Synergistic Hepatoprotective Effects of *Glycyrrhiza glabra* and Silymarin: Modulation of TGF-β/Smad Signaling Pathway in Experimental Liver Fibrosis

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Abstract

Liver fibrosis is a progressive pathological condition characterized by increased deposition of the extracellular matrix and deterioration of liver functions. The current study aimed to evaluate the hepatoprotective effects of the ethanolic and aqueous extracts of licorice roots (*Glycyrrhiza glabra*) compared to silymarin in carbon tetrachloride (CCl_4)-induced liver fibrosis in male rats. Twenty-five adult male Sprague–Dawley rats were used and randomly divided into five groups (5 rats per group). Liver fibrosis was induced by administering carbon tetrachloride (CCl_4) intraperitoneally for six weeks. After inducing fibrosis, the animals were orally treated with silymarin, ethanolic extract, or aqueous extract of licorice for 30 days. The levels of transforming growth factor beta (TGF-β), total protein, albumin, globulin, total bilirubin, as well as oxidative stress indicators including superoxide dismutase (SOD) enzyme, were evaluated, along with histological examination of liver tissues.

The CCl_4 group showed a significant increase ($P \leq 0.05$) in serum levels of TGF-β and bilirubin, accompanied by a significant decrease in total protein and albumin concentrations compared to the control group. The treatment with silymarin and licorice extracts led to a significant improvement in these biochemical changes. The aqueous extract also showed better efficacy in some indicators, particularly TGF-β levels and serum proteins. Histological examination confirmed these results, showing a decrease in hepatic degeneration, necrosis, inflammatory infiltration, and fibrosis in the treated groups compared to the untreated infected group. The results indicate that *Glycyrrhiza glabra* extracts possess liver-protective and anti-fibrotic effects, which are likely due to their antioxidant and anti-inflammatory properties thru the regulation of the TGF-β signaling pathway. The aqueous extract also showed relatively higher efficacy in improving liver functions and liver histological structure.

Keywords: Licorice (*Glycyrrhiza glabra*), Silymarin, Liver fibrosis, Transforming Growth Factor-beta (TGF-β), Hepatoprotection , CCl_4 , Oxidative Stress.

Introduction

Chronic liver disease is the eleventh leading cause of death and the fourteenth leading cause of morbidity worldwide, as the global burden of liver diseases continues to increase [1]. Liver cirrhosis is considered a progressive pathological condition and one of the main pathways leading to liver cirrhosis. It results from chronic liver injury caused by viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, autoimmune disorders, toxins, and chemical compounds [2,3]. Continuous liver damage leads to the excessive accumulation of extracellular matrix proteins, causing dysfunction in the normal structure and function of the liver.

Carbon tetrachloride (CCl_4) is considered one of the most commonly used experimental models for inducing liver fibrosis, due to its ability to cause severe oxidative stress and damage liver cells by generating reactive oxygen species and free radicals. These reactive compounds lead to damage of cellular membranes, stimulate inflammatory responses, and activate hepatic stellate cells, which play a pivotal role in the process of liver fibrosis [4].

Despite the advancements in medical treatments, the currently available therapies for liver fibrosis and cirrhosis remain limited and may be accompanied by undesirable side effects. Therefore, interest in using medicinal plants as alternative therapeutic agents has increased due to their relative safety, low cost, and diverse biological activities [5]. Medicinal plants are a rich source of bioactive compounds such as alkaloids, flavonoids, tannins, terpenes, steroids, and phenolic compounds, many of which possess antioxidant and anti-inflammatory properties, capable of reducing oxidative stress and cellular damage [6,7]. Antioxidants work by inhibiting oxidative reactions and neutralizing reactive free radicals, which contributes to protecting cells and tissues from damage [2].

Licorice (*Glycyrrhiza glabra L.)* is considered A perennial herbaceous plant belonging to the Fabaceae family, it has garnered significant scientific interest due to its pharmacological and therapeutic properties [8]. Licorice roots contain many bioactive compounds, including glycyrrhizin, flavonoids, polysaccharides, sterols, and phenolic compounds, which contribute to its therapeutic effects [8]. Previous experimental and clinical studies have shown that licorice extracts possess antioxidant, anti-inflammatory, antimicrobial, and antiviral properties, in addition to liver-protective and anti-diabetic effects [3,9]. Silymarin is also one of the most famous natural compounds that protect the liver and is widely used in studies related to liver injuries and fibrosis.

Therefore, the current study aimed to investigate the therapeutic effects of the aqueous and ethanolic extracts of licorice roots (*Glycyrrhiza glabra*) compared to silymarin in experimentally induced liver fibrosis in male rats. The study focused on evaluating biochemical and immunological parameters, including transforming growth factor beta (TGF-β), bilirubin, total protein, albumin, globulin, as well as the histopathological changes in liver tissues.

Material and Methods

Experimental Animals

Animals: The study was conducted on 25 adult male albino rats (Sprague Dawley strain), weighing 200–250g and their ages were approximately 12–14 weeks, obtained from the Animal House of the College of Veterinary Medicine/University of Tikrit from 1/10/2025-12/12/2025.

Animal accommodation and acclimatization, the animals were accommodated for two weeks before the experiment to confirm good health status, adaptation to new housing and environmental conditions and absence of any pre-existing diseases prior to the use of the animals in the study. The rats were randomly divided into a control and four experimental groups of five animals.

They were housed in cages measuring 60 cm length × 30 cm width × 25 cm height. The floors of the cages were covered with wood-shavings, and the cages with custom-made metal covers. Animals were maintained under standard laboratory conditions (12 h light/dark cycle, moderate temperature (25 ± 2 °C), ample ventilation). Strict hygiene conditions were maintained, with disinfection of all cages every three days (Dettol 90%; 70% ethanol) and replacement of bedding (wood shavings) every three days.

The rats received a basic laboratory diet, which consisted of wheat flour (35%), yellow corn (35%), soybean meal (20%) and concentrated animal protein (10%), for supplementation with barley, bran and limestone, supplemented with antifungal agents. Food and water were given ad libitum during the duration of the experiment. The animals were weighed after grouping in order to properly adapt the dose to be administered to each rat in each group [10].

Collection and preparation of plant materials

Licorice (*Glycyrrhiza glabra*) roots were purchased from local stores. The roots washed with adequate water to remove dust and other impurities and then air-dried in shade at 30–35 °C and continuously turned with proper ventilation placed on clean cloth.

Once fully dried, roots were powdered until fine with an electric blender. The sieved

powder was carefully sieved to result in particles of the same size (uniform size powder) and was stored in an airtight container until used in extraction.

Licorice Roots Ethanolic Extract

The dried and ground roots of the licorice plant (*Glycyrrhiza glabra*) were extracted using 70% ethanol as the extraction solvent. In summary, 100 grams of root powder were mixed with 400 ml of 70% ethanol (280 ml ethanol and 120 ml distilled water). Then, the mixture was subjected to soaking for 24 hours at room temperature with intermittent stirring. After the extraction process, the mixture was filtered using medical gauze followed by What man No.1 filter paper. After that, the filtrate was concentrated at a temperature of 40–45 °C using a rotary evaporator, then the resulting extract was dried and stored at a temperature below 20 °C until further use.

Licorice Roots Aqueous Extract

The aqueous extract of *Glycyrrhiza glabra* roots was prepared using a ratio of 100 grams of root powder to 400 ml of distilled water. The mixture was soaked for 24 hours at room temperature with intermittent stirring, then filtered using medical gauze and What man No.1 filter paper. After that, the filtrate was concentrated using a rotary evaporator at a temperature of 40 °C until a semi-solid extract was obtained. Finally, the resulting extract was stored at a temperature of 4 °C until use..

Dose Optimization Study

This experiment was done to find the effective concentration of licorice root extract by finding the concentration that produced the greatest hypoglycemic effect. The extract was applied at several concentrations for testing and the concentration determined to be the most effective physiologically was selected as the effective dose from tested concentrations (100, 150, 200, 300 mg/kg of body weight).

A total of two groups of animals (twenty rats) were randomized in numbers of five after a calculation which took into account the similarity of body weights. Each rat received an individual number for each experimental block to identify rats in pre- and post-treatment blood glucose levels [11]. The groups were as follows:

- Groups 1 (Control) 0.5 mL of distilled water was administered to rats (1–4).
- Group 2: Rats No. (5–8) were given a licorice root extract at a rate 50 mg/kg.
- Group 3Rats (9–12): received 100 mg/kg licorice root extract;
- Group 4: 200 mg/kg licorice root extract (rat number: 13−16).
- Group 5: Rats (17–20) were treated with an equivalent dose of licorice root extract (300 mg/kg).

After grouping, the extracts were given through diameter tube-gavage orally at doses of (50, 100, 200, and 300 mg/kg body weight) for characterizing the highest effective concentration to reduce blood glucose. An hour post-administration, glucose levels in the blood were measured using a glucometer (GlucoLab, USA), following collection of blood samples from the tail vein.

The findings of the dose determination experiment indicated the gradual decrease in blood glucose levels with each extract concentration increment. It exhibited the maximum efficacy as compared to other dosages with highest reduction at the 300 mg/kg dose. Accordingly, the concentration was chosen as the effective dose for further experiments within the framework of the study [11].

Experimental Design

To study the effect of licorice root extracts on liver fibrosis, twenty-five male albino rats were used and randomly divided into five groups, with five rats in each group having similar weights. During the experimental period, all animals were fed a standard laboratory diet. Liver fibrosis was induced by intraperitoneal injection of carbon tetrachloride (CCl_4) twice a week for six weeks. Carbon tetrachloride was diluted with olive oil in a 1:1 ratio and administered at a dose of 1 ml/kg body weight. Except for the negative control group, all experimental groups underwent liver fibrosis induction.

After inducing fibrosis, the animals were treated orally for 30 days with either the ethanolic extract or the aqueous extract of licorice roots (*Glycyrrhiza glabra*) or with silymarin..

The experimental groups were structured as follows:

- Group I (Normal Control): In this group, only olive oil was given throughout the experiment period.
- Group II (Positive Control): this group was treated with carbon tetrachloride (CCl_4) during the whole experimental period.
- Group III: CCl_4 -induced and then silymarin treated.
- Group IV: CCl_4 induced and treated with ethanolic extract of licorice roots.
- Group V: Induced with CCl_4 and received licorice roots aqueous extract.

Blood Sample Collection

One month after the start of the experiment, blood samples were obtained from all experimental animals. The rats were fasted for 12 hours but had free access to water before taking blood to avoid physiological and biochemical variations. Animals were anesthetized with intraperitoneal injection of a mixture of Ketamine and Xylazine at appropriate doses based on body weight to allow for adequate anesthetic depth and to minimize pain during blood collection. Blood samples were collected in gel-tubes and allowed to clot for 15 minutes at room temperature. Following this, serum was obtained by centrifugation at 3000 rpm for 15 min. The serum collected was transferred into clean tubes and stored at −20 °C until biochemical analysis. Lastly, animals were sacrificed immediately after blood collection and dissected tissues, such as liver and pancreas, of interest for histological analysis.

Immunological and Biochemical Parameter Measurement

Portions of TGF-β from rats (100 µl) were assayed by the use of Enzyme-Linked Immunosorbent Assay (ELISA) titres. It is used for the quantitative determination of TGF-β antigen in serum, plasma, cell culture media or other biological fluids. Total protein and albumin concentrations were determined in serum samples using an automated biochemical analyzer (Auto Biochemistry Analyzer). Total protein was analyzed by the Biuret method, in which proteins react with copper ions (Cu^{2+}) in alkaline medium to generate a violet-colored complex. The color intensity is directly proportional to the concentration of protein in the sample. Total bilirubin was determined in serum using a colorimetric method based on the Jendrassik and Gróf reaction from recent reports where bilirubin reacts with diazo reagents to produce a color (azobilirubin) which is then measured at 540 nm. Colour Intensity is directly proportional to the concentration of bilirubin. Analysis was done on Cobas analyzer according to the instruction of Roche Diagnostics kits. Globulin levels were indirectly calculated from total protein and albumin using the following equation [12]:

Globulin concentration = Total protein − Albumin

Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was performed to determine significant differences between groups at $P<0.05$ [13]

Results and Discussion

Determining the effective dose of the ethanolic extract of *Glycyrrhiza glabra* roots

Table (1) shows that a dose of 300 mg/kg body weight of the ethanolic extract of *Glycyrrhiza glabra* roots exhibited the best physiological response compared to the other tested doses (50, 100, 200, and 300 mg/kg body weight). Therefore, this dose was adopted as the effective dose to be used in subsequent experiments to evaluate the

protective effects of the ethanolic extract against experimentally induced liver fibrosis.

Determination of the effective dose of the aqueous extract of *Glycyrrhiza glabra* roots

As shown in Table (2), a dose of 300 mg/kg body weight of the aqueous extract of *Glycyrrhiza glabra* roots exhibited the best physiological response compared to the other tested doses (50, 100, and 200 mg/kg body weight). Accordingly, a dose of 300 mg/kg was selected as the effective dose to evaluate the physiological effects of the aqueous extract in subsequent liver fibrosis experiments.

Table (2): Determination of the effective dose of the aqueous extract of *Glycyrrhiza glabra* roots.

Effect of Licorice Extracts on Serum Transforming Growth Factor-β (TGF-β) Concentration in Male Rats

The outcomes described in Table (3) revealed that TGF-β serum level was remarkably higher ($P \leq 0.05$) in the carbon tetrachloride (CCl_4) group (48.93 ± 8.57^{a}) compared to the control group (10.98 ± 1.12^{c}). However, it was significantly lower ($P \leq 0.05$) in silymarin-treated group (8.57 ± 1.47^{c}) in comparison with the CCl_4 group (48.93 ± 8.57^{a}). In the same way, licorice treatment groups had significantly lower TGF-β compared with the CCl_4 group, where the ethanolic extract was (31.44 ± 6.52^{b}) and the aqueous extract (19.28 ± 1.79^{bc}). Our results show higher levels of TGF-β in the serum of animals with CCl_4 -intoxication than in the healthy control group, a trend that has already been described in 2 papers [14,15]. Carbon tetrachloride (CCl_4) is a toxic liver and renal carcinogen that acts via saturation of oxidative stress and inflammatory signaling, which leads to damage of many tissue types. In the liver, CCl_4 is metabolized through cytochrome CYP2E1 to produce free radicals and reactive oxygen species (ROS) that can cause hepatocyte membrane damage. Lipid peroxides and the excessive production of ROS induce hepatic injury and trigger the secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), aggravating liver damage [16]. Furthermore, enhanced oxidative stress and inflammatory signaling stimulates fibrogenesis via the activation of hepatic stellate cells (HSCs), which are the dominant source of TGF-β1 production [17], [18]. TGF-β1 is a strong profibrotic cytokine and one of the key players in liver fibrosis progression. Once again, that elevation of TGF-β levels in serum follow CCl_4 indicates a direct succession of events: beginning with oxidative stress, followed by inflammatory responses and activation of HSCs, leading to increased secretion of fibrogenic mediators such as TGF-β1 [19] . Experimental animals pre-treated with silymarin showed a marked decrease in TGF-β activity in comparison with the CCl_4 -treated group. Our these findings corroborates to the previous studies [20], [21],[22] . that illustrated the hepatoprotective and anti-fibrotic effects of Silymarin via antioxidant and anti-inflammatory action.

Biochemical Parameters

Male Rat Serum Albumin

Table (3) illustrates that serum albumin concentration (g/dL) was significantly ($P \leq 0.05$) lower in the carbon tetrachloride (CCl_4) group (3.03 ± 0.12^{b}) than the control group (3.50 ± 0.14^{a}).

In CCl_4 group, it (3.03 ± 0.12^{b}), however, in licorice-assisted groups, the difference was significantly higher, where the ethanolic licorice group increased (3.47 ± 0.23^{a}) and the aqueous licorice (3.45 ± 0.07^{a}). Moreover, there was an increase in albumin in the silymarin-treated group (3.27 ± 0.06^{ab}) compared to the CCl_4 group.

Total protein concentration in male rats.

The results of this analysis are summarized in Table (3) showing that total protein concentration (g/dL) decreased significantly ($P \leq 0.05$) in the CCl_4 group (4.50 ± 0.35^{c}) compared to the control group (6.65 ± 0.70^{a}).

Ethanolic extract (5.98 ± 1.38^{ab}) and aqueous extract (6.40 ± 1.77^{a}) both licorice-treated groups showed significant increase in comparison of CCl_4 group (4.50 ± 0.35^{c}). In a similar manner, total protein level was significantly increased (5.17 ± 0.03^{b}) in the silymarin-treated group when compared with that in the CCl_4 group.

Results The current study showed a significant ($P<0.05$) reduction in serum albumin and total protein in CCl_4 treated rats in comparison to the healthy control group. These results are consistent with other studies [23] , [24] , [25].

Hepatic protein levels are also diminished by oxidative damage due to lipid peroxidation generated by metabolism of carbon tetrachloride (CCl_4) into highly reactive free radicals like trichloromethyl (*CCl_4*) and trichloromethyl peroxyl (CCl_4 O)through cytochrome P450system. Lipid peroxidation is regarded as one of the characteristics of oxidative cellular injury. Free radicals are chemical species that have one or more unpaired electrons occupying atomic or molecular orbitals. These toxic radicals trigger chain reactions, and lipid peroxidation in phospholipid-rich membrane structures, mitochondria, and the endoplasmic reticulum. In this context, oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress caused by CCl_4 -induced lipid peroxidation. Its injury reduces protein synthesis as rough endoplasmic reticulum plays a role in the formation and synthesis of proteins [26]. Administration of silymarin also caused a rise in serum albumin and total protein levels when compared to the CCl_4 group. Silymarin from Silybum marianum is a strong free radical scavenger neutralizing the reactive oxygen species (ROS) generated from cellular metabolism and protecting the cells from oxidative stress damages hence presenting its cellular protective effects. It protects the hepatocytes and helps normalize the liver function through this mechanism [27]. Besides having direct antioxidant activity, silymarin improves endogenous antioxidant defense systems by upregulating the enzyme activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (. It also increases intracellular levels of glutathione (an important antioxidant for detoxification and protection of cells, which maintain membrane integrity and cell stability [28].

Serum Total Bilirubin Level in Male Rats

A comparison of the total bilirubin comparing CCl_4 (1.15 ± 0.07^{a} mg/dL) have significant increase ($P \leq 0.05$) compare with control group (0.93 ± 0.12^{b}) as shown in Table (3).The amount of silymarin (0.97 ± 0.06^{b} g/dL) and the aqueous extract of licorice roots (0.93 ± 0.29^{b} g/dL), were significantly decreased compared with the CCl_4 group (1.15 ± 0.07^{a} g/dL). Contrary to that, no significant difference was realized in comparison of the ethanolic extract-treated group and the CCl_4 group. The current results show a marked increase in serum bilirubin content in when compared to healthy control animals exposed to CC_4 as evinced by earlier studies [29]. Under physiological condition, hemoglobin is degraded to bilirubin and excreted into bile. Nevertheless, advanced hepatic injury disrupts the liver's capacity to takeup, conjugate and excrete bilirubin, resulting in hyperbilirubinaemia, which presents as a marker of hepatocellular damage and necrosis. An important mechanism behind these increased serum levels is the impaired ability of damaged liver tissue to continue perform bilirubin conjugation.

The serum bilirubin levels in the silymarin treated rats were significantly lower when compared with CCl_4 group. The possible mechanism for this effect may be related to the activation of nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway, which improves antioxidant enzyme expression and stabilizes mitochondrial membrane. Moreover, silymarin ameliorates the production of reactive oxygen species (ROS) by metal ion chelation and suppresses inflammatory responses. Moreover, it also maintains the integrity of the hepatocyte membrane, restricting toxins from entering this cell. Likewise, licorice aqueous extract tend to significantly lower bilirubin levels

from the CCl_4 group. This influence may be due to bioactive components of this plant and its effect on the production, conjugation and excretion of bilirubin by the liver [30].

Male Rat Serum Globulin Concentration

As demonstrated in Table (3), serum globulin concentration was significantly decreased ($P \leq 0.05$) in the CCl_4 group (1.47 ± 0.17^{c} mg/dL) when compared with the control group (3.15 ± 0.57^{a}). In particular, a markedly higher levels of the silymarin treatment (1.90 ± 0.04^{b} g/dL) compared to those levels of the CCl_4 group. Also, both licorice-treated groups exhibited a marked enhancement as the ethanolic extract (2.51 ± 1.15^{ab}) and aqueous extract (2.95 ± 1.30^{a}) compared with the CCl_4 group (1.47 ± 0.17^{c}). The current results showed between the control and CCl_4 groups a remarkable decrease in globulins levels, consistent with other studies [31]. The main cause of CCl_4 hepatotoxicity is the metabolic activation of CCl_4 by P450 system and the tri-chloromethyl free radicals, which are capable of surpassing lipid peroxidation. CCl_4 causes membrane damage in the hepatocyte, disrupting the proper functioning of the endoplasmic reticulum and mitochondria.Moreover, CCl_4 causes fat deposition within the liver, cellular fibrogenesis, and disruptions in lipid metabolism which are likely responsible for the altered levels of proteins and lipoproteins [32]. There was a significant difference in globulin level between CCl_4 group and licorice extracts treated groups. The potentially active properties of Liquorice might be due to its biologically active constituents such as glycyrrhizin, glycyrrhetinic acid, liquiritigenin, isoliquiritigenin, liquiritin, and glycycoumarin. New evidence indicates that these compounds exhibit hepatic protective effects via a combination of anti-steatotic, anti-oxidative, anti-immune, and anti-fibrotic actions. In addition, it has been also reported that licorice can inhibit the expression of pro-inflammatory cytokines from hepatocytes including IL-6, TNF-α, and NF-κB, and thus exert strong anti-inflammatory effects [33]. This indicates that licorice extract is beneficial in preventing hepatic oxidative and inflammatory cell injury.

Likewise, Silymarin treatment elevated regard of globulin in relation with CCl_4 group due to its anti-oxidative and anti-inflammatory properties with reducing of reactive oxygen species and inflammatory biomarkers. Silymarin also enhanced antioxidant defense systems (SOD, CAT, GPx, GR, GST, and GSH) and preserves near normal liver histoarchitecture [34] [35].

Histological Study Liver

In histological examination of liver sections from the control group, the hepatic architecture was normal with no pathological changes. Hepatocytes were arranged in radiating cellular cords extending towards the central vein with centrally located round nuclei and normal cytoplasm. Hepatic cords were organized between the hepatic sinusoids that are lined by fenestrated endothelial cells and without the continuous basement membrane that promotes interaction between the blood and hepatocytes. Hepatic structure was visualized using a standard histological method, hematoxylin and eosin (H&E) staining (40× magnification) for assessing liver architecture [36 [[37 [.

Conversely, liver sections in the carbon tetrachloride (CCl_4) group exhibited a spectrum of progressive histopathological changes that parallel the molecular and cellular mechanisms of hepatotoxicity described in the literature. For steatotic changes, microscopically, the sections demonstrated moderate steatosis with numerous lipid vacuoles in the cytoplasm of hepatocytes. These changes are attributed to the metabolic activation of CCl_4 to the trichloromethyl free radical (CCl_4 •) by the CYP2E1 enzyme which binds covalently to cell and induces a biphasic impairment of lipid metabolism. It includes the inhibition of hepatic lipid excretion and the simultaneous stimulation of lipid synthesis causing considerable intracellular lipid accumulation within substantial foamy vacuoles [38 [. In

the same vein, histological sections showed prominent hepatocellular swelling (hydropic degeneration), a direct morphological correlate of CCl_4 injury to plasma, lysosomal, and mitochondrial membranes. This alteration impairs sodium–potassium pumps which leads to water influx into the intracellular space [39[[[40.This cellular swelling was associated with marked nucleocytoplasmic pyknosis which is a recognized histological characteristic of irreversible cell injury and the onset of apoptotic (programmed) cell death. Subsequently, CCl_4 activates the pro-apoptotic proteins Bax and Bak to form pores in the outer mitochondrial membrane and leads to cytochrome c release to the cytosol. This event activates the caspase cascade wherein caspase-9 and caspase-3 are finally activated leading to DNA fragmentation and cell death.

The sections also revealed necrosis of vascular walls with marked proliferation of fibroblasts in the adjacent tissue in the fibrous work [41]. This represents a direct histological manifestation of hepatic fibrogenesis, whereby inflammatory signals released by injured cells especially transforming growth factor-beta (TGF-β) and tumor necrosis factor-alpha (TNF-α) typically induce activation of hepatic stellate cells (HSCs). When activated, these cells differentiate into myofibroblast-like cells which secrete collagen and extracellular matrix components [42].

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Table (1): Determination of the effective dose of ethanolic extract of *Glycyrrhiza glabra* roots on serum glucose concentration

Group	Glucose (mg/dl)
Control	104.00±3.61[b]
50 mg	114.00±3.61[a]
100 mg	106.33±7.64[b]
200 mg	291.67±7.64[c]
300 mg	76.67 ±6.51[d]

Values are expressed as mean ± standard error (SE).

The number of rats in each group was 3.

Different superscript letters within the same column indicate a significant difference at the probability level ($P \leq 0.05$).

Table (2): Effect of aqueous extract of *Glycyrrhiza glabra* roots on serum glucose level.

Group	**Glucose (mg/dl)**
Control	106.67±6.11[b]
50 mg	109.33±9.02[a]
100 mg	95.67±14.53[c]
200 mg	100.00±7.00[b]
300 mg	85 .00±5.00[d]

Values are expressed as mean ± standard error (SE).

The number of rats in each group was 3.

Different superscript letters within the same column indicate a significant difference at the probability level ($P \leq 0.05$).

Table 3. Serum proteins Parameters in Different Study Groups (Mean ± SE)

Group	TGF-β	Total Protein (g/dL)	Total Bilirubin	Globulin (g/dL)	Albumin (g/dL)
Control	10.98 ± 1.12^{c}	6.65 ± 0.70^{a}	0.93 ± 0.12^{b}	3.15 ± 0.57^{a}	3.50 ± 0.14^{a}
CCl_4	48.93 ± 8.57^{a}	4.50 ± 0.35^{c}	1.15 ± 0.07^{a}	1.47 ± 0.17^{c}	3.03 ± 0.12^{b}
Plant	8.57 ± 1.47^{c}	5.17 ± 0.03^{b}	0.93 ± 0.29^{b}	1.90 ± 0.04^{b}	3.27 ± 0.06^{ab}
Ethanol	31.44 ± 6.52^{b}	5.98 ± 1.38^{ab}	1.00 ± 0.26^{ab}	2.51 ± 1.15^{ab}	3.47 ± 0.23^{a}
Aqueous	19.28 ± 1.79^{bc}	6.40 ± 1.77^{a}	0.97 ± 0.06^{b}	2.95 ± 1.30^{a}	3.45 ± 0.07^{a}

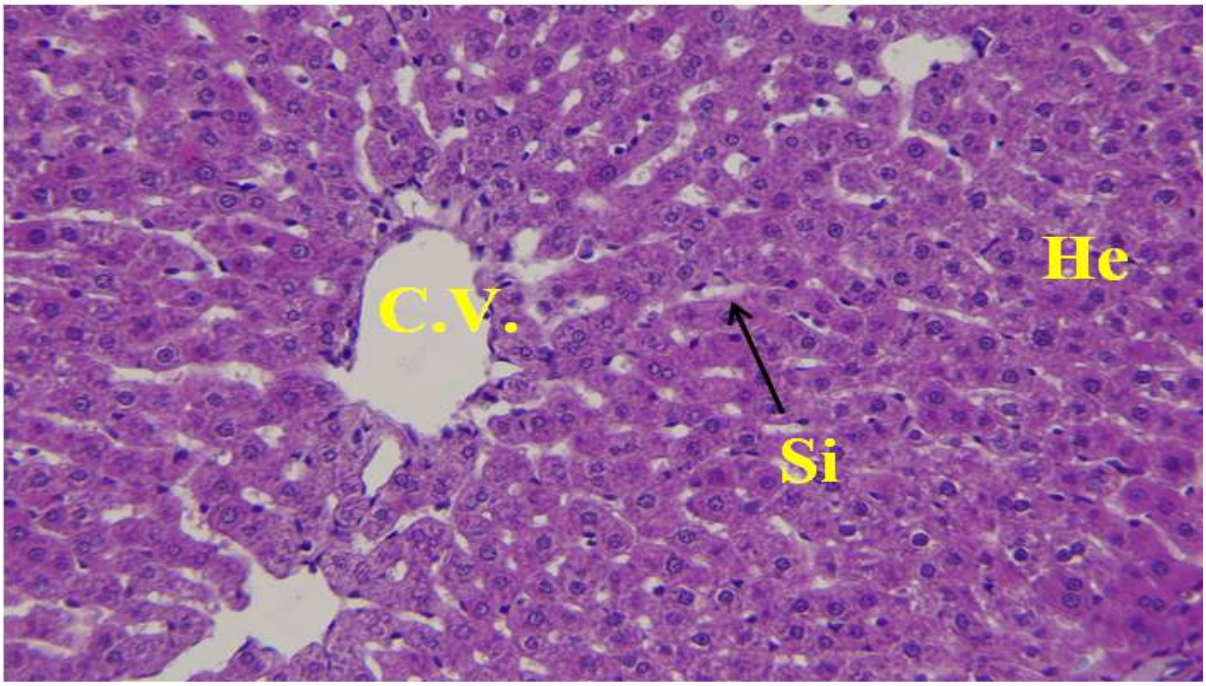

Figure (1): A transverse section of the liver from the control group showing normal hepatic architecture, where hepatocytes (He) are arranged in regular cords radiating around a central vein (C.V.). The hepatic sinusoids (Si) appear normal. (H&E, 40×).

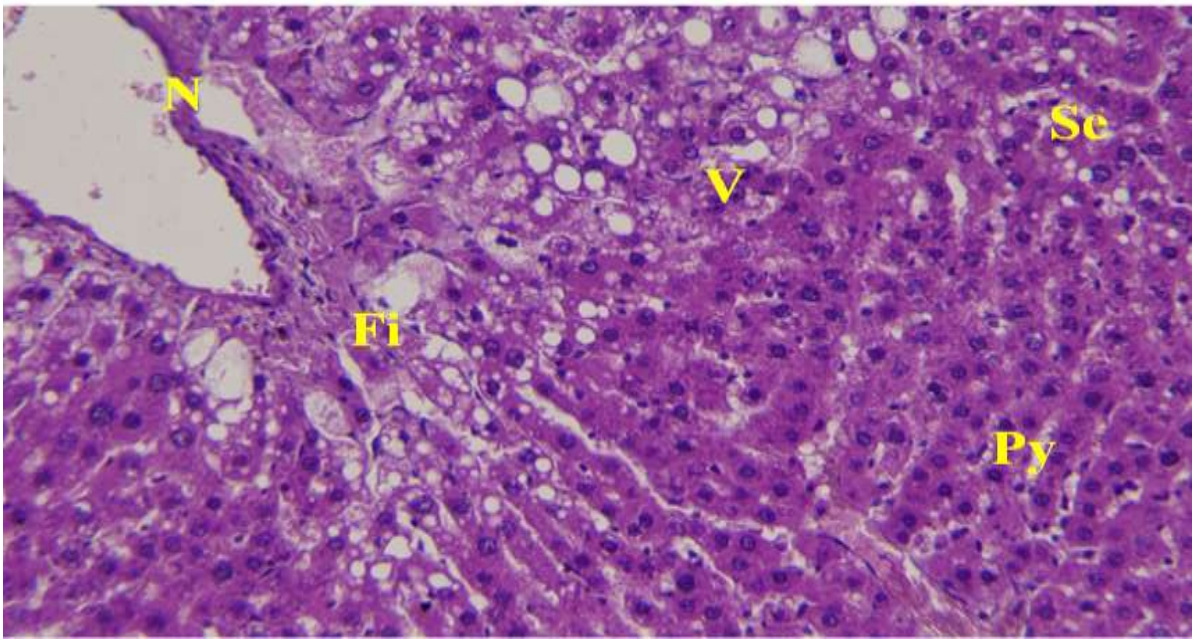

Figure (2): A transverse section of the liver from the CCl_4 -treated group showing moderate hepatic fatty degeneration (V), characterized by numerous lipid vacuoles within the cytoplasm. The hepatocytes also exhibit cellular swelling (Se) with nuclear pyknosis (Py), in addition to vascular layer necrosis and proliferation of fibroblasts beneath the blood vessels (Fi). (H&E, 40×.)

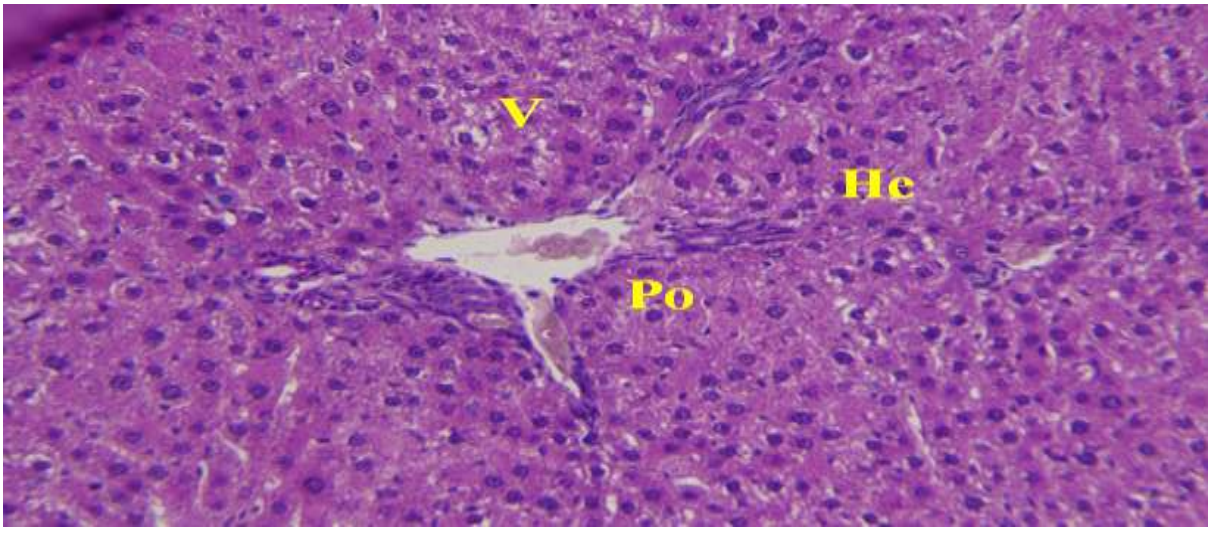

Figure (3): A transverse section of the liver from the silymarin-treated group showing preserved hepatic architecture with normal arrangement of hepatocytes around the portal area (Po). Some cytoplasmic vacuolations (V) were observed. No evidence of necrosis or fibrosis was detected, indicating only mild hepatic alterations. (H&E, 40×).

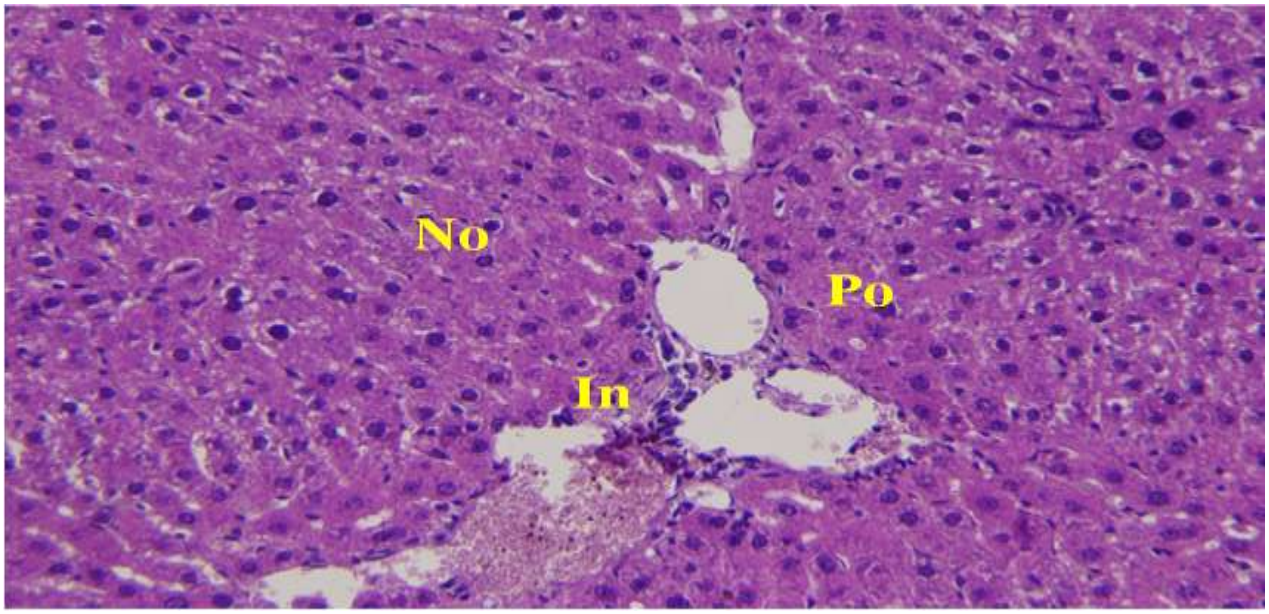

Figure (4): A transverse section of the liver from the group treated with the ethanolic extract of licorice roots showing preserved hepatic architecture with normal arrangement (No) of hepatocytes around the portal area (Po). The portal triad structures appeared intact with minimal inflammatory infiltration (In). No evidence of necrosis or fibrosis was observed, indicating normal hepatic tissue architecture. (H&E, 40×.).

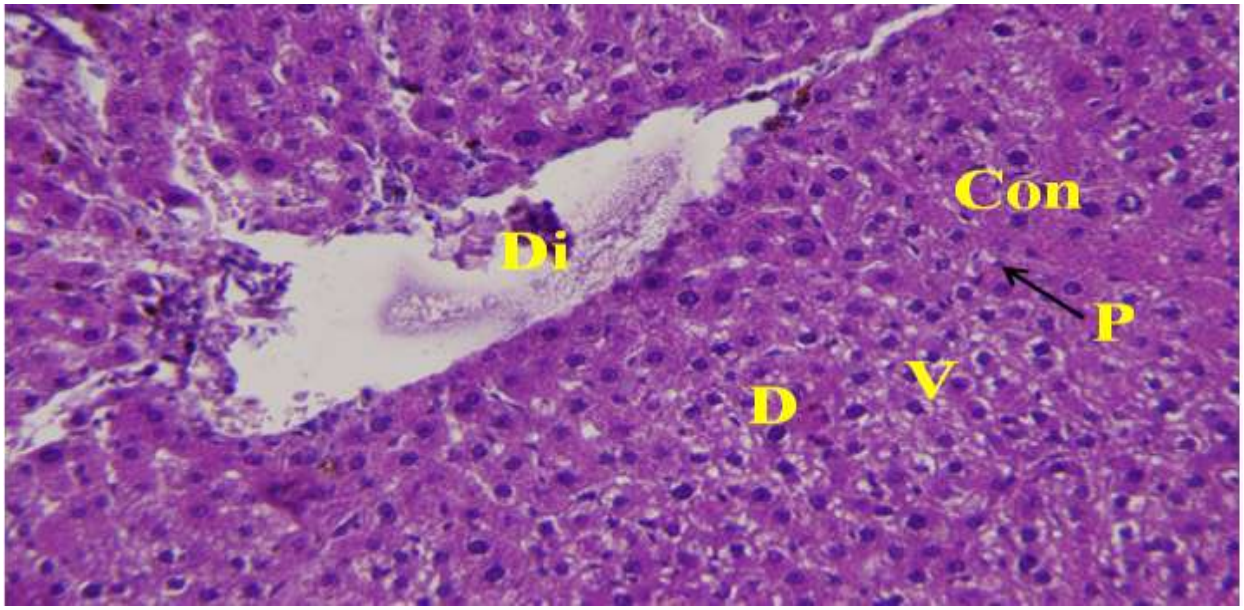

Figure (5): A transverse section of the liver from the group treated with the aqueous extract of licorice roots showing dilation (Di) of the central vein with sinusoidal congestion (Con). Hepatocytes exhibited cellular swelling and cytoplasmic vacuolization (V), indicating mild to moderate hepatic cellular degeneration (D), along with nuclear pyknosis (P) in hepatocytes. (Hematoxylin and Eosin stain, 40× magnification).

Conclusion

In conclusion, the present results showed that both ethanolic and aqueous extracts of *Glycyrrhiza glabra* roots have a distinct beneficial effect on the liver fibrosis caused by CCl_4 . The decrease in oxidative stress and inflammatory responses, the inhibition of transforming growth factor-beta (TGF-β), one of the major fibrogenesis mediators, are mechanisms by which it mediates this effect. The extracts were also able to furnish

biochemical liver function parameters and protein homeostasis in serum. In addition, licorice extracts also preserved the normal hepatic histoarchitecture and reduced the pathological changes in the liver tissues. Comparatively, these effects were similar to the silymarin-treated group, indicating that *Glycyrrhiza glabra* may be a potential natural hepatoprotective agent. Further studies must be performed to understand its mechanism of action and its clinical efficacy.

Acknowledgment: We extend our sincere gratitude to University of Kirkuk and the Department of Biology for their support and cooperation. This research did not receive any specific grant or financial support from funding agencies in the public, commercial, or non-profit sectors.

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