Swertianlarin, an Herbal Agent Derived from Swertia mussotii Franch, Attenuates Liver Injury, Inflammation, and Cholestasis in Common Bile Duct-Ligated Rats

Liangjun Zhang, Ying Cheng, Xiaohuang Du, Sheng Chen, Xinchun Feng, Yu Gao, Xiaoxue Li, Lin Liu, Mei Yang, Lei Chen, Zhihong Peng, Yong Yang, Weizao Luo, Rongquan Wang, Wensheng Chen, and Jin Chai

1 Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
2 Department of Traditional Chinese Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
3 Department of Pediatrics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
4 Chongqing Academy of Chinese Material Medicine, Chongqing 400065, China

Correspondence should be addressed to Jin Chai; chaijinsw@yahoo.com

Received 29 September 2014; Revised 23 December 2014; Accepted 24 December 2014

1. Introduction

Accumulation of toxic bile acids in hepatocytes and the amplification of inflammation in cholestasis are the major factors resulting in liver injury and fibrosis [1–3]. Multiple pathologies, that is, gallstone obstruction of the bile duct, biliary atresia, pancreas tumors, and drug toxicity, can cause persisting cholestasis which can lead to liver failure, fibrosis, cirrhosis, and death [4–6]. Ursodeoxycholic acid (UDCA) and its analogs can inhibit inflammation and enhance elimination of toxic bile acids. It is the only approved drug that is widely clinically used to treat cholestatic patients such as primary biliary cirrhosis (PBC) [7, 8]. However, one-third to two-thirds of cholestatic patients with PBC do not completely respond to UDCA [9, 10]. INT747, an FXR agonist, is a Phase II studies drug that exerts anticholestatic effects by altering bile acids metabolism in experiment models [11–14]. Therefore, the search for potential drugs that target inflammation and bile acids pools for the treatment of cholestasis is important.

Swertianlarin is an iridoid compound that is present in Swertia davidi Franch and Swertia mussotii Franch [15–18]. These two types of Swertia Franch are the traditional herbs used for the treatment of jaundice caused by viral hepatitis. It has been used for many centuries in southwest China and is supposed to protect the liver from injury [15, 18],
have anti-inflammatory properties, and ameliorate cholestasis. Swertianlarin from Enicostemma axillare is known to have antioxidant and hepatoprotective effects against D-galactosamine-induced acute liver damage in rats [19]. Previous studies have also reported that swertianlarin inhibits inflammation in adjuvant-induced arthritis and in interleukin IL-1β-induced rat fibroblast-like synoviocytes [20–23]. However, the anti-inflammation effect of swertianlarin on cholestasis remains to be clarified. Furthermore, whether swertianlarin alters the bile acid pool and reduces liver injury on cholestasis is unclear.

To address whether swertianlarin derived from Swertia mussotii Franch attenuates inflammation and liver injury on cholestasis, we assessed the liver injury, serum proinflammatory cytokine levels, and the concentrations of serum bile acids in a bile duct-ligated rat model treated with swertianlarin.

2. Materials and Methods

2.1. Chemicals. Swertianlarin, isolated from Swertia mussotii Franch as reported previously [24], was kindly provided by Chongqing Academy of Chinese Material Medical with a purity of 98% as analyzed by HPLC. All other chemicals were of analytical grade and purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA).

2.2. Animals and Treatments. The male Sprague-Dawley (SD) rats were provided by the Center of Laboratory Animals of Third Military Medical University, Chongqing, China. All experimental protocols were approved by the Ethics Committee of Third Military Medical University. Efforts were made to reduce animal suffering and minimize animal number used. Animals were housed in plastic cages with free access to food and water under a 12 h light/dark cycle. The normal rats were given swertianlarin in 4 groups: 0, 10, 50, and 100 mg/(kg/d), dissolved in 1% Tween-20 saline, n = 7, by gavage for 14 days. All rats survived and the serum biochemistries alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (TBIL), and direct bilirubin (DBIL) were normal in the study (data not shown). In this study, rats were randomly divided into three groups (n = 7 per group): sham saline, BDL without swertianlarin, and BDL with swertianlarin with each containing from five to seven animals. In the BDL swertianlarin group, rats were pretreated with swertianlarin 100 mg/(kg/d) dissolved in 1% Tween-20 by gavage for 1 d and then underwent BDL followed by continued administration of swertianlarin for 3, 7, and 14 d. In the BDL saline groups, rats underwent BDL after pretreatment with only 1% Tween-20 saline by gavage for 1 d and then continued treatment with only 1% Tween-20 saline for 3, 7, and 14 d. In the sham saline control group, rats were pretreated with 1% Tween-20 saline by gavage for 1 d and underwent a sham-operation followed by only 1% Tween-20 saline for 3, 7, and 14 d. After 3, 7, and 14 d, animals were sacrificed randomly between 9:00 am and 11:00 am. Blood was placed on ice for 60 min and centrifuged (8000 g, 10 min) to prepare serum. The serum was immediately stored at −80°C until used. The liver samples were immediately cut into small pieces and frozen in liquid nitrogen until used. The collected serum and liver samples were used for biochemistry and histopathology studies as described previously [25].

2.3. Serum Biochemistry. The concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acids (TBA), total bilirubin (TBIL), and direct bilirubin (DBIL) in serum samples were analyzed by standard enzymatic assays using commercial kits.

2.4. Determination of Serum Proinflammatory Cytokines TNFα, Interleukin-1β, and Interleukin-6. Serum samples from sham operated rats and bile duct-ligated rats with and without swertianlarin were collected before sacrifice (n = 7 per each group) and stored at −80°C until analysis. Serum cytokines, tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β), and IL-6 levels were determined by ELISA Kits (BlueGene Biotechnology, Shanghai, China) according to the manufacturer's instructions [25, 26].

2.5. Serum Bile Salts and Lipids Determination. The concentrations of serum bile acids chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDDCA), cholic acid (CA), taurocholic acid (TCA), deoxycholic acid (DCA), taurodeoxycholic acid A (TDC), tauroursodeoxycholic acid (TUDCA), tauro-alpha/beta-muricholic acid (Taα/βMCA), alpha-muricholic acid (αMCA), and beta-muricholic acid (βMCA) levels, serum lipid triglyceride hydrolase (Tgh), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by standard enzymatic assays using commercial kits purchased from BlueGene Biotech (Shanghai, China), according to the manufacturer's protocol as described previously [27, 28].

2.6. Histopathology. Rat liver samples were fixed in 4% formalin and subjected to standard histological procedures and paraffin embedding. Liver sections (5 μm in thickness) were stained with hematoxylin and eosin and evaluated for histological lesions.

2.7. Statistical Analysis. All values were expressed as mean ± standard deviation (SD). One-way analysis of variance followed by Dunnett’s multiple comparison post hoc test was used for statistical analysis of data using GraphPad Prism 6.01 (GraphPad Software Inc., San Diego, CA). A value of P < 0.05 was considered to be statistically significant.

3. Results

3.1. Swertianlarin Attenuates Liver Injury in Common Bile Duct-Ligated Rats. Figure 1(a) shows that serum ALT, AST, and ALP were significantly increased in both BDL rats treated with and those without swertianlarin for 3, 7, and 14 days, compared to sham group with saline (P < 0.05, Figure 1(a)).
Figure 1: Continued.
3.2. Swertianlarin Has Anti-Inflammatory Effect on BDL Rats. To investigate whether swertianlarin has anti-inflammatory effects, the concentration of serum proinflammatory cytokines TNFα, IL-1β, and IL-6 were measured by ELISA assay in the sham operated group and BDL groups with or without swertianlarin. The results showed that the serum TNFα levels for 3 and 7 d were dramatically increased while they were not significantly increased for 14 d in cholestatic rats without swertianlarin compared to sham operated rats (Figure 2(a)). However, the levels of serum TNFα in cholestatic rats with BDL for 3 and 7 d were significantly lower with swertianlarin treatment, 63% and 64% of saline BDL group, respectively ($P < 0.05$). However, they were not significantly different for 14 d, compared to the BDL group without swertianlarin (Figure 2(a)). Furthermore, serum IL-1β levels were significantly increased for 14 d while they were unchanged for 3 and 7 d in the BDL group with swertianlarin compared to those of the sham operated group (Figure 2(b)). However, the serum IL-1β levels for 3 and 14 d were noticeably lower in the swertianlarin BDL group (43% and 42% of BDL group without swertianlarin, resp.; $P < 0.05$). Moreover, the serum IL-1β levels were not altered by swertianlarin for 7 d (Figure 2(b)). Figure 2(c) shows that serum IL-6 levels were increased for 14 d but was decreased for 3 d ($P < 0.05$) and was not different for 7 d in BDL group without swertianlarin compared to the sham operated group (Figure 3(c)). Serum IL-6 in the swertianlarin BDL group for 14 d was 61% of saline BDL group ($P < 0.05$), whereas the serum IL-6 levels were not affected by swertianlarin for 3 and 7 d (Figure 3(c)). These results demonstrated that swertianlarin treatment reduced levels of serum proinflammatory cytokines TNFα, IL-1β, and IL-6 in this rat model.

3.3. Swertianlarin Alters the Concentration of Serum Bile Salts in Rats with Bile Duct Ligation. The accumulation of bile salts is the major factor resulting in the liver injury in cholestasis [1, 3, 21]. Because an apparent decrease in liver injury in cholestatic rats was associated with swertianlarin treatment, it was determined if swertianlarin alters the concentration of serum bile salts in cholestasis. The results showed that CDCA was lower in BDL rats without swertianlarin for 3, 7, and 14 days compared with sham operated rats ($P = 0.31$, $P < 0.05$, and $P < 0.05$, Figure 3(a)). In contrast, the TCDCA levels were increased in the BDL rats without swertianlarin for 7 d but were unaltered after 3 and 14 d (Figure 3(a)). The administration of swertianlarin decreased CDCA levels after 7 and 14 d and reduced TCDCA levels after 7 d in BDL rats ($P < 0.05$, Figure 3(a)). The concentration of serum CA was unchanged in BDL rats without swertianlarin, compared to the sham operated group. However, the serum CA levels were...
Figure 2: Alterations of serum proinflammatory cytokines TNFα, IL-1β, and IL-6 levels by swertianlarin in BDL rats. (a) The changes of serum TNFα in the sham operated, BDL with swertianlarin, and BDL without swertianlarin groups for 3, 7, and 14 d. (b) The alterations of serum IL-1β in sham operated, BDL with swertianlarin, and BDL without swertianlarin groups for 3, 7, and 14 d. (c) The determination of serum IL-6 in sham operated rats and BDL rats treated with or without swertianlarin after 3, 7, and 14 d. Data were analyzed as described in Materials and Methods. ∗P < 0.05 versus sham group with saline; #P < 0.05 versus BDL group with saline.

...
Figure 3: Continued.
group after 3 and 7 d but were higher in the BDL group without swertianlarin compared to the sham group for 14 d ($P < 0.05$). However, serum $\alpha$MCA levels were decreased in the BDL group with swertianlarin for 3, 7, and 14 d ($P < 0.05$), compared with the BDL group without swertianlarin (Figure 3(f)). Serum $\beta$MCA levels were lower in the BDL group without swertianlarin for 3 d ($P < 0.05$) while they were not significantly changed for 7 d and 14 d, compared with the sham group. Administration of swertianlarin for 14 d significantly decreased serum $\beta$MCA levels in the BDL rats ($P < 0.05$, Figure 3(f), right). These results indicated that swertianlarin administration was associated with lower levels of conjugated and unconjugated bile salts in BDL rats.

### 3.4. Swertianlarin Does Not Affect Lipids Levels in BDL Rats

Serum Tgh, TG, and LDL-C levels for 3 and 7 d were higher, but unchanged for 14 d in BDL saline rats, compared with the sham group ($P < 0.01$, Figures 4(a), 4(b), and 4(d)). However, the HDL-C levels were increased for 3 d but decreased for 14 d in BDL rats (Figure 4(c)). Furthermore, there was no difference in serum HDL-C in BDL rats after 7 d (Figure 4(c)). The results showed that levels of Tgh, TG, HDL-C, and LDL-C were not significantly different in BDL rats with or without swertianlarin (Figure 4). These results suggest that lipid metabolism was not affected by swertianlarin in this model.

### 4. Discussion

Persisting cholestasis caused by bile duct obstruction (i.e., gallstones and pancreas tumors), hepatitis, and drugs can lead to liver failure, fibrosis, and cirrhosis [1–6]. The accumulation of bile acids in hepatocytes and hepatic chronic inflammation plays key roles in cholestatic liver injury [1–3]. The drugs that enhance elimination of toxic bile acids, such as UDCA and INT747 are beneficial for cholestatic patients [7–14]. In the present study, it was observed that swertianlarin attenuates liver injury and inflammation and is associated with lower concentrations of bile salts in BDL rats.

Models of cholestasis can be produced by administration of some drugs, for example, $\alpha$-naphthylisothiocyanate (ANIT) (intrahepatic cholestasis), or by bile duct ligation (extrahepatic cholestasis) [27–29]. The presence of toxic bile acids is the major factor resulting in liver injury in cholestasis [1, 2]. The present study showed that certain conjugated and unconjugated bile acids in serum were reduced in BDL rats by swertianlarin treatment for 3, 7, and 14 d, implying that swertianlarin attenuates cholestasis. Serum bile acid levels were reduced in BDL rats treated with swertianlarin for 14 d. These data may explain why levels of serum markers of liver injury, ALT, and AST, as well as liver necrosis determined by histopathology, were significantly decreased in BDL rats treated with swertianlarin for 14 d. Many studies have demonstrated that inhibition of bile acid synthetic enzymes (e.g., Cyp7a1), upregulation of detoxification enzymes (e.g., Cyp3a11 and Ugt2b), and bile acids transporters (e.g., Mrp3 and Mrp4) contribute to the repression of bile acid synthesis, the reduction of bile salt toxicity. This may occur by increasing water solubility and elimination of bile acids, alleviating cholestasis and liver injury in cholestatic animal models and in human cholestatic patients using UDCA and INT747 [1, 2, 25]. The differences in timing of alterations of serum bile acids levels in BDL rats may be due to changes in hepatic bile acid transporters, synthetic and detoxification enzymes regulated by swertianlarin. However, this hypothesis
Figure 4: The lipids Tgh, TG, HDL-C, and LDL-C levels were unaffected by swertianlarin in bile duct-ligated rats. (a) Serum Tgh, (b) TG, (c) HDL-C, and (d) serum LDL-C levels were measured in sham operated rats, BDL rats without swertianlarin, and BDL rats with swertianlarin for 3, 7, and 14 d. Data were analyzed as described in Materials and Methods. *P < 0.01; #P < 0.05, versus sham group with saline. n = 7 per group. Saline, 1% Tween-20 saline; swertianlarin dissolved in 1% Tween-20 saline.

needs to be tested by more in-depth studies in future. Moreover, it was found that the lipid levels Tgh, TG, HDL-C, and LDL-C were not different in swertianlarin-treated BDL rats compared to BDL rats without swertianlarin. However, serum lipid levels were severely disturbed in all of BDL rats. These results suggested that BDL not only affected bile acid homeostasis, but also interfered with lipid homeostasis. In addition, one recent study also reported that swertianlarin from Enicostemma axillare had antioxidant and hepatoprotective effects against D-galactosamine-induced acute liver damage in rats [19]. These support the protective role of swertianlarin on cholestasis.

We also demonstrated that elevated serum levels of proinflammatory cytokines TNFα for 3 and 7 d, IL-1β, and IL-6 for 14 d in BDL rats were significantly reduced by swertianlarin treatment, implying that swertianlarin has an anti-inflammatory effect on cholestatic process. These observations in BDL rats with swertianlarin treatment are similar to those previously described in adjuvant-induced arthritis and IL-1β-induced rat fibroblast-like synoviocyte models [20, 21]. A recent study reported that inflammation plays an important role in the progression of the pathology of cholestasis [3]. Knockout of TNFα in a cholestatic mouse model reduced liver injury and fibrosis [30]. Therefore, swertianlarin may also alter the progression of cholestasis by inhibiting inflammation. However, whether swertianlarin exerts an anti-inflammatory effect on cholestasis by the activation of NF-κB and JAK signaling, as reported previously [20], remains to be clarified.

Although the swertianlarin-treated BDL rats had less liver injury, inflammation, and cholestasis than those without swertianlarin, these results are from one cholestasis model. Whether swertianlarin also exerts the protective role in other cholestatic models (e.g., lipopolysaccharide-induced
cholestasis) or human cholestasis (e.g., primary biliary cirrhosis) needs further studies. Moreover, the molecular mechanism by which swertianlarin affects cholestasis remains to be determined.

5. Conclusions

The results demonstrated that swertianlarin from *Swertia mussotii* Franch attenuates liver injury, inflammation, and cholestasis in BDL rats. The findings of the present study contribute to understanding the protective role of swertianlarin in cholestasis and provide preliminary data for the development of potential drugs for the treatment of cholestasis.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Liangjun Zhang and Ying Cheng equally contributed to this study.

Acknowledgments

This work was supported by National Natural Science Foundation of China (81070320, 81100280, and 81470880), Scholarship Foundation of China Scholarship Council (CSC no. 81070320, 81100280, and 81470880), Scholarship Foundation of Third Military Medical University (2013).

References

[1] M. Trauner, P. J. Meier, and J. L. Boyer, "Mechanisms of disease: molecular pathogenesis of cholestasis," *The New England Journal of Medicine*, vol. 339, no. 17, pp. 1217–1227, 1998.

[2] M. Wagner, G. Zollner, and M. Trauner, "New molecular insights into the mechanisms of cholestasis," *Journal of Hepatology*, vol. 51, no. 3, pp. 565–580, 2009.

[3] B. L. Woolbright, D. J. Antoine, R. E. Jenkins, M. L. Bajt, B. K. Park, and H. Jaeschke, "Plasma biomarkers of liver injury and inflammation demonstrate a lack of apoptosis during obstructive cholestasis in mice," *Toxicology and Applied Pharmacology*, vol. 273, no. 3, pp. 524–531, 2013.

[4] W. R. Kim, J. Ludwig, and K. D. Lindor, "Variant forms of cholestatic diseases involving small bile ducts in adults," *The American Journal of Gastroenterology*, vol. 95, no. 5, pp. 1130–1138, 2000.

[5] R. Poupon, O. Chazouilleres, and R. E. Poupon, "Chronic cholestatic diseases," *Journal of Hepatology*, vol. 32, no. 1, supplement, pp. 129–140, 2000.

[6] H. Yang, D. J. Antoine, U. Andersson, and K. I. Tracey, "The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis," *Journal of Leukocyte Biology*, vol. 93, no. 6, pp. 865–873, 2013.

[7] G. Paumgartner and T. Puhl, "Medical treatment of cholestatic liver disease," *Clinics in Liver Disease*, vol. 12, no. 1, pp. 53–80, 2008.

[8] P. Fickert, M. Wagner, H.-U. Marschall et al., “24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice,” *Gastroenterology*, vol. 130, no. 2, pp. 465–481, 2006.

[9] A. Parés, L. Caballeria, and J. Rodés, “Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic acid,” *Gastroenterology*, vol. 130, no. 3, pp. 715–720, 2006.

[10] C. Corpechot, L. Abenavoli, N. Rabahi et al., “Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis,” *Hepatology*, vol. 48, no. 3, pp. 871–877, 2008.

[11] Y. Liu, J. Binz, M. J. Nemerick et al., “Hepatoprotection by the farnesoid X receptor agonist GW40646 in rat models of intra- and extrahepatic cholestasis,” *The Journal of Clinical Investigation*, vol. 112, no. 11, pp. 1678–1687, 2003.

[12] M. Trauner, M. Wagner, P. Fickert, and G. Zollner, "Molecular regulation of hepatobiliary transport systems: clinical implications for understanding and treating cholestasis," *Journal of Clinical Gastroenterology*, vol. 39, no. 4, supplement 2, pp. S11–S124, 2005.

[13] L. Huang, A. Zhao, J. L. Lew et al., “Farnesoid X receptor activates transcription of the phospholipid pump MDR3,” *The Journal of Biological Chemistry*, vol. 278, no. 51, pp. 51085–51090, 2003.

[14] A. Moschetta, A. L. Bookout, and D. J. Mangelsdorf, “Prevention of cholesterol gallstone disease by FXR agonists in a mouse model,” *Nature Medicine*, vol. 10, no. 12, pp. 1352–1358, 2004.

[15] H. Yang, C. Ding, Y. Duan, and J. Liu, “Variation of active constituents of an important Tibetan folk medicine *Swertia mussotii* Franch. (Gentianaceae) between artificially cultivated and naturally distributed,” *Journal of Ethnopharmacology*, vol. 98, no. 1–2, pp. 31–35, 2005.

[16] C. Tian, T. Zhang, L. Wang, Q. Shan, and L. Jiang, “The hepatoprotective effect and chemical constituents of total iridoids and xanthones extracted from *Swertia mussotii* Franch,” *Journal of Ethnopharmacology*, vol. 154, no. 1, pp. 259–266, 2014.

[17] R. L. Gao, L. Wang, Y. Yang et al., “Simultaneous determination of oleanolic acid, ursolic acid, quercetin and apigenin in *Swertia mussotii* Franch by capillary zone electrophoresis with running buffer modifier,” *Biomedical Chromatography*, vol. 29, no. 3, pp. 402–409, 2015.

[18] G. Fan, W.-Z. Luo, S.-H. Luo et al., “Metabolic discrimination of *Swertia mussotii* and *Swertia chirayita* known as ‘Zangyinchen’ in traditional Tibetan medicine by $^1$H NMR-based metabolomics,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 98, pp. 364–370, 2014.

[19] V. Jaishree and S. Badami, “Antioxidant and hepatoprotective effect of swertiamarin from *Enicostemma axillare* against d-galactosamine induced acute liver damage in rats,” *Journal of Ethnopharmacology*, vol. 130, no. 2, pp. 465–481, 2010.

[20] S. Saravanavan, V. I. Hairul Islam, N. Prakash Babu et al., “Swertiamarin attenuates inflammation mediators via modulating kappaB $\beta$/kappaB and Jak2/Stat3 transcription factors in adjuvant induced arthritis,” *European Journal of Pharmaceutical Sciences*, vol. 56, no. 1, pp. 70–86, 2014.

[21] S. Saravanavan, V. I. H. Islam, K. Thirugnanasambantham et al., “Swertiamarin ameliorates inflammation and osteoclastogenesis intermediates in IL-1beta induced rat fibroblast-like synoviocytes,” *Inflammation Research*, vol. 63, no. 6, pp. 451–462, 2014.
[22] K. S. Park, B. H. Kim, and I.-M. Chang, “Inhibitory potencies of several iridoids on cyclooxygenase-1, cyclooxygenase-2 enzymes activities, tumor necrosis factor-α and nitric oxide production in vitro,” Evidence-Based Complementary and Alternative Medicine, vol. 7, no. 1, pp. 41–45, 2010.

[23] H. B. Vaidya, S. Giri, M. Jain, and R. K. Goyal, “Decrease in serum matrix metalloproteinase-9 and matrix metalloproteinase-3 levels in Zucker fa/fa obese rats after treatment with swertiamarin,” Experimental and Clinical Cardiology, vol. 17, no. 1, pp. 12–16, 2012.

[24] J. Vaijanathappa and S. Badami, “Antiedematogenic and free radical scavenging activity of swertiamarin isolated from Enicostemma axillare,” Planta Medica, vol. 75, no. 1, pp. 12–17, 2009.

[25] J. Chai, Y. He, S. Y. Cai et al., “Elevated hepatic multidrug resistance-associated protein 3/ATP-binding cassette subfamily C 3 expression in human obstructive cholestasis is mediated through tumor necrosis factor alpha and c-jun NH2-terminal kinase/stress-activated protein kinase-signaling pathway,” Hepatology, vol. 55, no. 5, pp. 1485–1494, 2012.

[26] H. He, A. Mennone, J. L. Boyer, and S.-Y. Cai, “Combination of retinoic acid and ursodeoxycholic acid attenuates liver injury in bile duct-ligated rats and human hepatic cells,” Hepatology, vol. 53, no. 2, pp. 548–557, 2011.

[27] Y. Wang, L. Jing, X.-M. Zhao et al., “Protective effects of hydrogen-rich saline on monocrotaline-induced pulmonary hypertension in a rat model,” Respiratory Research, vol. 12, no. 4, article 26, 2011.

[28] Y. Tanaka, L. M. Aleksunes, Y. J. Cui, and C. D. Klaassen, “ANIT-induced intrahepatic cholestasis alters hepatobiliary transporter expression via Nrf2-dependent and independent signaling,” Toxicological Sciences, vol. 108, no. 2, pp. 247–257, 2009.

[29] B. L. Copple, H. Jaeschke, and C. D. Klaassen, “Oxidative stress and the pathogenesis of cholestasis,” Seminars in Liver Disease, vol. 30, no. 2, pp. 195–204, 2010.

[30] A. Geier, C. G. Dietrich, M. Trauner, and C. Gartung, “Extrahepatic cholestasis downregulates Oatp1 by TNF-α signalling without affecting Oatp2 and Oatp4 expression and sodium-independent bile salt uptake in rat liver,” Liver International, vol. 27, no. 8, pp. 1656–1665, 2007.