1016/j.apsb.2020.11.006

35. Elflner C, Goeppert B, Longerich T, Scherr AL, Stindt J, Nanduri LK, et al. Osteopontin is induced by hedgehog pathway activation and promotes fibrosis in biliary atresia. *Pediatr Res.* (2020) 88:106892. doi: 10.1016/j.pedres.2020.106892

36. Noël P, Von Hoff DD, Saluja AK, Velagapudi M, Borazanci E, Han H. Triptolide and its derivatives as cancer therapies. *Trends Pharm Sci.* (2019) 40:327–71. doi: 10.1016/j.tips.2019.03.002

37. Yuan Z, Zhang H, Hasmat M, Ding J, Chen X, Liang P, et al. New perspective of triptolide-associated hepatotoxicity: liver hypersensitivity upon LPS stimulation. *Toxicology.* (2019) 414:45–56. doi: 10.1016/j.tox.2019.01.005

38. Wang X, Jiang Z, Cao W, Yuan Z, Sun L, Zhang L. Th17/Treg imbalance in triptolide-induced liver injury. *Fibrotropia.* (2014) 9:245–51. doi: 10.1016/j.fibro.2014.01.006

39. Wang X, Jiang Z, Xing M, Fu J, Su Y, Sun L, et al. Interleukin-17 mediates triptolide-induced liver injury in mice. *Food Chem Toxicol.* (2014) 71:33–41. doi: 10.1016/j.fct.2014.06.004

40. Ghonem NS, Assis DN, Boyer JL. Fibrous and cholestasis. *Hepatology.* (2015) 62:623–43. doi: 10.1002/hep.27744

41. Yang M, Ramachandran A, Yan HM, Woolbright BL, Copple BL, Fickett P, et al. Osteopontin is an initial mediator of inflammatory and liver injury during obstructive cholestasis after bile duct ligation in mice. *Toxicon Lett.* (2014) 224:186–95. doi: 10.1016/j.toxlet.2013.10.030

42. Whittington PF, Malladi P, Melin-Aldana H, Azran M, Rackl CI, Sahai A. Expression of osteopontin correlates with portal biliary proliferation and fibrosis in biliary atresia. *Pediatr Res.* (2005) 57:837–44. doi: 10.1203/01.PDR.0000161414.99181.61

43. Syn WK, Choi SS, Liaskou E, Karaca GF, Agboola KM, Oo YH, et al. Osteopontin is induced by hedgehog pathway activation and promotes fibrosis progression in nonalcoholic steatohepatitis. *Hepatology.* (2011) 53:106–15. doi: 10.1002/hep.23998

44. Nuñez-García M, Gomez-Santos B, Buqué X, García-Rodriguez JL, Romero MR, Marin JJG, et al. Osteopontin regulates the cross-talk between phosphatidylincholine and cholesterol metabolism in mouse liver. *J Lipid Res.* (2017) 58:1903–15. doi: 10.1194/jlr.M078980.2014.01.006

45. Wang X, Aoki H, Yang J, Peng K, Liu R, Li X, et al. The role of sphingosine-1-phosphate receptor 2 in bile-acid-induced cholangioocyte proliferation and cholestasis-induced liver injury in mice. *Hepatology.* (2017) 65:2005–18. doi: 10.1002/hep.29076

46. Coombes JD, Swiderska-Syn M, Döllé L, Reid D, Eksteen B, Claridge L, et al. Osteopontin neutralisation abrogates the liver progenitor cell response and fibrogenesis in mice. *Gut.* (2015) 64:1120–31. doi: 10.1136/gutjnl-2013-306844

47. Hou KH, Wei CW, Su YR, Chou T, Lin YL, Yang FC, et al. Upregulation of ReβL in the mll-122 knockout mice contributes to increased levels of proinflammatory chemokines/cytokines in the liver and macrophages. *ImmunoLett.* (2020) 226:22–30. doi: 10.1016/j.imlet.2020.06.015