Meta Analysis of Traditional Chinese Medicine in Treating Cholecystolithiasis and Network Pharmacological Analysis of High-Frequency Drug Pairs

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Abstract: Objective to explore the clinical efficacy of Traditional Chinese medicine (TCM) in the treatment of gallstones, and to study the mechanism of high-frequency drug pairs (Bupleurum chinense and Lysimachia charistinae) in the treatment of gallstones. Methods: A computer search was conducted to search The Cochrane Library, CNKI, VIP, Wanfang, CBM, PubMed, and Web of Science databases for meta-analysis of randomized controlled studies on TCM treatment of gallstones in recent ten years. According to the results of the heterogeneity test, appropriate models were selected to make the forest map and funnel map, and the literature quality was evaluated. Network pharmacologic analysis was performed for the drug pairs (Bupleurum chinense and Pennisetum chinens) that appeared most frequently in the included literature. Chemical components and targets of the two traditional Chinese medicines in the treatment of gallstones were screened out, GO analysis and KEGG analysis were performed, and the potential mechanism of action of the two traditional Chinese medicines in the treatment of gallstones was discussed. Results: Meta-analysis showed that there was a significant difference in the effective rate between traditional Chinese medicine and conventional medicine in the treatment of gallstones. After network pharmacological analysis, there were 14 chemical components and 90 targets in the treatment of gallstones by S. chinensis and Bupleurum. GO analysis revealed 578 biological processes, 53 cell compositions, and 104 molecular functions. KEGG analysis showed that the biological pathway of Bupleurum and Radix monstris in the treatment of gallstones involved cancer pathway and TNF signaling pathway. Conclusion: TCM treatment of gallstones can improve clinical efficacy, and TCM treatment of gallstones has important clinical significance. Gallstone is a common biliary system disease in China, and its incidence is increasing year by year [1]. Gallstones can lead to gallbladder cancer [2]. Gallbladder cancer is a highly malignant cancer. Therefore, attention should be paid to the treatment of gallstones. However, there are some sequelae in the surgical treatment of gallstones, such as the recurrence of gallstones, bile duct injury, postoperative infection, etc. [3]. Ursodeoxycholic acid, anti-inflammatory cholagogue tablets, Dankang capsules, and other commonly used drugs in the clinical treatment of gallstones are difficult to solve the problem of the root. The author believes that the treatment based on syndrome differentiation of traditional Chinese medicine, combined with the patient's physique, can not only achieve the effect of dissolving and removing

stones, but also eliminate and prevent the recurrence of stones from the root. Now the author uses statistical analysis to evaluate the clinical efficacy of traditional Chinese medicine in the treatment of cholecystolithiasis, and studies the potential mechanism of high-frequency traditional Chinese medicine based on the results of meta-analysis, to broaden new ideas for clinical conservative treatment of cholecystolithiasis.

1. Data and Methods

1.1. Literature Search

Method: RCTS related to gallstone published on The Cochrane Library, CNKI, VIP, Wanfang, cbm, PubMed, and Web of science databases in the past ten years from January 1, 2012 to April 21, 2022; The Chinese key words are: gallstone, Chinese medicine, liver soothing, cholagogic, stone removing, stone dissolving, fossil, and stone removing. The English search terms are: gallstone, schoolholithiasis, traditional Chinese medicine, something live gallblader, separating staked life qi for promoting bill flow, express gallstone, discharge, gallblader stones, resolution of gallstone, termination of gallstone, resolve gallstone, and resolve gallstone. The retrieval strategy of "subject words+free words" is used for retrieval. Taking CNKI as an example, the publication time was between2012-01-012022-04-21, the subject word=cholecystolithiasis, and free words=Chinese medicine or liver soothing or cholagogic or stone removing or stone dissolving or fossil or stone removing were searched in the results.

1.2. Inclusion Criteria

① Subjects were all patients with gallstones with diameter ≤ 1.0cm or without symptoms confirmed by imaging; ② Included clinical studies were randomized controlled trials; ③ The control group was only treated with conventional drugs; the test group was only treated with Chinese herbal decoction or Chinese herbal formula granules; ④ The evaluation standard of treatment effect is the comprehensive effect. The comprehensive effect includes the number and size of gallbladder stones, as well as the symptoms and signs of patients. It is classified orderly by cure, effectiveness (including significant effects) and ineffectiveness.

1.3. Exclusion Criteria

① Nonconformity with inclusion criteria; ② Clinical study of patients with bile duct stones; ③ Patients with gallstone and other chronic diseases; ④ Repeated published literature; ⑤ The control group was larger than 2 groups.

1.4. The Process of Literature Screening

Two researchers will screen the literature independently, and the third party will negotiate for a resolution in case of disagreement. Use Noteexpress software to screen out duplicate documents; after reading the title, abstract, and full text, we excluded the literature reviews, animal experiment, meta-analysis, network pharmacology, cases, famous Chinese medicine experience, clinical research on the treatment of gallstones and bile duct stones with integrated traditional Chinese and

western medicine, clinical research on surgery combined with traditional Chinese medicine, non randomized controlled trials, etc.

1.5. Statistical Method

The quality evaluation and heterogeneity test of the included literature were conducted by using the literature-specific analysis software Revman 5.3. When the result of heterogeneity check is I, $\leqslant$ 50% indicates that there is no heterogeneity among the included literatures, and the fixed effect model should be used; I $\geqslant$ at 50%, it indicates that there is heterogeneity among the included literatures. After analyzing the reasons for heterogeneity, a random effects model is used. I ^{2}The smaller the value of is, the better the homogeneity of the included literature is. $P < 0.05$ indicates that the difference between the control group and the treatment group is statistically significant. Use Revman 5.3 to make a summary map of bias risk, a forest map, and a funnel map. The summary chart of bias risk shows the quality of the included literature, the forest chart shows the results after the combined effect amount, and the funnel chart shows whether the published literature is biased.

1.6. Network Pharmacological Analysis

According to the frequency statistics of all drugs in the 8 included Chinese herbal prescriptions, the two Chinese herbal medicines (Bupleurum chinense and Lysimachia christinae) with the highest frequency in the 8 prescriptions were obtained, and their network pharmacological analysis was carried out. The TCMSP data platform was used to obtain the active chemical components and target genes of Herba Lysimachiae and Radix Bupleuri, and the oral bioavailability $\geq$ 30% and drug-like property $\geq$ 0.18 were used as the main screening conditions to obtain the effective chemical active components and target genes of these two drugs. In the Uniprot database, "Human" and "Reviewed" are used as screening conditions to convert the target genes of these two drugs into simplified gene IDs. With "cholelithiasis" as the key word, relevant targets of gallbladder stones were collected in the databases of Digest, CTD, and GeneCards and sorted out. The online mapping software makes the Wayne map and screens out the common targets of effective drug component targets and disease targets. The common targets are the potential targets of Radix Bupleuri and Herba Lysimachiae for the treatment of gallbladder stones. At the same time, the main chemical components of Herba Lysimachiae and Radix Bupleuri in the treatment of gallstones were screened, and the visual data network structure diagram of "drugs - main chemical components - common target - disease" was constructed. The gene ID of the common targets of Radix Bupleuri and Herba Lysimachiae in the treatment of gallstones was imported into DAVID database for GO analysis and KEGG analysis. Use the online platform of "Weishengxin" to make bubble diagrams.

2. Results

2.1. Overview of Document Retrieval

After searching, 5913 related articles were obtained, 2071 duplicate articles were excluded, 3824 articles were excluded after reading the title and abstract, and 18 potentially relevant reports were obtained. After reading the full text, 8 randomized controlled trials were screened according to the inclusion criteria, with a total of 600 patients. See the characteristics table (Table 1) for the details of the included literature.

Table 1: The characteristics table

Clinical research	Sample size		Course of treatment	Interventions	
	Treatment	Control		Treatment	Control
Huang dunhuang 2016	45	43	6 months	Self made spleen strengthening and gallbladder regulating decoction	Ursodeoxycholic acid
Ji chengfeng 2012	36	36	2 months	Self made shugan lidan decoctio	Ursodeoxycholic acid
Zhang zhuhao 2014	40	40	1 month	Qinggan lidan paishi decoction	Shudantong tablets+xiaoyan lidan tablets
Kong lin 2018	30	30	3 months	Modified sini sanjin soup	Ursodeoxycholic acid
Wang xiaoyun 2016	45	45	3 weeks	Jinhu granules	Inflammation-resolving gall-bladder-excreting tablet
Wang dandan 2013	30	30	3 months	No. 1 prescription for gall diseases	Dankang capsule
Wang ping 2017	36	36	2 months	Cholagogue granule	Ursodeoxycholic acid
Yan shaozhuo 2021	45	45	6 weeks	Self prepared chen jin pai shi tang	Danshu capsules

2.2. Meta Analysis Results

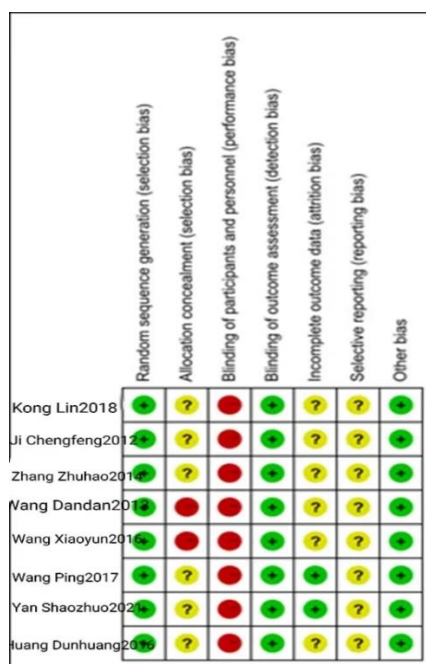## 2.2.1. Results of Bias Risk Summary Chart

Figure 1: The risk map of literature quality bias

The RCT bias risk assessment tool in 5.3.0 of the Cochrane Manual was used to evaluate the bias of the eight included literatures [4-11] from six aspects: generation of random sequences, allocation concealment, implementation of blind methods, and integrity of result data, reporting bias, and other bias. Because traditional Chinese medicine was compared with conventional drugs such as Chinese patent medicine and western medicine, the 8 randomized controlled trials could not be double-blind. Wang Dandan [4] and Wang Xiaoyun [5] used an open random number table method, which caused high-risk distribution bias. The bias of the remaining 6 articles is unknown. Except for Yan Shaozhuo [6] and Wang Ping [7] who reported the test objects falling off, the other six papers [8-11] did not make relevant statements on this. Whether there is a selective report on 8 articles [4-11] is mentioned. See the risk map of literature quality bias (Figure 1) for details.

2.2.2. Forest Map Results

The results after inspection are as follows: I ^{2}= 47%<50%, indicating that there is no heterogeneity in the included clinical studies, and the fixed effect model is used. After the fixed effect model combined the effect amount, the results showed that RR=1.37, 95% CI: 1.37 (1.24,1.52), P<0.00001, Z=6.13. It shows that the difference between the treatment group and the control group is statistically significant. It can be seen from the forest map (Figure 2) that the diamond is located to the right, indicating that the treatment group has a higher efficiency than the control group.
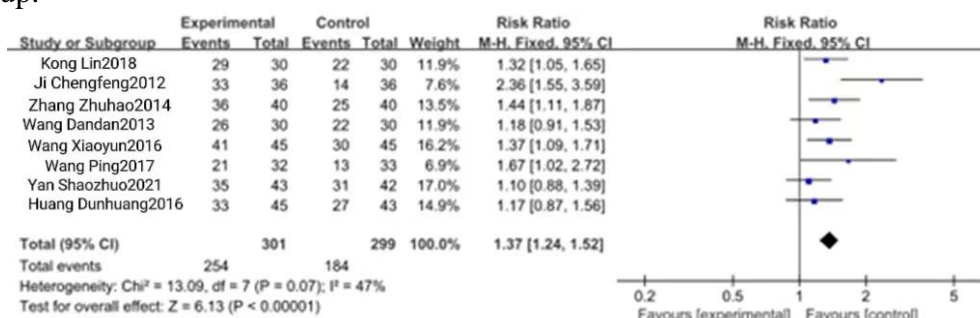

Figure 2: The forest map

2.2.3. Funnel Chart Results

It can be seen from the funnel chart that the distribution of the 8 included studies is asymmetric. It may be related to the small sample size included, or the negative results of some tests are not given, or the intervention measures of the treatment group and the control group in each clinical study are different. As shown in Figure 3.
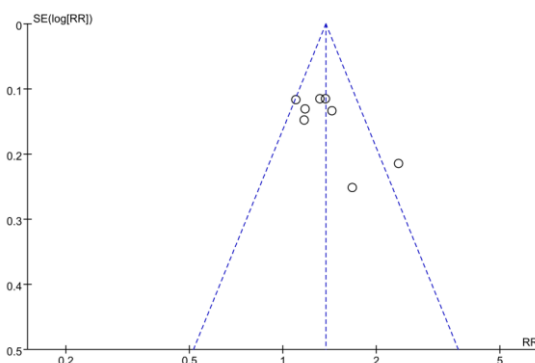

Figure 3: Funnel plot

2.3. Results of Network Pharmacological Analysis

2.3.1. Composition and Target

The TCMSP database was used to search for two traditional Chinese medicines, Lysimachia christinae and Bupleurum chinense. According to the OB (oral bioavailability) and DL (drug-like property) values in "1.6", 10 effective active chemical components of Lysimachia christinae, 174 targets, 17 effective active chemical components of Bupleurum chinense and 178 targets were screened. With the keyword "cholystolithiasis", 156 targets were found in the Digest database, 1109 targets were found in the CTD database, and 60 targets were found in the GeneCards database. After sorting out and removing the weight, 1252 gallbladder stone targets were obtained. 90 common targets of cholecystolithiasis, Lysimachia christinae, and Bupleurum chinense were screened by Wayne diagram (Fig. 4). At the same time, the main chemical components of Lysimachia christinae and Bupleurum chinense for the treatment of gallstone were also screened as Kaempferol, Quercetin, Stigmasterol, acacetin, hesperetin, epicatechin, isorhamnetin, baicalin and other 14 kinds of chemical components, Make a picture of "medicine - main chemical components - common target - disease", see Figure 5 for details. Among them, yellow represents Bupleurum chinense and Lysimachia christinae, red represents disease, pink represents common components of Bupleurum chinense and Lysimachia christinae, orange represents the chemical components of Lysimachia christinae in the treatment of gallbladder stones, green represents the chemical components of Bupleurum chinense in the treatment of gallbladder stones, and blue represents the potential target of Bupleurum chinense and Lysimachia christinae christinae in the treatment of gallbladder stones.
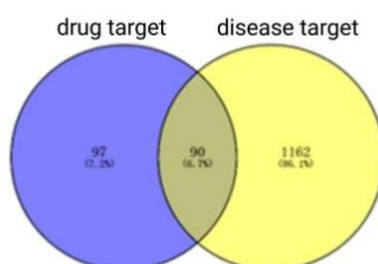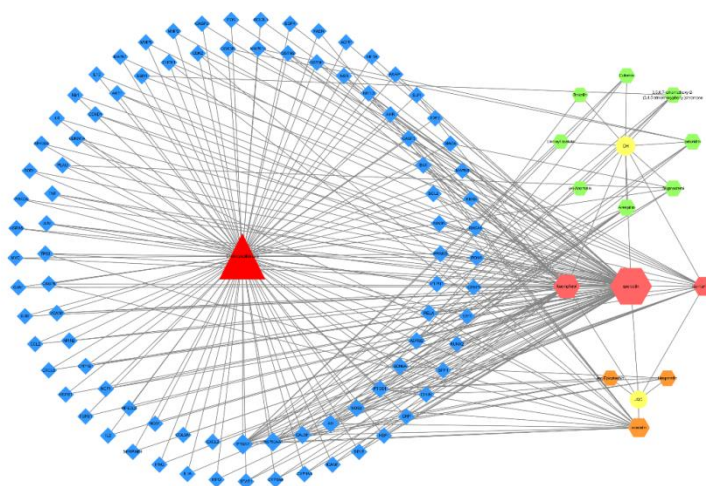

Figure 4: Wayne diagram

Figure 5: Medicine - main chemical components - common target – disease

2.3.2. Bubble Chart of Go Analysis and KEGG Analysis

The gene ID of the target was imported into the DAVID database for GO analysis and KEGG analysis. Through GO analysis, 578 biological processes, 53 cell compositions, and 104 molecular functions were obtained. According to the order of P values, the top ten results of biological processes (BP), molecular functions (MF), and cell components (CC) were selected to draw a bubble chart (Figure 6). It can be seen that the main biological processes involved include the positive regulation of RNA polymerase II promoter transcription, the positive regulation of DNA template transcription, positive regulation of gene expression, inflammatory response, etc. The cell components involved mainly include nucleus, cytoplasm, cytoplasmic matrix, nucleus and cytoplasm, extracellular space, etc. The main molecular functions involved are protein binding, protein binding, enzyme binding, etc. Through KEGG analysis, 169 signal pathways were obtained, and the top 20 signal pathways with the highest enrichment were selected to draw a bubble chart (Figure 7). Among them, the mechanism of Bupleurum chinense and Lysimachia christinae in treating cholecystolithiasis is related to cancer pathway, TNF signal pathway, IL-17 signal pathway, AGE RAGE and other signal pathways.
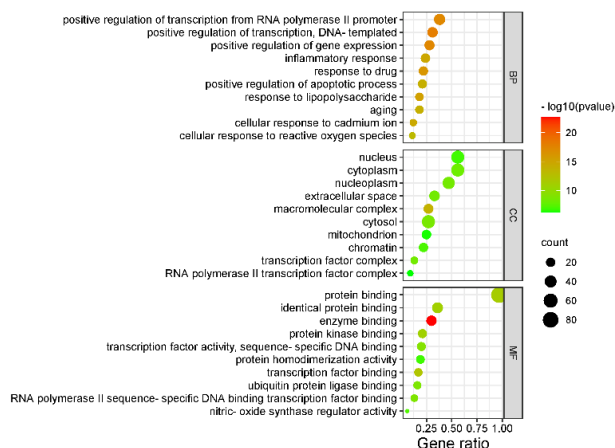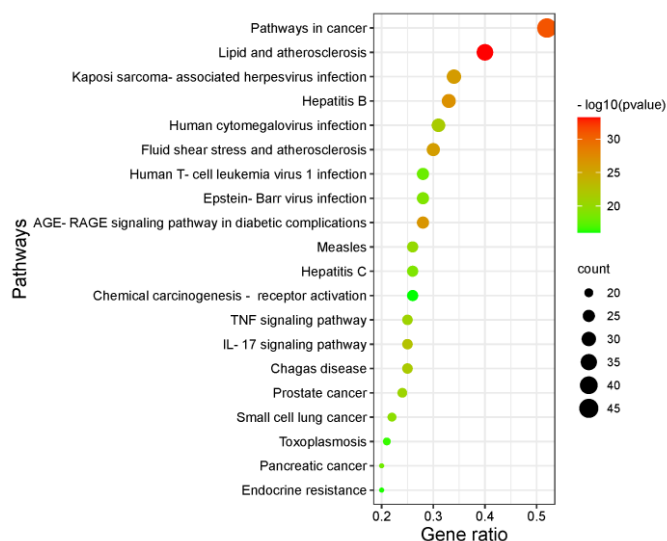

Figure 6: Bubble chart 1

Figure 7: Bubble chart 2

3. Discussion

Cholecystolithiasis belongs to the categories of "hypochondriac pain", "jaundice", and "gall distension" in traditional Chinese medicine. The sufferers of this disease are mostly caused by exogenous damp heat, evil, emotional disorders, eating disorders, physical factors, infection, and insect accumulation. The disease is located in the liver and gallbladder, with stagnation of qi and qi in the liver, loss of liver drainage, or the evil of damp heat and phlegm and blood stasis invading the gallbladder, leading to cholestasis, poor drainage, and long-term accumulation of stones. Constitution is an important factor leading to gallstones. Some clinical studies have shown that people with damp heat, phlegm dampness, qi depression, and qi deficiency constitution are more likely to get gallstones [12]. Traditional Chinese medicine (TCM) treats gallbladder stones by soothing the liver and regulating qi, clearing heat and removing dampness, nourishing yin and soothing the liver, clearing heat and detoxifying, promoting blood circulation and removing blood stasis [13], and adds and subtracts traditional Chinese medicine such as cholagogue and removing stones. Many famous TCM doctors have rich clinical experience in the treatment of cholecystolithiasis. Qiu Jianxing [14], the first famous Chinese medicine doctor in China, believed that most of the patients with gallstones in Lingnan area mainly had damp heat of liver and gallbladder combined with stagnation of liver qi. In clinical practice, prescriptions were selected according to the severity of damp heat and stagnation of liver qi, and good clinical effects were achieved. The data mining results show that the common syndrome types of cholelithiasis treated by Zhou Lao include catharsis, disharmony between gallbladder and stomach, dampness and heat, disharmony between liver and stomach, spleen dysfunction, etc. Zhou Lao has flexibly responded to various syndrome types through the combination of four diagnostic methods, syndrome differentiation, and treatment, and achieved good results [15]. Xie Xushan, a famous old Chinese doctor, mainly treats acute cholecystolithiasis by attacking evil and unblocking the fu organs, while he mainly treats static cholecystolithiasis by dissolving and expelling stones [16].

3.1. Research Significance and Clinical Effectiveness

Meta analysis shows that Chinese medicine has obvious advantages in the treatment of gallstones. For patients with asymptomatic cholecystolithiasis who cannot be treated surgically, who are unwilling to be treated surgically, or whose diameter is less than 2cm, or who have cholecystolithiasis with gallbladder wall thickening less than or equal to 4mm, traditional Chinese medicine can be used as the preferred treatment scheme [13]. However, western medicine and Chinese patent medicine commonly used in clinical practice have limitations. For example, ursodeoxycholic acid is only effective for cholesterol stones, and it is easy to relapse once the drug is stopped [17-18]. Most Chinese patent medicines only treat gallstones of a specific syndrome type, for example, Xiaoyan Lidan Tablet is more inclined to treat hepatobiliary wet heat gallstones; Danshu capsule is more likely to be used in patients with cholelithiasis of liver depression and qi stagnation type. The clinical efficacy of Chinese medicine in treating cholecystolithiasis is obvious to all, and it has become a consensus that the recurrence rate and side effects of Chinese medicine in treating cholecystolithiasis are small. Based on the results of meta-analysis, it was found that Bupleurum chinense and Lysimachia christinae were common traditional Chinese medicines for the treatment of gallbladder stones, and the two herbs with the highest frequency of use by the elderly Zhou were also Bupleurum chinense and Lysimachia christinae [15]. Chaihu enters the Liver and Gallbladder Meridian to regulate the liver qi, clear the gallbladder heat of Shaoyang, reconcile Shaoyang, and treat the gallbladder from the liver. Herba Lysimachiae can clear away heat and damp, promote gallbladder and jaundice, detoxify, and detumescence. According to Shaanxi Traditional Chinese Medicine, Herba Lysimachiae is mainly used to treat cholelithiasis and

jaundice. Bupleurum chinense and Lysimachia christinae both complement each other and treat both symptoms and root causes. The further study and discussion on the pharmacological effects of Bupleurum chinense and Lysimachia christinae have important clinical significance for the majority of traditional Chinese medicine practitioners in the treatment of gallbladder stones.

3.2. Analysis of Potential Action Mechanism

Lysimachia christinae has the pharmacological effects of cholagogic and lithotriptic, inhibiting the formation of stones, anti-inflammatory and analgesic. The water and alcohol extracts of Lysimachia christinae can promote bile secretion, smooth muscle relaxation, and gallbladder emptying, to achieve the lithotriptic effect [19-20]. Wu Haijuan et al. [21] found that the water decoction of Lysimachia christinae can reduce the number of gallstones and gallstones by increasing the cholecystokinin receptor in serum and reducing the level of vasoactive intestinal peptide. Bupleurum chinense has the pharmacological effects of protecting the liver, anti-inflammation, anti-tumor, lowering cholesterol, and so on, and has a good effect on the treatment of cholesterol calculi [22-23]. Dachaihu decoction, which takes Chaihu as the king drug, has the pharmacological effect of cholagogic and lithotriptic, and inhibits the formation of stones [24].

The most important pathway of Bupleurum chinense and Lysimachia christinae in the treatment of gallbladder stones is the cancer pathway. If the gallbladder stones are not cured for a long time, they will evolve into gallbladder cancer. Bupleurum chinense and Lysimachia christinae inhibit the expression of relevant cancer genes in the treatment of gallbladder stones and then prevent the disease from further worsening, which also fully reflects the idea of preventing the disease in traditional Chinese medicine [25]. According to relevant reports, local cholecystitis caused by bacterial infection is an initiating factor for gallstone formation [26]. TNF is a polymorphic hormone. When the body is infected, the content of TNF in the body increases, becoming an important factor in the local inflammation of the gallbladder. The effective chemical components of Bupleurum chinense and Lysimachia christinae can reduce biliary inflammation by inhibiting and blocking TNF signaling pathway [27]. Some studies [28] found that AGE RAGE signaling pathway can activate the reverse transcription factor NF-κB(nuclear factor-κB) By stimulating the nucleus to release a large number of adhesion factors, proinflammatory cytokines (IL6, TNF-α and so on), inhibit the expression of inflammatory factors to achieve the purpose of anti inflammation. IL-17 signaling pathway can also mediate the inflammatory response. Interleukin-17 (IL-17) is a kind of inflammatory factor produced by T-helper cell Th17, which can directly or indirectly induce a variety of inflammatory factors and chemokines to mediate the inflammatory responses. It can be seen from the KEGG analysis chart that, in addition to the cancer pathway, TNF signal pathway, and AGE-RAGE signal pathway, it is also closely related to other pathways, indicating that the treatment of cholecystolithiasis by Herba Lysimachiae and Radix Bupleuri is coordinated through a variety of metabolic pathways, but its specific mechanism remains to be further explored. There are many targets for Bupleurum chinense and Lysimachia christinae, among which EGFR can effectively promote mitosis, mediate inflammatory reactions, and repair the regeneration of peripheral nerves and blood vessels, thus improving the pathological state of gallbladder tissue to achieve the purpose of treating gallstone [29-30]. Target AKTI1 can promote apoptosis of local lesion cells of the gallbladder to treat calculous cholecystitis [31].

Abnormal metabolism of bilirubin, cholesterol, phospholipid, and bile acids can lead to gallstones [32]. Quercetin, a common component of Lysimachia christinae and Bupleurum chinense, not only has anti-inflammatory effects, but also regulates the metabolism of glycerol phospholipids and cholesterol, and can inhibit the formation of cholesterol gallstones. The anti-inflammatory

effect of quercetin is mainly achieved by inhibiting tumor necrosis factor (TNF), interleukin (IL) and other inflammatory factors [33-36]. Quercetin also has the effect of enhancing the immune system of the body to prevent the normal flora of the body from becoming pathogenic bacteria and causing local cholecystitis when the immune system is reduced [37]. The treatment of cholecystolithiasis with kaempferol is mainly anti-inflammatory and analgesic, and relevant experimental studies show that kaempferol has no obvious toxic and side effects on the body [38]. Isorhamnolicin can inhibit inflammatory reaction by blocking NF kB signaling pathway, reduce local inflammation of the gallbladder, promote gallbladder contraction, and expel stones [39]. Stigmasterol can inhibit the expression of related proinflammatory mediators by downregulation of NF kB P65, thereby increasing the expression of IL-10 (anti-inflammatory cytokine) and achieving the cholagogic effect [40]. Albiquitin has the effects of immune regulation, anti-inflammatory and bacteriostasis, and inhibition of tumor cells. Its anticancer mechanism is to inhibit the expression of MMP2 and MMP9 [41]. Baicalin can promote gallbladder contraction and expel stones [42]. Hesperidin and epicatechin have anti-inflammatory and cholagogic effects [43].

To sum up, the specific mechanism of Bupleurum chinense and Lysimachia christinae in treating cholecystolithiasis is closely related to their anti-inflammatory pharmacological effects.

3.3. Deficiencies and Suggestions for Future Research

This paper combines meta-analysis and network pharmacology. Although the results of meta-analysis and network pharmacology are of clinical significance, there are still many shortcomings: ①, the inclusion of eight clinical studies can not achieve double-blind trials; ② There are 6 articles [8-11] that do not report whether there are patients with abscission, and the reasons for abscission and related treatment methods; ③ There is no uniform standard for the course of medication; ④ The sample size included in the 8 clinical studies is small, which may affect the test results; ⑤ In the eight clinical studies included, Bupleurum chinense and Lysimachia christinae were used as two traditional Chinese medicines, but because the number of studies included was too small, there were some limitations in using Bupleurum chinense and Lysimachia christinae as high-frequency drug pairs; ⑥ The results of network pharmacology only stay at the theoretical level, and many chemical components, targets and pathways have not been verified by experiments.

Although there are many shortcomings in this study, it still has a certain reference value for the treatment of cholecystolithiasis, especially for patients who are unable or unwilling to operate. In future research, if we can expand the sample size and truthfully report the number of shed people, the test results will be more convincing. Unify the treatment course standard with the included literature, and describe the hidden distribution in detail, then the results of meta-analysis will have more clinical reference value. Future experiments can further study the relationship between other pathways and gallstones, as well as the mechanism of other components in the treatment of gallstones, and provide a strong experimental demonstration for the treatment of gallstones with traditional Chinese medicine.

4. Summary

This paper used statistical software to prove that traditional Chinese medicine has a good clinical efficacy in the treatment of gallstones, and that the treatment of gallstones with Bupleurum chinense and Lysimachia christinae is related to the anti-inflammatory pharmacological effect. This study has some shortcomings and limitations, but it still has the value of guiding clinical TCM doctors to choose prescriptions and drugs. The treatment of cholecystolithiasis with traditional Chinese

medicine has a bright clinical prospect and is willing to promote it to the clinic and benefit the vast number of patients with cholecystolithiasis.

References

[1] Wang Qihan, Zhang Zhongwen, et al. Epidemiological survey of cholecystolithiasis in Shanghai. Gastroenterology, 2018, 23 (3): 252-257.

[2] Koshiol J, Gao YT, Dean M, et al. Association of Aflatoxin and Gallbladder Cancer. Gastroenterology, 2017, 153(2): 488-494. e1.

[3] Zhang Kai. Meta analysis of related factors of bile duct injury in laparoscopic cholecystectomy. Taiyuan: Shanxi Medical University, 2020.

[4] Wang Dandan. Clinical Study on Gall Disease No. 1 in Treating 60 Patients with Gallstone of Liver gallbladder Damp heat Type. Harbin: Heilongjiang University of Traditional Chinese Medicine, 2013.

[5] Wang Xiaoyun. Chen Guangyu. Observation on the efficacy of Jinhu Granule in the treatment of early gallstone. Hainan Medical Journal, 2016, 27 (9): 1511-1512.

[6] Yan Shaozhuo. Clinical observation of Chen Jin Paishi Decoction in the treatment of gallbladder stones of hepatobiliary damp heat type. Harbin: Heilongjiang College of Traditional Chinese Medicine, 2021.

[7] Wang Ping. Clinical Study on Lidan Fossil Recipe in Treating Cholecystolithiasis of Liver Depression and Spleen Deficiency. Taiyuan: Shanxi College of Traditional Chinese Medicine, 2017.

[8] Huang Dunhuang, Wu Yaonan 45 Cases of Gallstone Treated with Jianpi Lidan Decoction. Guangming Traditional Chinese Medicine, 2016, 31 (3): 350-351.

[9] Ji Chengfeng. 36 Cases of Gallstone Treated with Shugan Lidan Decoction. Chinese Journal of Integrated Digestion of Traditional Chinese and Western Medicine. 2012, 20 (7): 328-329.

[10] Kong Lin, Clinical Study on Modified Sini Sanjin Decoction in Treating Cholecystolithiasis of Qi Stagnation and Phlegm Obstruction Type. Jinan: Shandong University of Traditional Chinese Medicine, 2018.

[11] Zhang Zhuhao, Wu Zhaohuai, Cai Weijie, etc Clinical Observation on 40 Cases of Gallstone Treated with Qinggan Lidan Paishi Decoction. Medical Aesthetics and Beauty, (12): 688-689.

[12] Yi Xilei, Guo Lv.120 Cases of cholelithiasis: Investigation and analysis of TCM constitution differentiation. Journal of Modern Integrated Traditional Chinese and Western Medicine, 2013, 22 (4): 382-383

[13] Li Junxiang, Chen Xuan, Liang Jian, etc Consensus on Diagnosis and Treatment of Cholelithiasis with Integrated Traditional and Western Medicine (2017). Chinese Journal of Integrated Digestion of Traditional and Western Medicine, 2018, 26 (2): 132-138

[14] Zhang Jianyi, Liao Jinping, Zheng Qijin. Experience of Qiu Jianxing, the First National Famous Chinese Medicine, in Treating Cholelithiasis. Shizhen Traditional Chinese Medicine, 2020, 31 (10): 2519-2521

[15] Jiang Mengting. Research on the syndrome and treatment of cholelithiasis by Professor Zhou Zhongying based on data mining, Nanjing University of Traditional Chinese Medicine: 2019

[16] Chen Mengmeng, Xie Xushan. Professor Xie Xushan's experience in treating gallstones. Asia Pacific Traditional Medicine, 2015, 11 (8): 56-57

[17] Rabenstein T, Radespiel T, Radespiel-Troger M, Hopfner L, et al.Ten years experience with piezoelectric extracorporeal shockwave lithotripsy of gallbladder stones..Eur J Gastroenterol Hepatol, 2005, 17(6):629-639.

[18] Guarino M P,Cocca S,Altomare A,et al,Ursodeoxycholic acid therapy in gallbladder disease, a story not yet cpmpleted..World J Gastroenterol, 2013, 19(31):5029-5034.

[19] Fang BJ, Shen JY.Zhang H. et a1.Effect of emodin on mobility signal transduction system of gallbladder smooth muscle in Guinea pig with cholelithiasis.Asian Pac J Trop Med.2016.9(10):991—996.

[20] Li Jun, Cai Hong, Wang Junming, et al. Chemical constituents, pharmacological effects and clinical application of Lysimachia christinae. Journal of Gerontology, 2017, 37 (12): 6262-6264

[21] Wu Hongjuan, Li Chunlan, Ma Chunlan, et al. The effect of Lysimachia christinae on serum cholecystokinin receptor and vasoactive intestinal peptide levels in patients with gallstone. Journal of Modern Integrated Chinese and Western Medicine, 2017, 26 (25): 2793-2795

[22] Wu Haiyan. Pharmacological study and clinical application of Bupleurum chinense. Clinical rational drug use, 2018,11 (9A): 100-102

[23] Yan Meiling, Yang Liu, Hou Ejiao. Research progress on chemical constituents and pharmacological effects of Bupleurum chinense. Information on Traditional Chinese Medicine, 2018, 35 (5): 103-109

[24] Song Xiaoxue, Huang Jinfeng, Tian Ming, et al. Pharmacology and clinical application of Dachaihu decoction. Journal of Traditional Chinese Medicine, 2019, 47 (4): 112-116

[25] Gao Yunxiao, Li Ze, Yang Liu, et al. Exploring the mechanism of Chaihu rhubarb on the prevention and treatment of cholelithiasis based on network pharmacology and molecular docking. World Journal of Traditional Chinese

Medicine, 2022, 17 (14): 447-454.

[26] Moazeni—Bistgani M, Imani R.Bile bacteria of patients with choleli—thiasis and theirs antibiogram.Acta Med Iran, 2013, 51(1 1):779—783.

[27] Zhang Tao. Research on the mechanism of Dachaihu decoction in treating calculous cholecystitis based on network pharmacology. Beijing, Beijing University of Traditional Chinese Medicine: 2021

[28] Yang Chaomao, Yang Zhixin, Ma Xiaoling. The mechanism of AGEs RAGE signaling pathway in diabetes nephropathy and research progress in traditional Chinese medicine. Journal of Traditional Chinese Medicine, 2019, 34 (9): 1864-1868

[29] Zhang Tao, Yang Chengcheng, Zhang Shaohui, et al. Study on the network pharmacology of Jiawei Dachengqi Decoction in the treatment of intestinal obstruction by activating blood circulation Chinese Journal of Integrated Traditional and Western Medicine Surgery, 2020,26 (05): 838-849

[30] Daniela Marino, Yvonne Angehrn, Sarah Klein, et al. Activation of the epidermal growthfactor receptor promotes lymphangiogenesis in the skin. Joumal of DemaoloialScience, 2013, 71(3).

[31] Zhao Yanmei, Zhang Jiankang, Cai Zhaobin, et al. Clinical research progress of Akt inhibitors   China Modern Applied Pharmacy, 2017,34 (04): 625-630

[32] Wang Cheng, Sun Peilong. Research progress on the causes of cholelithiasis. Journal of Hepatobiliary Surgery, 2018, 26 (2): 157-160

[33] Li Yao, Liu Hongnan, Yin Yulong Quercetin Scientific Observation, 2020, 15 (6): 67-70

[34] Chunlian T, Xin L, Yu C,et al. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. South African Journal of Botany, 2021, 137:257-264.

[35] Ma Na, Li Yajing, Fan Jiping. Research progress on pharmacological effects of quercetin. Journal of Liaoning University of Traditional Chinese Medicine, 2018, 20 (8): 221-224

[36] Ye Qing, Li Junnian, Yang Dongmei, et al. Effect of plant quercetin on the immune function of voles. Acta Veterinaria Sinica, 2019, 39 (1): 77-83

[37] Tian Ruixue, Sun Yaozong, Yao Youhao, et al. Effect of quercetin on immune function of immunocompromised mice. Chinese Journal of Modern Medicine, 2019, 21 (9): 13 - 16

[38] Chen Dan. Study on anti-inflammatory and analgesic effects of kaempferol and its mechanism. Nanjing: Nanjing University of Traditional Chinese Medicine, 2021

[39] Kim S,Jin C Y,Kim C,et al. Isorhamnetin alleviates lipopolysaccharide-induced inflammatory responses in BV2 microglia by inactivating NF- κ B, blocking the TLR4 pathway and reducing ROS generation. International Journal of Molecular Medicine, 2018, 43(2):682-692.

[40] Ahmad Khan M, Sarwar A, Rahat R, et al. Stigmasterol protects rats from collagen induced arthritis by inhibiting proinflammatory cytokines. International Immunopharmacology, 2020, 85: 106642.

[41] Ma Na, Li Yajing, Fan Jiping Progress in pharmacological research of acacia. China Modern Applied Pharmacy, 2018, 35 (10): 1951-1595.

[42] Wang Yutong, Lu Guanhua Research progress in the treatment of cholelithiasis with traditional Chinese medicine. Journal of Liaoning University of Traditional Chinese Medicine, 2021, 23 (2): 138-141.

[43] Tong Guanzhen, Fu Xiaoping, Yang Yan, etc Research progress on distribution and pharmacological activity of epicatechin. Journal of Yunnan Agricultural University, 2018, 33 (2): 343-349.