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Evaluate the effect of licorice on anti-liver fibrosis: a systematic review and meta-analysis

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Abstract

To investigate the effect of licorice on anti-liver fibrosis actions, we present systematic review and meta-analysis via systematic literature between 2010 and 2020 from the electronic databases, PubMed, Cochrane Library, Embase, ISI. A software program (Endnote X8) has been utilized for managing the electronic titles. Searches were performed with mesh terms. Mean differences between two groups (Glycyrrhizic acid and control group) with 95% confidence interval (CI), fixed effect model and Inverse-variance method were calculated. Random effects were used to deal with potential heterogeneity and I^2 showed heterogeneity. The Meta analysis and forest plots have been evaluated with the Stata V16. A total of 184 potentially relevant titles and abstracts were found during the electronic and manual search. Finally, a total of four publications fulfilled the inclusion criteria required for this systematic review. TGF-β1 mRNA expression significantly decreased following Glycyrrhizic acid relative to the control group and Glycyrrhizic acid significantly decreased expression of TGF-β1 mRNA, which was about 2.90 times that in the control group. Mean difference between Glycyrrhizic acid and control group was -18.12 U/L (MD, -18.12 U/L 95% Ca, -6.22 U/L. P = 0.00). Glycyrrhizic acid reduced TGF-β1, Smads mRNAs, hypdroxyproline, alanine aminotransferase.

Keywords: licorice; glycyrrhizic acid; liver fibrosis.

Practical Application: Results of this meta-analysis suggest Glycyrrhizic acid combination remarkably reduced TGF-β1, Smad2 and Smad3 mRNAs and protein expression. Furthermore, Glycyrrhizic acid reduced hypdroxyproline, alanine aminotransferase and combination could reverse hepatic fibrosis by suppressing HSC activation, inhibiting the TGF-β1 signaling pathway and activating general liver wound-healing.

1 Introduction

Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases (Pellicoro et al., 2014; Lodyga & Hinz, 2020). Advanced hepatic fibrosis can lead to liver architecture distortions and the formation of fibrous septa, which contains pseudo lobules or nodules, and then eventually develops into liver cirrhosis, which is a kind of irreversible and incurable liver disease. Therefore, attenuating or reversing hepatic fibrosis is a crucial strategy for preventing liver cirrhosis (Krenkel & Tacke, 2017; Zhou et al., 2016). Activation of quiescent hepatic stellate cells (HSCs) during the development of hepatic fibrosis can produce α-smooth muscle actin (α-SMA) positive, my fibroblast like cells that are the main cause of increased muscle ECM (Moreira, 2007; Park et al., 2020). In HSCs TGF-β1 signaling, transforming growth factor-β receptor I (TGF-βRI) binds its ligand which leads to receptor-activation of Smads (Smad2 and Smad3) via direct serine phosphorylation. This activation then induces Smad2 and Smad3 association with Smad4 and the formed complex translocates into the nucleus where the transcription of specific genes, such as collagens, are impacted (Nagaraja et al., 2012; Zhu et al., 2019). Therefore, inhibition of HSC TGF-β1 signaling should be considered as an important strategy in the prevention or treatment of liver fibrosis. Recent studies have shown that significant advances have been made in understanding the pathogenicity of liver fibrosis, however effective anti-fibrotic therapies have not been reported (Morales-Ávila et al., 2020). Huang Qi decoction (HQD) is a traditional Chinese medical formula. It is used in the treatment of diseases such as fatigue, heart palpitations, etc., it can also be used in the treatment of chronic liver diseases, including liver cirrhosis (Liu et al., 2012). Total astragalus saponins (AST) is a main component of Radix Astragali and glycyrrhizic acid (GA) is a main component of Radix Glycyrrhizae (Yan et al., 2017; Sun et al., 2016). Previously studies showed that HQT could inhibit the hepatic fibrosis progression in dimethyl nitrosamine (DMN)-induced hepatic fibrosis models (Shang et al., 2017). Also, the four substances astragalus saponins, astragalus flavonoids, glycyrrhizae acid, and glycyrrhizae flavonoids can affect the progression of liver fibrosis in animal models, even more effectively than HQD. According to importance of subject the aim of present systematic review

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and meta-analysis was investigating the effect of licorice on anti-liver fibrosis actions.

2 Method

2.1 Search strategy

From the electronic databases, PubMed, Cochrane Library, Embase, ISI have been used to perform a systematic literature between 2010 and 2020. Therefore, a software program (Endnote X8) has been utilized for managing the electronic titles. Searches were performed with mesh terms:

("Liver Cirrhosis"[Mesh]) AND ("Glycyrrhizic Acid/administration and dosage"[Mesh] OR "Glycyrrhizic Acid/adverse effects"[Mesh] OR "Glycyrrhizic Acid/pharmacology"[Mesh] OR "Glycyrrhizic Acid/toxicity"[Mesh]) OR ("Glycyrrhiza"[Mesh] OR "licorice acid" [Supplementary Concept]) AND ("Transforming Growth Factor beta1"[Mesh]) AND ("Smox protein, Drosophila" [Supplementary Concept]) AND ("Alanine Transaminase"[Mesh]) AND ("Hepatic Stellate Cells"[Mesh]).

This systematic review has been conducted on the basis of the key consideration of the PRISMA Statement–Preferred Reporting Items for the Systematic Review and Meta-analysis (Liu et al., 2012).

2.2 Selection criteria

Inclusion criteria

1. In vitro studies and in vivo studies
2. Animal model
3. Used glycyrrhizic acid
4. Control group
5. in English

2.3 Data extraction and method of analysis

The data have been extracted from the research included with regard to the study, years, animals, sample size, Drugs, liver fibrosis and treatment. For Data extraction, two reviewers blind and independently extracted data from abstract and full text of studies that included. Mean differences between two groups (Glycyrrhizic acid and control group) with 95% confidence interval (CI), fixed effect model and Inverse-variance method were calculated. Random effects were used to deal with potential heterogeneity and I^2 showed heterogeneity. Chi-square (I^2) tests for homogeneity were done to quantify the extent of heterogeneity (P-value below 0.1 considered statistically significant). I^2 values above 50% signified moderate-to-high heterogeneity. The Meta analysis and forest plots have been evaluated with the Stata V16.

3 Results

According to the research design, 184 potentially important research abstracts and titles have been discovered in our electronic searches. At the first phase of the study selection, 179 research have been with regard to the topics and abstracts. Therefore, we fully assessed the complete full-text papers of the rest 23 studies in the second stage so that we excluded 19 publications due to the lack of the defined inclusion criteria. Then, four papers remained in agreement with our inclusion criteria required (Figure 1). Table 1 reports the individual studies in this meta-analysis.

3.1 Sample size

Therefore, four studies (in vitro) have been included to evaluate the effect of Glycyrrhizic acid on liver fibrosis. The information for each study is shown in Table 1. The sample size of Glycyrrhizic acid and control group a total was 35 and 41, respectively.

3.2 Effects of glycyrrhizic acid on hypdroxyproline

In two studies, Moher et al. (2009) evaluated effects of Glycyrrhizic acid on hypdroxyproline. Mean difference between Glycyrrhizic acid and control group was -94.68 μg/g (MD, 94.68 μg/g 95% CI -130.04 μg/g, -59.32 μg/g. P = 0.00) among two studies and heterogeneity found (I^2 = 0.0%) (Figure 2). This result showed, when examining hypdroxyproline levels in the liver tissue, levels were significantly increased in the control group relative to the Glycyrrhizic acid. The Glycyrrhizic acid group showed a decreased in hypdroxyproline levels.

3.3 Effects of glycyrrhizic acid on alanine aminotransferase (ALT)

In four studies, Qu et al. (2015) evaluated the Effects of Glycyrrhizic acid on ALT. Mean difference between Glycyrrhizic acid and control group was -18.12 U/L (MD, -18.12 U/L 95% CI 30.02 U/L, -6.22 U/L. P = 0.00) among two studies and heterogeneity found (I^2 = 99.07%) (Figure 3). This result showed, ALT activity was significantly increased in the control group relative to the Glycyrrhizic acid group. Alternatively, ALT

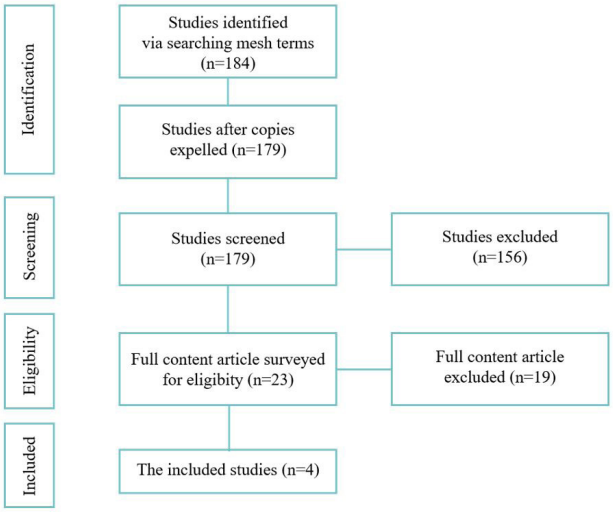

Figure 1. Study attrition.

Table 1. Studies selected for systematic review and meta-analysis (evaluate the effect of glycyrrhizic acid on liver fibrosis).

Study years	Animals	Drugs	Sample size		Liver fibrosis and treatment
			Control group	GA group	
Zhou et al. (2016) (4)	Wistar male rats	GA (purity: 95%, Batch NO: ZL20141011GCS)	2	4	Liver fibrosis was induced by intraperitoneal injection with 0.5% DMN in saline (2 mL/kg body weight) for three consecutive days per week for up to four weeks.
Liang et al. (2015) (16)	Male Sprague-Dawley rats	GA and α-smooth muscle actin (SMA) antibody were purchased from Sigma-Aldrich (St Louis, MO, United States).	15	15	Rats were also treated with a 40% solution of CCl4 by hypodermic injection at a dose of 3 mL/kg plus 0.2% GA solution in water (3 mL) by intraperitoneal injection three times weekly, beginning at the first week, following a previously published method. Rats in the control group were treated with the same is volumetric dose of olive oil and water. Animals were sacrificed 24 h after the last injection.
Qu et al. (2015) (17)	Male Sprague-Dawley (SD) rats	Glycyrrhizin (GL)	8	12	Fibrosis was induced by subcutaneous injection of 0.2 mL/100 g CCl4 in olive oil twice weekly for 8 consecutive weeks (the first dose was doubled). In control group, olive oil was subcutaneously injected. In liver fibrosis group, animals were intraperitoneally treated with normal saline (NS).
Moro et al. (2008) (18)	Transgenic mice harboring	GA	10	10	Sections prepared from the excised liver were subjected to Azan–Mallory staining.

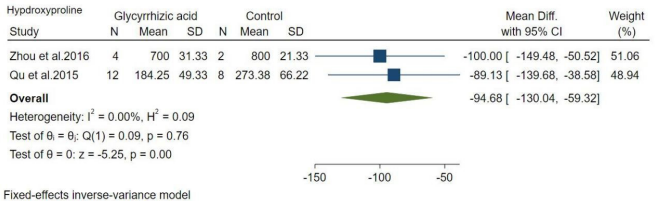

Figure 2. Forest plot showed the effects of glycyrrhizic acid on hypdroxyproline.

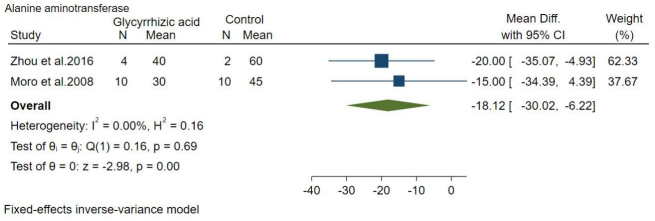

Figure 3. Forest plot showed the effects of glycyrrhizic acid on alanine aminotransferase (ALT).

activity was significantly decreased in the GA group relative to the control group.

3.4 Cytotoxicity of glycyrrhizic acid

One study, evaluated the Cytotoxicity of Glycyrrhizic acid. Lactate dehydrogenase (LDH) is a type of protein, known as an enzyme. Higher medium LDH levels indicate higher cytotoxicity. The result of this study showed, no cytotoxic effects were noted for Glycyrrhizic acid.

3.5 Effects of glycyrrhizic acid on mRNA expression of TGF-β1

In four studies, Moro et al. (2008) evaluated the expression levels of TGF-β1 mRNA expression using real-time PCR. Mean difference between Glycyrrhizic acid and control group was 2.90 (MD, 2.90 95% CI 2.82, 2.99. P = 0.00) among 4 studies and heterogeneity found (I^2 = 96.89%) (Figure 4). TGF-β1 mRNA expression significantly decreased following Glycyrrhizic acid relative to the control group and Glycyrrhizic acid significantly decreased expression of TGF-β1 mRNA, which was about 2.90 times that in the control group. Furthermore, a decrease in either mRNA of the above TGF-β1 signaling components was noted after treatment. These results suggest that GA combination treatment blocks TGF-β1 signaling.

3.6 Effects of glycyrrhizic acid on mRNA expression of Smads

In three studies, evaluated the mRNA expression level of Smads using real-time PCR. Mean difference between Glycyrrhizic acid and control group was -0.22 (MD, -0.22 95% CI -1.08, 0.64. P = 0.00) among 3 studies and heterogeneity found (I^2 = 0.0%) (Figure 5). There was no statistically significant difference between Glycyrrhizic acid and control group to mRNA expression levels of Smad2 (Figure 4). But Smads mRNA expression decreased following Glycyrrhizic acid combination treatment relative to the control group.

4 Discussion

Liver fibrosis is characterized by excessive deposition and qualitative extracellular matrix (ECM) changes in the liver, with these changes associated with general liver wound-

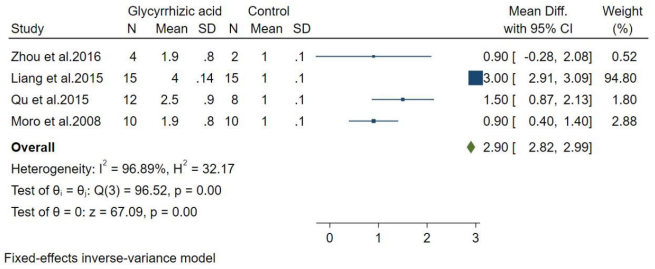

Figure 4. Forest plot showed the effects of glycyrrhizic acid on mRNA expression of TGF-β1.

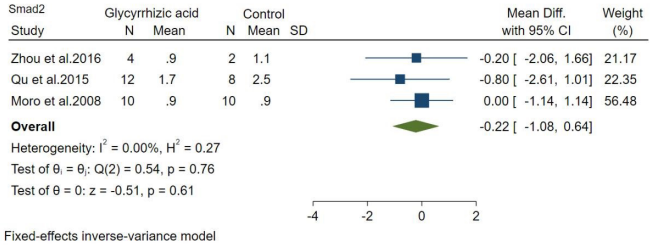

Figure 5. Forest plot showed the effects of glycyrrhizic acid on mRNA expression of Smads.

healing regardless of origin. While intense research has led to considerable improvements in the understanding of liver fibrosis pathogenesis, effective antifibrotic therapies are still lacking. In China, Huangqi decoction (HQD) was reported for the first time. This plant consists of Chinese herbs, Radix Astragali (Astragalus membranceus (Fisch.) Bge. Root, Huangqi) and Radix Glycyrrhizae (Glycyrrhiza uralensis Fisch., root and rhizome, Gancao). This plant has been used to treat patients with syndromes such as fatigue, heart palpitations, dry mouth and sallow complexion, and is commonly used for chronic liver disease, including liver cirrhosis (Liang et al., 2015). According to previous studies, the present study was performed to evaluate the effect of licorice on liver fibrosis.

In present systematic review and meta-analysis the Glycyrrhizic acid showed a decreased in hypdroxyproline levels. Studies showed similar result to present study, Glycyrrhizic acid can significantly improve the pathological changes associated with hepatic fibrosis, to include, significantly reducing liver hypdroxyproline levels (Li et al., 2019; Lin et al., 2005). Also In present systematic review and meta-analysis ALT activity was significantly decreased in the Glycyrrhizic acid group relative to the control group. Furthermore, a decrease in either mRNA of the above TGF-β1 signaling components was noted after treatment. These results suggest that Glycyrrhizic acid combination treatment blocks TGF-β1 signaling Glycyrrhizic acid combination remarkably reduced TGF-β1, Smad2 and Smad3 mRNAs and protein expression. These results suggest that the Glycyrrhizic acid combination could reverse hepatic fibrosis by inhibiting the TGF-β1 signaling pathway. TGF-β1 has been identified as the most profibrotic cytokine, and can elevate the expression of collagen I in hepatic stellate cell. Other studies show similar results to the present study (Milanini et al., 1998; Cassiman et al., 2002; Gomes et al., 2012; Zhang et al., 2016; Yamashita et al., 2017). Abdel-Wahab et al. (2021) and Chen et al. (2017) investigated the effect of licorice roots extract on some metabolic pathways and their regulating miRNAs in hepatocellular carcinoma cells, the result various beneficial effects of licorice roots extract including induction of apoptosis and cell cycle arrest, upregulating tumor suppressor miRNAs; let7a-3p, miR-34c-5p, miR-122-5p, miR-126-3p, miR195-5p, miR-199a-5p, miR-206, and miR326-5p and inhibiting HIF1a, PI3K and C-Myc and activating AMPK and p53. Notably, mirnas have been shown to be involved in the development of fibrosis (Abdel-Wahab et al., 2021; Zheng & Wang, 2020). Fourth, inhibiting enzymes of glycolysis; HK-2, LDH-A and PK-M2. He et al. (2019) investigate the critical role of uptake transporters mediating the transport of aconitum alkaloids into the liver, the result showed liquorice might lower the toxicity of aconite by reducing its exposure in the liver through inhibition of uptake transporters. Also Salawu et al. (2019) showed that the composite blends Moringa leaf + Licorice root at a regulated dose, could be explored as functional food in the provision of nutritionally important minerals, and the management of stress-related diseases.

5 Conclusion

Meta-analysis showed the Glycyrrhizic acid reduced TGF-β1, Smads mRNAs, hypdroxyproline, alanine aminotransferase and combination could reverse hepatic fibrosis by suppressing HSC activation and inhibiting the TGF-β1 signaling pathway.

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