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Experimental Study on the Treatment of Gallbladder Distention by Dahuang Lingxian Prescription

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Abstract

Objective: To investigate the mechanism of inhibiting the secretion of "lithogenic bile" and treating gallbladder distention with the TCM prescription Dahuang Lingxian. Methods: Forty SPF-grade SD male mice were randomly divided into blank control group, model group, ursodeoxycholic acid group and TCM group, 10 mice in each group. The blank control group was kept normally, and the model group, ursodeoxycholic acid group and TCM group were established as gallbladder distention models. The blank control group and the model group were given 1.5 ml of saline by gavage daily; the ursodeoxycholic acid group was given 100 mg/kg/d of a mixture of saline and ursodeoxycholic acid capsules by gavage daily; the TCM group was given 39 g/kg/d of aqueous decoction of Dahuang Lingxian Prescription by gavage daily. After 6 weeks of continuous intervention, all mice were executed and the lithogenesis rate, ultrastructure and mRNA expression were observed. Results: The lithogenesis rate of mice in the TCM group was significantly reduced ($P < 0.0083$). The cell ultrastructure showed that the nuclear membrane was intact, the endoplasmic reticulum and mitochondrial content were significantly increased, and the expression of key mRNAs in the TGF-β1/Smads pathway was significantly improved ($P < 0.05$), which could achieve the same therapeutic effect as ursodeoxycholic acid capsules. Conclusion: Dahuang Lingxian Prescription can reduce the secretion of "lithogenic bile" by inhibiting the inflammatory reaction and fibrosis of biliary system. Dahuang Lingxian Prescription has certain advantages in the treatment of gallbladder distention.

Keywords

Gallbladder Distention, Dahuang Lingxian Prescription, Cell Ultrastructure, Signal Transduction, Traditional Chinese Medicine

1. Introduction

Gallbladder distention is a disease caused by the abnormal secretion of bile in the biliary system, resulting in pathological deposits [1]. The key to the treatment of gallbladder distention is how to inhibit the secretion of "lithogenic bile" and make the bile secretion smooth [2]. A large number of scholars have conducted a lot of basic research on the treatment mechanism of gallbladder distension, and how to inhibit the secretion of "lithogenic bile" by regulating the transduction effect of signaling pathways is the current hot spot of research [3]. The treatment of gallbladder distension can be divided into surgical treatment and non-surgical treatment. The surgical treatment usually chooses cholecystectomy, partial hepatectomy, minimally invasive cholelithotomy and so on. In non-surgical treatment, stone soluble drugs such as tauroursodeoxycholic acid are often selected for prevention and treatment. Surgical treatment and non-surgical treatment can minimize the damage of the body, preserve the functional gallbladder, and effectively improve the quality of life of patients [4]. However, the operation cost is more expensive, and long-term use of stone dissolution drugs will also produce certain adverse reactions, so traditional Chinese medicine has a good advantage in the treatment of bile distension. Chinese medicine has been increasingly used in the treatment of gallbladder distension, and it can improve the metabolic function of bile acids by activating the signaling pathways, such as farnesoid X recptor (FXR)-mediated transport effect [5], bile salt exportpump (BSEP) and cholesterol 7a-hydroxylase (cholesterol 7a-hydroxylase). (CYP7A1) mediated bile acid synthesis and excretion [6], transforming growth factor beta 1 (TGF-β1) mediated fibrosis [7], interleukin, IL)-mediated inflammatory response [8], etc., have been shown to be effective in inhibiting gallstone formation and treating gallbladder distension [9]. Therefore, it is important to investigate the effect of signaling pathways to inhibit the secretion of "lithogenic bile" for the treatment of gallbladder distension. How can TCM inhibit the secretion of "lithogenic bile" through the regulation of signaling pathways to achieve the purpose of treating gallbladder distension? It is still an urgent problem to be solved.

Gallbladder distension is the name of a disease in Chinese medicine, firstly found in the Yellow Emperor's Classic of Internal Medicine, which refers to the gallbladder disease with distension and pain in the right upper abdomen, accompanied by fever and jaundice. In modern medicine, gallstone disease and calculous cholecystitis belong to the category of gallbladder distension [10]. There is no uniform standard in Chinese medicine for establishing animal models of gallbladder distension. The study showed that bile acid is the most

important substance affecting the secretion of "lithogenic bile", and the abnormal secretion of bile acid in patients with gallbladder distension can affect the physiological function of FXR [11]. FXR was the first nuclear receptor identified to be closely linked to the physiological function of bile acids, regulating the enterohepatic circulation of bile acids and inhibiting gallstone formation [12].

Our preliminary study showed that the Chinese medicine, Dahuang Lingxian Prescription, can improve the symptoms of local inflammatory reaction and fibrosis due to gallstone disease and calculous cholecystitis. By mediating the effects of FXR on the synthesis, secretion, and transport of bile acids, which in turn regulate the physiological secretion of bile, promote the normal metabolism of bile, and inhibit the formation of gallstones [13], FXR, an important transcriptional regulator of bile acid metabolism, regulates the bile salt exportpump (BSEP), cholesterol 7a (cholesterol 7 alpha-hydrolase, CYP7A1) mediated signaling effects, and exert the ability to synthesize and excrete bile acids [14]. The conduction pathway mediated by BSEP and CYP7A1 can prevent tissue fibrosis and promote normal bile excretion by regulating transforming growth factor beta 1 (TGF-β1). Fibrosis is closely related to inflammatory response, and IL-mediated inflammatory response can also overexpress TGF-β1, leading to local symptoms such as pain, fever and jaundice [15]. TGF-β1 is the most direct target gene leading to fibrosis in the liver and biliary system and the most critical cytokine in inhibiting gallstone formation. Activated TGF-β1 phosphorylates type I cytokine receptors on the cell membrane surface, specifically recognizes and phosphorylates downstream Smads factors, exerts a regulatory inflammatory response and inhibits local fibrosis effect [16]. In our study, we used ursodeoxycholic acid capsules for control with Dahuang Lingxian Prescription, which is currently the drug of choice for gallbladder distension in China [17] [18]. Therefore, we investigated the mechanism of TCM in inhibiting gallstone formation, improving cell structure, and regulating TGF-β1/Smads signaling pathway, and investigated the mechanism of TCM in preventing fibrosis in the biliary system and inhibiting "lithogenic bile" secretion by using Dahuang Lingxian Prescription to intervene in a mouse model of gallbladder distension compared with ursodeoxycholic acid capsules. We will investigate the mechanism of Chinese medicine to prevent fibrosis and inhibit "lithogenic bile" secretion in biliary system.

Since the clinical manifestations produced by gallbladder distension in Chinese medicine include all manifestations including cholelithiasis and calculous cholecystitis, we combined the mechanism of action of Chinese medicine intervention in animal models of cholelithiasis and calculous cholecystitis and applied this research approach to the study of gallbladder distension to investigate the mechanism by which the transduction effect of Chinese medicine mediated signaling pathways prevents fibrosis in the biliary system and inhibits gallstone formation, which can better clarify the mechanism of action of Chinese medicine in treating gallbladder distension.

2. Materials and Methods

2.1. Experimental Animals

Forty SPF-grade SD male mice, with a mass of about 20 g, were housed in the experimental animal center provided by Guangxi University of Traditional Chinese Medicine, animal license number: SYXK Gui 2019-0001. The environmental humidity was controlled at 55% and constant temperature of 24˚C. The experiments were conducted after 1 week of acclimatization.

2.2. Experimental Reagents

The PCR reaction kit was produced by ABI, USA. Ursodeoxycholic acid capsules (0.25 g/25 capsules/box, approval number H20150365) were produced by Hawker, Germany. The lithogenic feeds (cholesterol, sucrose, cod liver oil) and Dahuang Lingxian Prescription (raw rhubarb, wei lingxian, mannitol, moneywort, heliotrope shell, zelenium, bupleurum, yujin, chicken neijin, magnet, astragalus, licorice) required for modeling were provided by the central pharmacy of the First Affiliated Hospital of Guangxi University of Chinese Medicine.

2.3. Experimental Methods

2.3.1. Establishment of Model Mice

We have successfully established animal models of cholelithiasis and stone cholecystitis, so in this paper, based on the LPS injection method [19] and animal cholelithiasis modeling method [20] developed by Professor Tang Qianli, we used high cholesterol and high calorie lithogenic feeds to induce a mouse model of gallbladder distension that meets the characteristics of both models.

2.3.2. Mouse Grouping and Drug Intervention

Forty mice were randomly selected as 10 mice for the blank control group, and the remaining 30 mice were firstly established as gallbladder distension model, and then randomly divided into model group, ursodeoxycholic acid group and TCM group respectively, with 10 mice in each group. According to our previous study, using the equivalent dose conversion method between pharmacological test animals and humans [21], the blank control group and model group were given 1.5 ml of saline by gavage daily; the ursodeoxycholic acid group was given 100 mg/kg/d of a mixture of saline and ursodeoxycholic acid capsules by gavage daily; the TCM group was given 39 g/kg/d of aqueous decoction of Dahuang Lingxian Fang (The TCM group was given a daily dose of 39 g/kg/d of Dahuang Lingxian Prescription water decoction (Dahuang Lingxian Prescription added to distilled water to make Dahuang Lingxian water decoction) for gavage. After 6 weeks of continuous intervention, all mice were executed.

2.3.3. Separation of Bile Duct and Gallbladder to Observe Stone Formation

After the mice were anesthetized, heparin at a dose of 100 U/100g was injected intravenously to prevent excessive coagulation, followed by portal vein puncture,

and 37˚C perfusion solution was pumped at a rate of 10 ml - 15 ml/min for 15 min. The bile ducts and gallbladder were separated by surgical dissection to observe the stone formation in the ducts and gallbladder. And the bile duct tissues were stored in −80˚C refrigerator, and a part of them were to be analyzed by electron microscopy and a part of them were to be detected by real-time fluorescent quantitative PCR.

2.3.4. Collection of Bile Ducts for Electron Microscopy

Bile duct tissues of approximately 1 mm × 1 mm × 1 mm in size were collected and placed in 2% glutaraldehyde for fixation. The bile duct tissues were washed in buffer and fixed in 1% osmium acid solution for 1 hour. Dehydration was performed with different concentrations of acetone solution in the order from low to high (usually 30% - 50% - 70% - 90% - 100%). Finally, embedding agent infiltration was performed so that the embedding agent gradually penetrated into the tissue cells. At the end of infiltration, the tissues were placed in a high temperature environment for fusion synthesis, and the cell structures were respectively examined after staining with lead citrate and uranyl acetate.

2.3.5. Extracted RNA, Designed Primers

The bile duct tissues were homogenized and centrifuged by adding lysate according to the reaction system, and the reaction solution was added sequentially and detected by UV spectrophotometer, and the extracts with OD values between 1.8 - 2.0 were selected as the extracted RNA. The NCBI database was searched for the full-length sequences of mouse-related genes, and Primer Express 5.0 software was used to design primers. TGF-β1 (F: 5'-ACTGGAGTTGTACGGCAGTG-3', R: 5'-TTTGGGGCTGATCCCGTTGATT-3', bp: 123);
Smad2 (F: 5'-GGGGCTGATCCCGTTGATT-3',
R: 5'-GCCCGGTAAATCTACCCAGAA-3', bp: 125);
Smad4 (F: 5'-TGTGGCTTCCACAAGTCAGC-3',
R: 5'-GTCCAGGTGGTAGTGCTGTT-3', bp: 153);
Smad7 (F: 5'-TCTCAAACCAACTGCAGGCT-3',
R: 5'-TTGGGAATCTGAAAGCCCCC-3', bp: 97); reference gene β-actin (F: 5'-CACTGTCGAGTCGCGTCC-3', R: 5'-GTCATCCATGGCGAACTGGT-3', bp: 90).

2.3.6. Real-Time Fluorescence Quantitative PCR

The RNA was added to the mixture according to the reaction system, RNase freed H_2O was added, shaken and mixed and briefly centrifuged, incubated at 37˚C for 60 minutes to synthesize the first strand of cDNA, and finally the RT-PCR reaction was measured, and the relative expression of each gene was calculated sequentially according to the Ct value, and the results were calculated by the fluorescence relative quantitative analysis ($2^{-\Delta\Delta Ct}$ method).

2.3.7. Statistical Treatment

SPSS 24.0 statistical software was used for data analysis. Count data were ex-

pressed by chi-square test, and measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Kruskal-Wallis H test was used for intra-group comparison, and differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Stone Formation in Each Group of Mice

Table 1. Lithogenesis in the biliary system of mice in each group after 6 weeks of intervention.

Group	Cases	Stone formation			χ^2	P
		No stones	With stones	Effective (%)		
Blank control group	10	10	0	100	25.916	0.000
Model group	10	0	10	0*		
Ursodeoxycholic acid group	10	8	2	80		
TCM group	10	8	2	80		

Note: Compared to the rest of the groups, *$P < 0.0083$.

3.2. Ultrastructure of Mouse Cells in Each Group (10,000×)

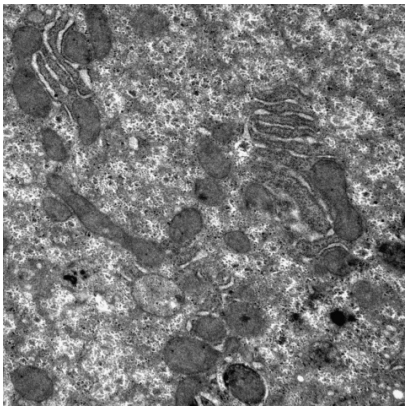

Figure 1. Model group.

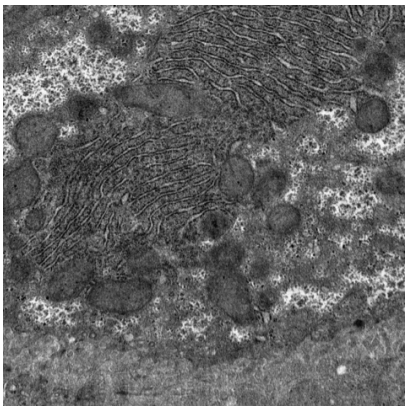

Figure 2. Blank control group.

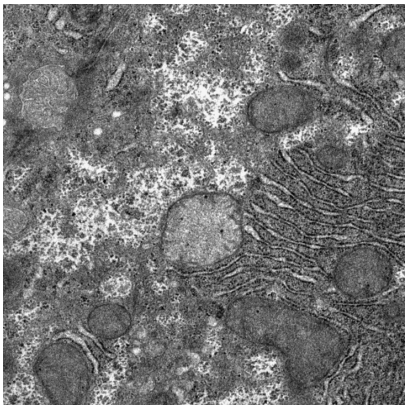

Figure 3. Ursodeoxycholic acid group.

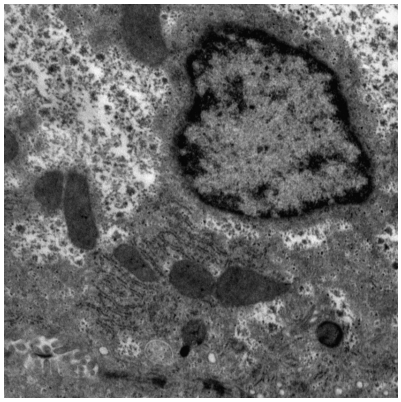

Figure 4. TCM group.

3.3. Expression of mRNA in Each Group of Mice

Table 2. Amplification of mRNA/β-actin in each group of mice after 6 weeks of intervention.

Group	n	TGF-β1	Smad2	Smad3	Smad4	Smad7
Blank control group	10	0.104 ± 0.013	0.103 ± 0.011	0.121 ± 0.029	0.516 ± 0.022	0.118 ± 0.031
Model group	10	0.410 ± 0.032*	0.255 ± 0.023*	0.378 ± 0.032*	0.190 ± 0.036*	0.030 ± 0.011*
ursodeoxycholic acid group	10	0.167 ± 0.034$^{*\Delta}$	0.139 ± 0.036$^{\Delta}$	0.114 ± 0.040$^{\Delta}$	0.391 ± 0.027$^{*\Delta}$	0.099 ± 0.039$^{\Delta}$
TCM group	10	0.175 ± 0.042$^{*\Delta}$	0.131 ± 0.021$^{\Delta}$	0.147 ± 0.035$^{\Delta}$	0.397 ± 0.034$^{*\Delta}$	0.102 ± 0.038$^{\Delta}$

Note: Compared with the blank control group, $^{*}P < 0.05$; Compared with the model group, $^{\Delta}P < 0.05$.

4. Discussion

Our study showed that Dahuang Lingxian Prescription has a good effect in improving the cell structure and inhibiting "lithogenic bile". As shown in **Table 1**, Dahuang Lingxian Prescription can significantly reduce the formation of gallstones. The cell structure also shows that under the intervention of Dahuang Lingxian Prescription, the nuclear membrane of the nucleus is intact, and the content of endoplasmic reticulum and mitochondria is significantly increased compared with those in the model group shown in **Figures 1-4** showed that the

number of mitochondria increased greatly, indicating that the injured cells of mice in the ursodeoxycholic acid group and TCM group were in the repair stage. Ursodeoxycholic acid capsule and Dahuang Lingxian Prescription both improved the cell microenvironment and promoted the recovery of damaged tissues. The PCR results in **Table 2** showed that down-regulation of TGF-β1 expression, bidirectional regulation of the expression of four subtypes of Smad family, inhibition of fibrosis, and achieved the same therapeutic effect as ursodeoxycholic acid group. These results all indicate that Dahuang Lingxian Prescription can restore the cell structure damage caused by cholestasis and biliary tract inflammation. Dahuang Lingxian Prescription can improve the cell structure in the biliary system, promote the normal secretion and excretion of bile, regulate the inflammatory response, and inhibit gallstone formation.

Our study has shown that the Dahuang Lingxian Prescription can inhibit the secretion of "lithogenic bile" by improving the morphology of biliary cells, affecting the metabolic function of cells, and regulating the transduction effect of signaling pathways for the treatment of gallbladder distension. However, bile synthesis and excretion are not only influenced by biliary cells but also by hepatocytes [22]. We speculate that Dahuang Lingxian Prescription can affect the function of hepatocytes and biliary cells, and play a role in inhibiting gallstone formation. Since our study is still in the preliminary stage, the number of experimental mice and specimens were small, and no side effects of Dahuang Lingxian Prescription were found. However, compared with the surgical treatment and non-surgical treatment, Dahuang Lingxian Prescription has less cost and clear efficacy, and no adverse reactions were found in animal experiments. Therefore, we believe that Dahuang Lingxian Prescription can better prevent gallbladder distension. We hope that the morphology and function of hepatocytes, as well as the signaling effects mediated by cytokines, can be investigated in depth in the next step.

Related studies have shown that TCM can improve cellular ultrastructure and restore normal function to damaged tissues by influencing the synthesis of biosynthetic silver nanoparticles and using the properties of nanosilver to regulate inflammatory responses and modulate signaling pathways [23]. TCM can also play a regulatory role by affecting TGF-β1: TGF-β1 positively regulates Smad2 and Smad3 and negatively regulates Smad4 and Smad7, and plays an important role in inducing cellular targeted aggregation, maintaining cellular growth cycle, and regulating apoptosis [24]. By specifically increasing the expression of Smad2 and Smad3, TGF-β1 can prevent local inflammatory reaction and fibrosis, improve the metabolic function of hepatocytes, and then prevent the production of "lithogenic bile" [25]. Smad4 and Smad7 are both inhibitory receptors, which can prevent Smad3 from forming heterologous complexes and block signal transduction [26]. Therefore, the TGF-β1/Smads signaling pathway can down-regulate Smad4 and Smad7 to further inhibit the inflammatory response and fibrosis, and improve cell activity by inhibiting the inflammatory response and fi-

brosis. Our study also showed that rhubarb lingxian can improve cell structure, metabolic function and regulate TGF-β1/Smads signaling effect. It can reduce inflammation and fibrosis in the biliary system, inhibit the secretion of "lithogenic bile", and enable the smooth synthesis and discharge of bile, thus achieving the purpose of treating gallbladder distension.

5. Conclusion

In summary, we speculate that the possible mechanism of the treatment of gallbladder distension by Dahuang Lingxian Prescription is to improve the metabolic function of hepatocytes and biliary cells by bidirectional regulation of TGF-β1/Smads signal transduction, so as to reduce the secretion of "lithogenic bile" and achieve the purpose of treating gallbladder distension. A large number of medical practitioners have explored the therapeutic mechanisms of gallbladder distension and certain research results have been made, but many areas still deserve in-depth exploration and research. Therefore, we will continue to explore the mechanism of Dahuang Lingxian Prescription in the treatment of gallbladder distension and investigate the mechanism of Chinese medicine in treating the disease by improving cell structure and cell function. The results of this study will provide a theoretical basis for research on the treatment of gallbladder distension and hopefully achieve new results.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Gallaher, J.R. and Charles, A. (2022) Acute Cholecystitis: A Review. *Journal of the American Medical Association*, **327**, 965-975. https://doi.org/10.1001/jama.2022.2350

[2] Latenstein, C.S.S. and de Reuver, P.R. (2022) Tailoring Diagnosis and Treatment in Symptomatic Gallstone Disease. *The British Journal of Surgery*, znac154. https://doi.org/10.1093/bjs/znac154

[3] Reddy, S., Jagtap, N., Kalapala, R., *et al.* (2021) Choledocholithiasis in Acute Calculous Cholecystitis: Guidelines and Beyond. *Annals of Gastroenterology*, **34**, 247-252. https://doi.org/10.20524/aog.2020.0562

[4] Chen, J.M., Yu, Y. and Wu, H.S. (2020) Research Progress in the Prevention and Treatment of Recurrence of Gallstones after Gallbladder-Preserving Cholecystolithotomy with Chinese Medicine. *Clinical Journal of Traditional Chinese Medicine*, **32**, 1388-1391.

[5] Li, Y., Gao, X.-X. and Qin, X.-M. (2020) Advances in Research of Traditional Chinese Medicine for Promoting Bile Secretion and Excretion. *China Journal of Chinese Materia Medica*, **45**, 1287-1296.

[6] Guan, B.Y., Tong, J.L., Hao, H.P., *et al.* (2022) Bile Acid Coordinates Microbiota Homeostasis and Systemic Immunometabolism in Cardiometabolic Diseases. *Acta Pharmaceutica Sinica B*, **12**, 2129-2149. https://doi.org/10.1016/j.apsb.2021.12.011

[7] Navarro-Corcuera, A., Ansorena, E., Montiel-Duarte, C., *et al.* (2020) AGAP2: Modulating TGFβ1-Signaling in the Regulation of Liver Fibrosis. *International Journal of Molecular Sciences*, **21**, Article No. 1400. https://doi.org/10.3390/ijms21041400

[8] Lozano Ruiz, B. and González Navajas, J.M. (2020) The Emerging Relevance of AIM2 in Liver Disease. *International Journal of Molecular Sciences*, **21**, 6535-6535. https://doi.org/10.3390/ijms21186535

[9] Tang, Q.L. (2021) Basic Research and Review on Clinical Prevention and Treatment of Cholelithiasis. *Chinese Youjiang Medical Journal*, **49**, 1-6.

[10] Liu, J., Yang, H.J., Zeng, Q.Q., *et al.* (2020) Research Progress of Chinese Herbs on Regulating MAPK Pathway in the Treatment of Chronic Prostatitis. *Acta Chinese Medicine and Pharmacology*, **48**, 72-76.

[11] Di Ciaula, A., Garruti, G., Wang, D.Q.-H., *et al.* (2018) Cholecystectomy and Risk of Metabolic Syndrome. *European Journal of Internal Medicine*, **53**, 3-11. https://doi.org/10.1016/j.ejim.2018.04.019

[12] Cariello, M., Piccinin, E., Garcia-Irigoyen, O., *et al.* (2018) Nuclear Receptor FXR, Bile Acids and Liver Damage: Introducing the Progressive Familial Intrahepatic Cholestasis with FXR Mutations. *Biochimica et Biophysica Acta (BBA)—Molecular Basis of Disease*, **1864**, 1308-1318. https://doi.org/10.1016/j.bbadis.2017.09.019

[13] Zhuo, C.-Y., Bao, C.-C., Feng, S., *et al.* (2021) Effect of Dahuang Lingxian Capsule on Farnesol X Receptor in Gallstone Mouse Model. *Chinese Journal of Integrated Traditional and Western Medicine*, **41**, 836-841.

[14] Zhou, Y., Zhuo, C.Y., Tang, Q.L., *et al.* (2020) Dahuang Lingxian Capsule in Regulation of BSEP and NTCP Gene Expressions. *Journal of Youjiang Medical University for Nationalities*, **42**, 401-405.

[15] Xu, Y., Wang, B., Tang, Q.-L., *et al.* (2021) Mechanism of Dahuang Lingxian Granule on the Formation of Hepatolithiasis Based on NF-κB Signaling Pathway. *Lishizhen Medicine and Materia Medica Research*, **32**, 2074-2078.

[16] Gan, Y.-R., Gui, X.-B., Yu, Y., *et al.* (2022) Dahuang Lingxian Prescription Regulates Effect of TAK1 and TRAF6 Interaction and Co-Localization on Inflammatory Response of Bile Duct Cells. *Chinese Journal of Experimental Traditional Medical Formulae*, **28**, 92-99.

[17] Patel, R., Portone, G., Lambert, J.A., *et al.* (2021) Disease-Modifying Therapies and Symptomatic Management for Primary Biliary Cholangitis. *British Journal of Hospital Medicine*, **82**, 1-9. https://doi.org/10.12968/hmed.2021.0247

[18] Zhuo, C.Y., Zhou, Y., Feng, S., *et al.* (2020) Mechanism of Dahuang Lingxian Cap-

sule Regulating the Gallbladder Motor Function to Prevent Gallstone in Mice. J*ournal of Youjiang Medical University for Nationalities*, **42**, 539-542.

[19] Zhang, M., Dai, J.Y., Yu, Y., *et al.* (2022) Effects of Dahuang Lingxian Prescription on Wnt5a-Ca_2^+-PKC Signal Trans-Duction Pathway and Inflammatory Response in Rats with Intrahepatic Bile Ductinjury Induced by LPS. *Chinese Journal of Immunology*, **38**, 39-44.

[20] Tang, Q.-L., Lv, Z., Yu, Y., *et al.* (2016) Mechanism of Dahuang Lingxian Capsule for Regulating and Controlling Expression of Hepatocyte Transporters and Bile Metabolism Spectrum in Gallstone Mice. *Chinese Journal of Integrated Traditional and Western Medicine*, **36**, 953-959.

[21] Li, C.-J., Yu, Y., Gan, Y.-R., *et al.* (2022) Effects of LPS-Induced Cholangitis on the Cytoskeleton Morphology of Bile Duct Epithelium and the Intervention Mechanism of Dahuang Lingxian Formula for These Changes. *Journal of Hainan Medical University*, **35**, 623-625.

[22] Wu, H.S., Yu, Y., Chen, J.M., *et al.* (2021) Effect of DHLX on Alleviating the Inflammatory Response of Bile Duct Cells: An Analysis Based on TLR4/MyD88 Signaling Pathway. *Acta Chinese Medicine and Pharmacology*, **48**, 9-13.

[23] Islam, R., Sun, L.M. and Zhang, L.B. (2021) Biomedical Applications of Chinese Herb-Synthesized Silver Nanoparticles by Phytonanotechnology. *Nanomaterials*, **11**, 2757-2757. https://doi.org/10.3390/nano11102757

[24] Zou, L.-L., Li, J.-R., Li, H., *et al.* (2021) TGF-β Isoforms Inhibit Hepatitis C Virus Propagation in Transforming Growth Factor Beta/SMAD Protein Signalling Pathway Dependent and Independent Manners. *Journal of Cellular and Molecular Medicine* **25**, 3498-3510. https://doi.org/10.1111/jcmm.16432

[25] Dewidar, B., Meyer, C., Dooley, S., *et al.* (2019) TGF-β in Hepatic Stellate Cell Activation and Liver Fibrogenesis. *Cells*, **8**, Article No. 1419. https://doi.org/10.3390/cells8111419

[26] Su, Q., Fan, M.Y., Wang, J.J., *et al.* (2019) Sanguinarine Inhibits Epithelial-Mesenchymal Transition via Targeting HIF-1α/TGF-β Feed-Forward Loop in Hepatocellular Carcinoma. *Cell Death & Disease*, **10**, Article No. 939. https://doi.org/10.1038/s41419-019-2173-1