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### Methodology

# Ameliorative effect of liquorice extract versus silymarin in experimentally induced chronic hepatitis: A biochemical and genetical study

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### ARTICLE INFO

#### Article history:

Received 24 September 2018

Accepted 17 October 2018

Available online 7 November 2018

#### Keywords:

Chronic hepatitis

Liquorice extract

Silymarin

### SUMMARY

Chronic hepatitis is major health problem that affect liver function with high morbidity and mortality in developing countries. Macerated liquorice root used since several decades as one of traditional drink in Egypt. We aimed in this study to evaluate the hepatoprotective effect of liquorice extract versus silymarin in chronic hepatitis rat model and explain the possible mechanism of hepatoprotection in such disease. To achieve our aim fifty male albino rats were used and divided into 5 equal groups, control group, chronic hepatitis group, chronic hepatitis group protected with liquorice extract, chronic hepatitis group protected with silymarin, normal group administrated liquorice extract. The results of the study exhibits the hepatoprotective effects of liquorice extract against chronic hepatitis as well as silymarin through there antiinflammatory and antioxidants mechanism.

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## 1. Introduction

Chronic hepatitis is a slowly progressive long term inflammation of liver tissues. Chronic hepatitis may be asymptomatic in early stages of the disease appearance and detected by laboratory investigation. It can be developed to cirrhosis and/or fibrosis and even increase risk for hepatocellular carcinoma in sever stages. Chronic hepatitis can caused by several factors vary from metabolic, ischemic, infection to genetic factors [1].

Milk thistle (*Silybum marianum*) family Asteraceae one of most commonly used medicinal plants among centuries. It has been used to treat a range of liver and gallbladder disorders, including hepatitis, cirrhosis and jaundice, and to protect the liver against poisoning from chemical and environmental toxins, including snakebites, insect stings, mushroom poisoning and alcohol [2].

Milk thistle contain a number of hepatoprotective bioactive molecule such as lipids, in the form of linoleic, oleic and palmitic acid and flavonoids including quercetin, taxifolin, eriodictyol and chrysoeriol. However, the constituents responsible for the activity are flavanolignans (flavanone derivatives) initially isolated as a mixture of addition products of a coniferyl alcohol, phenylpropanoid alcohol, and a 2,3-dihydroflavonol, taxifolin. This mixture, known as silymarin and consists of silybin isosilibin, silychristin and silydianin, as well as silimonin, isosilychristin, isosilibinin [3].

Liquorice (*Glycyrrhiza glabra*) family Leguminosae has been widely used in folk medicine in treatment of several diseases like infection, inflammation and constipation. Liquorice contain coumarins (glycyrin, heniarin, liqcoumarin and umbelliferone), flavonoids (flavonols and isoflavones including formononetin, glabrin, glabrol, glabrone, glyzarin, glycyrol, glabridin and derivatives, kumatakenin, licoflavonol, licoisoflavones A and B, licoisoflavanone, licoricone, liquiritin and derivatives) and terpenoids (glycyrrhizic or glycyrrhizinic acid) all these compounds are responsible for pharmacological activity of liquorice [4].

The aim of the study was to evaluate the hepatoprotective effect of liquorice extract in comparison with silymarin (as the most bioactive agent in milk thistle) in experimentally induced chronic hepatitis and outline molecular mechanism through its effect on liver function, inflammatory biomarkers and antioxidant system.

## 2. Materials and methods

### 2.1. Preparation of liquorice plant extract

Liquorice (whole material root) was obtained from commercial source to be used for preparation of crude extraction. The aerial parts of liquorice plant were collected from our environment and the identified plant parts were refluxed in running tap water then with distilled water shade dried at room temperature and coarsely crushed using a pestle and mortar.

Extracts were prepared by macerating a weighted amount of the crushed plant parts 500 gm. in a known volume 1 L of water/organic (distilled water: absolute ethanol 70:30 v/v). Maceration continued for 72 h in refrigerator with internment shaking. The hydroethanolic extract was then strained through muslin mish (cotton), filtered through whattman paper #1.

The obtained filtrate was then concentrated using a shaking water bath at 70°C in a wide mouthed containers and the residue obtained (yield) was then weighed and used by dissolving in a measured amount of hydroethanol (70:30 v/v) solution [5]. The obtained liquorice extract was administrated orally at a high concentration dose of 400 mg/kg body weight [6].

### 2.2. Carbon tetrachloride and induction of chronic hepatotoxicity

CCL<sub>4</sub> used in the present study was kindly gifted from SIGMA pharmaceuticals, Quesna, Egypt. It was obtained as a pure concentration 100% CCL<sub>4</sub>. Rats were received the dosage rate of 20% of CCL<sub>4</sub> by intra peritoneal injection where each 20 ml of CCL<sub>4</sub> were dissolved in 80 ml of olive oil [7].

### 2.3. Laboratory animals

Fifty male rats aging 6–8 weeks of approximate weights 180–210 gm. were used in this study. Rats were attained from the animal house, Benha University, kept in separate metal cages and allowed to a plenty of water and diets (its composition is explained below) at room temperature. Rats were kept at constant environmental and nutritional conditions throughout the period of the experiment. The animals were left for one week for acclimatization before the beginning of the experiment. Rats received different treatments as explained below in the study design.

The animals were fed on basal ration throughout the course of the experiment in the form of concentrated diet composed of carbohydrates 58%, protein 17.5%, Lipid 3.4%, Cellulose 3.1%, Minerals 1.49%, Calcium 0.9%, Phosphorus 0.59% and moisture 12%.

### 2.4. Experimental design

Acclimatized rats were divided into five groups, each consists of ten rats. To assess the aim of the present work, groups are treated differently as follows:

#### 2.4.1. Group I (control group)

Rats were fed on normal diet and received 0.5 ml corn oil/rat/i.p/twice weekly and 1 ml saline solution/rat/orally/daily for 8 weeks of the experiment.

#### 2.4.2. Group II (chronic hepatitis group)

Rats were fed on normal diet and received 0.5 ml CCl<sub>4</sub>/rat/i.p/twice weekly and 1 ml saline solution/rat/orally/daily for 8 weeks of the experiment.

#### 2.4.3. Group III (silymarin protected group)

Rats were fed on normal diet and received 0.5 ml ccl<sub>4</sub>/rat/i.p/twice weekly and 1 ml silymarin/rat/orally/daily for 8 weeks of the experiment which is equivalent to a dosage rate of 100 mg/kg body weight of silymarin.

#### 2.4.4. Group IV (liquorice extract protected group)

Rats were fed on normal diet and received 0.5 ml CCl<sub>4</sub>/rat/i.p/twice weekly and high concentrated dose 1 ml LE/rat/orally/daily for 8 weeks of the experiment which is equivalent to a dosage rate of 400 mg/kg body weight of liquorice.

#### 2.4.5. Group V (control group with liquorice extract)

Rats were fed on normal diet and received 0.5 ml corn oil/rat/i.p/twice weekly and high concentrated dose 1 ml LE/rat/orally/daily for 8 weeks of the experiment which is equivalent to a dosage rate of 400 mg/kg body weight of liquorice.

### 2.5. Sampling

#### 2.5.1. Blood sampling for biochemical and hematological analysis

Blood for serum was collected after 4th and 8th weeks from the start of the experiment. Samples were collected from the venous plexus located at the medial canthus of the eye by means of heparinized capillary tubes. The collected blood was allowed to clot at room temperature for an hour; and then refrigerated for further an hour for clot retraction. Clear sera were separated by centrifugation at 3000 r.p.m. for 10 min and then collected in Eppendorf's tubes using automatic micropipettes. Part of the sample were used immediately for measuring the activity of the following biochemical and hematological parameters to assess the hepatic injuries; Complete blood picture (CBC), Alanine amino-transferase (ALT), Aspartate aminotransferase (AST) and Gamma glutamyl transferase ( $\gamma$ GT).

The rest of the amount serum was kept in deep freezer (–20 °C) for analysis of the following biochemical parameters; Total bilirubin, Direct bilirubin, Indirect bilirubin, Total protein, Albumin, Globulin, A/G ratio, and Alkaline phosphatase (ALP).

### 2.5.2. Liver tissue samples for biochemical analysis (liver tissue)

**2.5.2.1. Preparation of hepatic tissue.** At the end of the each experimental period, rats were sacrificed by cervical decapitation. The livers specimens were quickly removed and weighted, then perused with cold saline to exclude the blood cells and then blotted on filter paper, and stored at 20 °C for subsequent biochemical analysis.

Briefly, hepatic tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 min at 4 °C. The resultant supernatant was directly used for the determination of the following biochemical parameters: Lipid peroxidation marker; Malondialdehyde (L-MDA), Glutathione peroxidase (GSH).

### 2.5.3. Tissue samples for molecular analysis (liver tissue) for molecular investigation and reactive oxygen species (ROS) analysis

Liver tissues were collected from all animal groups, put in eppendorfs tube and were immediately kept in liquid nitrogen and stored at –80°C until RNA extraction for determination of the following parameters; Catalase (CAT), COX-2, L-Malondialdehyde (L-MDA), Super Dismutase (SOD), Glutathione Peroxidase (GPX), Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interlukin-10, Interleukine-1- $\beta$  (IL-1- $\beta$ ), Necrosis factor KAPPA- $\beta$  (NF-KB)

## 3. Results

Our results showed that the mean value of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Gamma glutamyl transferase ( $\gamma$ GT), A/G ratio and (Total, Direct and Indirect bilirubin) were significantly increased in chronic hepatitis group in comparison with control group while the mean value of serum total protein (TP), Albumin and Globulin were significantly decreased in chronic hepatitis group in comparison with control group.

The mean value of serum  $\gamma$ GT, ALP, ALT and AST were significantly decreased in Liquorice extract protected group in comparison with chronic hepatitis group while the mean value of serum TP, Albumin, Globulin and A/G were significantly increased in liquorice extract protected group in comparison with chronic hepatitis group.

Related to hematological profile, our results showed that, the mean value of plasma WBC's, Hb% and Granulocytic count levels were significantly increased in chronic hepatitis group in comparison with control group while the mean value of plasma RBC's, Hematocrit, RDW and lymphocytic count levels were significantly decreased in chronic hepatitis group in comparison with control group.

The mean value of plasma WBC's, Granulocytic count and RDW levels were significantly decreased in liquorice extract protected group in comparison with hepatitis group while the mean value of plasma RBC's, Hb%, Hematocrit and lymphocytic count levels were significantly increased in Liquorice extract protected group in comparison with chronic hepatitis group.

Reactive oxygen species parameters revealed a significantly increase in serum MDA and significantly decrease in serum GSH in chronic hepatitis group compared to control groups and shown a significantly decreased with serum MDA and a significantly increased with serum GSH in Liquorice extract protected group comparing to chronic hepatitis groups.

The molecular results showed a significantly up regulated with IL-1 $\beta$ , TNF- $\alpha$ , NFKB and COX-2 gene expression levels and a significantly down regulation with IL-10, GPX, SOD and CAT in rats liver tissues in chronic hepatitis group compare to control group (see [Tables 1 and 2](#)).

There is a significant increase with IL-10, SOD, CAT, IL-1 $\beta$  gene expression levels and a significantly decrease with TNF- $\alpha$ , NFKB and COX-2 gene expression levels in Liquorice extract protected group comparing to chronic hepatitis groups (see [Figs. 1–13](#)).

## 4. Discussion

Chronic hepatitis is considered as a serious disease that affect liver tissue and widely distributed in developing countries especially in Egypt. It reduce patients life quality and leading to more hazardous

**Table 1**  
Effect of liquorice extract administration on the bio-statistical analysis.

| Parameters         | Measurement points | Group 1         | Group 2          | Group 3                   | Group 4         | Group 5                    |
|--------------------|--------------------|-----------------|------------------|---------------------------|-----------------|----------------------------|
| ALT                | 4th week           | 65.00 ± 12.17   | 118.67 ± 1.73    | 77.0 <sup>ns</sup> ± 1.73 | 58.0 ± 1.53     | 77.33 <sup>ns</sup> ± 8.51 |
|                    | 8th week           | 90.33 ± 1.76    | 111.33 ± 11.14   | 94.67 ± 1.76              | 100.0 ± 6.43    | 102.67 ± 7.51              |
| AST                | 4th week           | 116.67 ± 21.84  | 239.33 ± 7.51    | 222.0 ± 16.17             | 252.0 ± 15.01   | 132.67 ± 5.70              |
|                    | 8th week           | 101.33 ± 1.76   | 196.0 ± 11.14    | 232.0 ± 18.04             | 256.0 ± 11.14   | 259.33 ± 7.86              |
| Total Protein      | 4th week           | 8.10 ± 0.36     | 5.80 ± 0.25      | 5.60 ± 0.76               | 5.90 ± 0.50     | 6.42 ± 0.12                |
|                    | 8th week           | 7.50 ± 0.09     | 5.10 ± 0.15      | 6.6 ± 0.13                | 6.40 ± 0.00     | 6.60 ± 0.24                |
| Serum albumin      | 4th week           | 3.55 ± 0.19     | 3.00 ± 0.23      | 3.24 ± 0.34               | 3.19 ± 0.11     | 3.36 ± 0.28                |
|                    | 8th week           | 3.40 ± 0.10     | 3.10 ± 0.17      | 3.56 ± 0.29               | 3.4 ± 0.10      | 3.65 ± 0.11                |
| Serum Globulin     | 4th week           | 4.55 ± 0.21     | 2.26 ± 0.21      | 2.36 ± 0.24               | 2.71 ± 0.21     | 3.04 ± 0.40                |
|                    | 8th week           | 4.5 ± 0.12      | 2.04 ± 0.12      | 3.04 ± 0.18               | 2.42 ± 0.18     | 2.95 ± 0.18                |
| A/G Ratio          | 4th week           | 0.78 ± 0.17     | 1.56 ± 0.25      | 1.3 ± 0.17                | 1.2 ± 0.15      | 1.16 ± 0.28                |
|                    | 8th week           | 0.66 ± 0.07     | 1.5 ± 0.13       | 1.17 ± 0.24               | 1.64 ± 0.20     | 1.23 ± 0.25                |
| ALP                | 4th week           | 434.667 ± 12.13 | 781.333 ± 7.06   | 710.33 ± 175.14           | 465.33 ± 8.82   | 468.67 ± 1.76              |
|                    | 8th week           | 553.33 ± 74.05  | 899.333 ± 114.78 | 899.33 ± 96.47            | 566.67 ± 125.86 | 728.67 ± 199.52            |
| γGT                | 4th week           | 2.20 ± 0.67     | 3.3 ± 0.58       | 2.66 ± 0.88               | 2.66 ± 0.67     | 2.9 ± 0.33                 |
|                    | 8th week           | 2.90 ± 0.88     | 4.3 ± 0.58       | 3.10 ± 0.67               | 3.0 ± 0.58      | 3.11 ± 0.58                |
| Total Bilirubin    | 4th week           | 0.21 ± 0.01     | 0.33 ± 0.01      | 0.25 ± 0.02               | 0.122 ± 0.00    | 0.21 ± 0.04                |
|                    | 8th week           | 0.24 ± 0.01     | 0.37 ± 0.01      | 0.30 ± 0.01               | 0.20 ± 0.03     | 0.22 ± 0.08                |
| Direct Bilirubin   | 4th week           | 0.11 ± 0.01     | 0.19 ± 0.01      | 0.14 ± 0.02               | 0.11 ± 0.00     | 0.12 ± 0.04                |
|                    | 8th week           | 0.13 ± 0.01     | 0.21 ± 0.01      | 0.16 ± 0.01               | 0.14 ± 0.03     | 0.14 ± 0.08                |
| Indirect Bilirubin | 4th week           | 0.10 ± 0.01     | 0.21 ± 0.01      | 0.14 ± 0.05               | 0.10 ± 0.01     | 0.13 ± 0.03                |
|                    | 8th week           | 0.11 ± 0.03     | 0.23 ± 0.01      | 0.16 ± 0.01               | 0.12 ± 0.05     | 0.15 ± 0.05                |

Data are presented as (Mean ± S.E). S.E = Standard Error.

**Group 1:** control group, **Group 2:** chronic hepatitis group, **Group 3:** chronic hepatitis protected with silymarin, **Group 4:** chronic hepatitis protected with liquorice extract, **Group 5:** control group with liquorice extract.

complication. Treatment of chronic hepatitis is costly and large number of patients not respond appropriately with therapies [8].

Liquorice possess a variety of pharmacologic action such as anti-inflammatory, antiviral, antioxidant, immunomodulatory, hepatoprotective and cardioprotective effect [9].

Our results was in accordance with [10] who reported that, The structural similarity between glycyrrhetic acid and mineralocorticoid hormones secreted by the adrenal cortex and gluco-corticoid activity of glycyrrhizic acid, licorice exhibit steroid-like anti-inflammatory activity similar to hydrocortisone. This leads to inhibition of phospholipase A<sub>2</sub> activity, which is responsible for numerous inflammatory processes. In vitro research has also demonstrated glycyrrhizic acid inhibits cyclooxygenase activity and prostaglandin formation as well as indirectly inhibiting platelet aggregation, all factors in the inflammatory process.

In addition [11] found that, aspartate aminotransferase released by hepatocytes incubation with anti-liver cell membrane antibody plus complement can be significantly decreased after adding glycyrrhizin to this system.

Also [12], induced leakage of lactic dehydrogenase and glutamic oxaloacetic transaminase in a monolayer culture of rat hepatocytes by exposing these cells to carbon tetrachloride (CCl<sub>4</sub>). This enzyme leakage was caused by a change in membrane permeability. The addition of glycyrrhizin caused a dose-dependent reduction of enzyme release; it was slightly effective at 25 µg/ml and maximally effective at 200 µg/ml. Glycyrrhizin probably acted by a direct protective effect on the hepatocyte membranes by preventing a change in cell permeability.

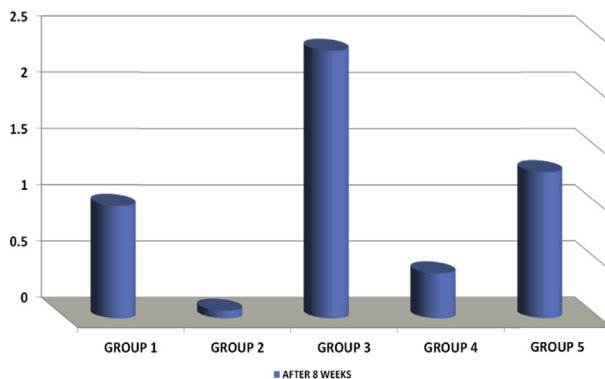
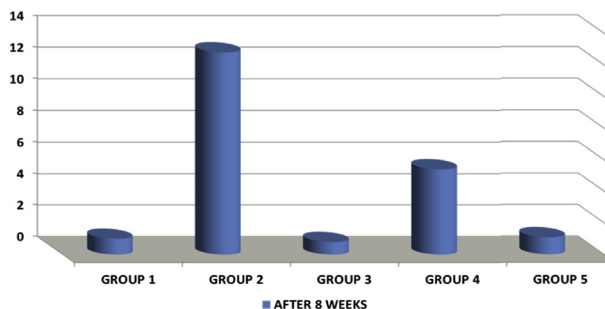
In addition [13] compared the hepatoprotective action of glycyrrhizin and glycyrrhetic acid. They found that glycyrrhetic acid was effective in decreasing the transaminases in a concentration of 5 µg/ml and glycyrrhizin was only effective at a dosage of 1000 µg/ml. This study suggests that glycyrrhetic acid is a better hepatoprotective drug than glycyrrhizin. After ischaemia-reperfusion damage of the liver in rat models the serum levels of aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase and lipid peroxides in liver tissue increased, while the liver glutathione concentration decreased significantly. Pretreatment of the rats with subcutaneous glycyrrhizin for 10 days suppressed

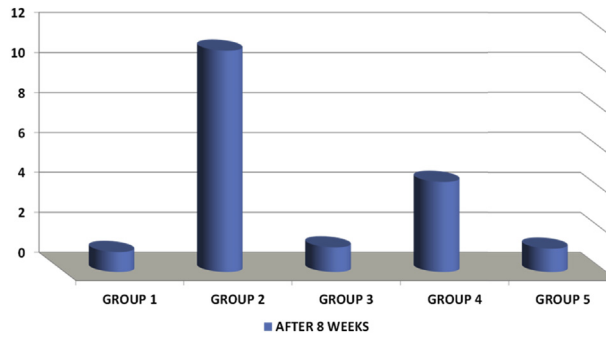
**Table 2**

Effect of liquorice extract treatment on the bio-statistical analysis.

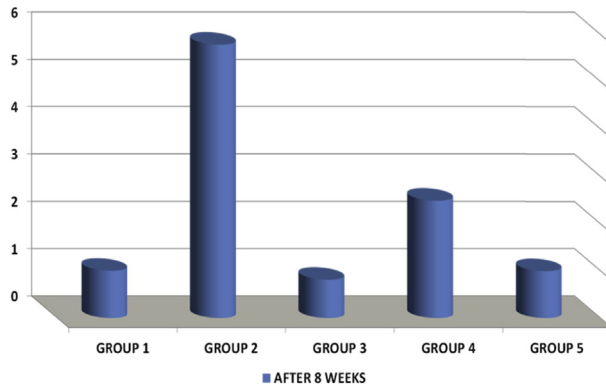
| Parameters         | Measurement points | Group 1       | Group 2       | Group 3      | Group 4      | Group 5      |
|--------------------|--------------------|---------------|---------------|--------------|--------------|--------------|
| Plasma WBC's       | 4th week           | 12.87 ± 2.63  | 11.9 ± 1.37   | 10.23 ± 1.88 | 12.87 ± 0.56 | 12.2 ± 0.50  |
|                    | 8th week           | 7.5 ± 0.12    | 10.0 ± 0.86   | 8.07 ± 0.64  | 8.57 ± 0.58  | 9.0 ± 0.81   |
| Plasma RBC's       | 4th week           | 2.03 ± 0.15   | 2.09 ± 0.11   | 3.54 ± 0.39  | 4.31 ± 0.41  | 4.5 ± 0.42   |
|                    | 8th week           | 3.29 ± 0.29   | 2.83 ± 0.25   | 2.76 ± 0.28  | 2.75 ± 0.26  | 3.9 ± 0.35   |
| (Hb%)              | 4th week           | 9.2 ± 0.50    | 9.17 ± 0.19   | 9.47 ± 0.23  | 9.57 ± 0.19  | 9.7 ± 0.32   |
|                    | 8th week           | 0.90 ± 0.23   | 9.03 ± 0.15   | 9.0 ± 0.35   | 9.57 ± 0.13  | 9.17 ± 0.49  |
| (HCT)              | 4th week           | 2.03 ± 0.15   | 2.09 ± 0.11   | 3.54 ± 0.39  | 4.31 ± 0.41  | 4.5 ± 0.42   |
|                    | 8th week           | 3.29 ± 0.29   | 2.83 ± 0.25   | 2.76 ± 0.28  | 2.75 ± 0.26  | 3.9 ± 0.50   |
| Lymphocytic count  | 4th week           | 3.35 ± 0.80   | 2.55 ± 0.11   | 2.54 ± 0.47  | 3.10 ± 0.19  | 2.43 ± 0.09  |
|                    | 8th week           | 2.75 ± 0.40   | 2.29 ± 0.20   | 2.32 ± 0.17  | 2.46 ± 0.09  | 2.34 ± 0.31  |
| Granulocytic Count | 4th week           | 8.26 ± 1.70   | 8.09 ± 1.03   | 6.55 ± 1.23  | 8.22 ± 0.36  | 7.89 ± 0.42  |
|                    | 8th week           | 3.69 ± 0.57   | 6.42 ± 0.49   | 5.96 ± 0.41  | 5.32 ± 0.61  | 5.63 ± 0.52  |
| (RDW)              | 4th week           | 79.88 ± 10.98 | 47.78 ± 22.84 | 72.0 ± 1.00  | 71.0 ± 0.00  | 67.0 ± 0.00  |
|                    | 8th week           | 66.0 ± 2.08   | 53.33 ± 8.25  | 46.0 ± 8.19  | 39.0 ± 2.65  | 67.0 ± 0.00  |
| (MDA)              | 4th week           | 33.67 ± 0.88  | 65.47 ± 0.88  | 30.53 ± 0.38 | 44.17 ± 1.13 | 33.17 ± 0.44 |
|                    | 8th week           | 33.17 ± 0.84  | 70.3 ± 1.18   | 29.71 ± 0.40 | 41.33 ± 1.20 | 32.37 ± 0.84 |
| (GSH)              | 4th week           | 33.73 ± 0.91  | 10.4 ± 0.30   | 38.77 ± 0.29 | 26.40 ± 0.83 | 33.51 ± 0.86 |
|                    | 8th week           | 35.0 ± 0.58   | 9.51 ± 0.25   | 40.0 ± 0.58  | 28.0 ± 0.58  | 34.17 ± 0.44 |

Data are presented as (Mean ± S.E). S.E = Standard Error.

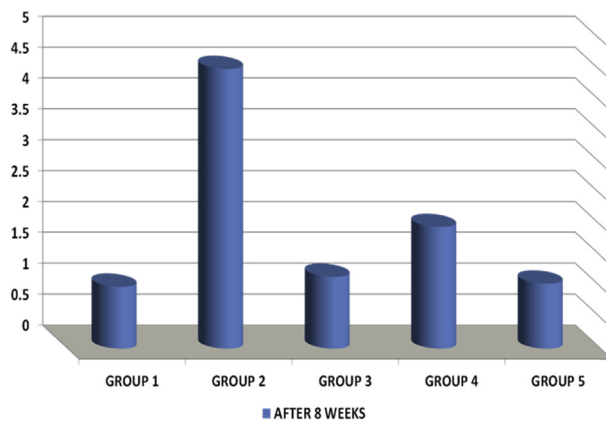
**Group 1:** control group, **Group 2:** chronic hepatitis group, **Group 3:** chronic hepatitis protected with silymarin, **Group 4:** chronic hepatitis protected with liquorice extract, **Group 5:** control group with liquorice extract.**Fig. 1.** Changes in relative expression of IL-10 gene in liver tissues on oral administration of 1 ml 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis.**Fig. 2.** Changes in relative expression of IL-1β gene in liver tissues on oral administration of 1 ml LE of 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis.



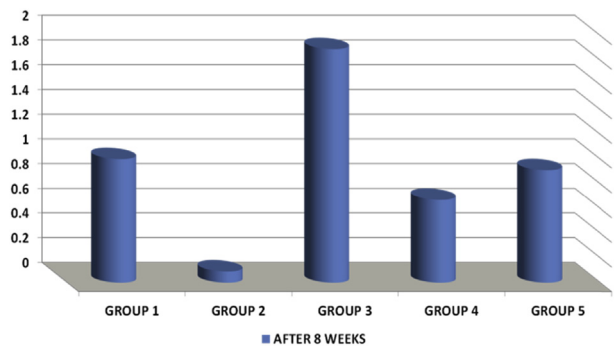
**Fig. 3.** Changes in relative expression of TNF- $\alpha$  gene in liver tissues on oral administration of 1 ml LE 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis.



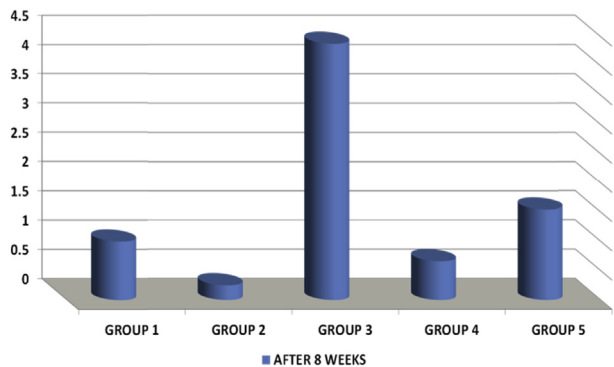
**Fig. 4.** Changes in relative expression of NFKB gene in liver tissues on oral administration of 1 ml LE of 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



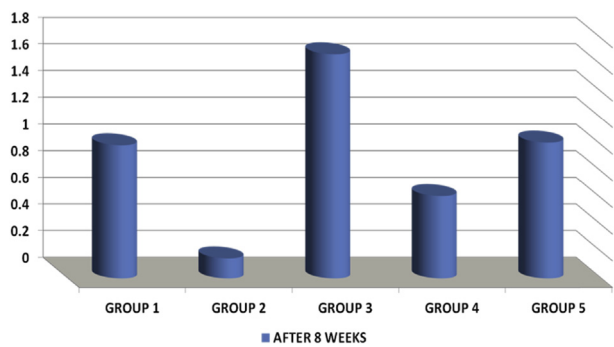
**Fig. 5.** Changes in relative expression of COX-2 gene in liver tissues on oral administration of 1 ml LE 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis.



**Fig. 6.** Changes in relative expression of GPX gene in liver tissues on oral administration of 1 ml LE 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis.



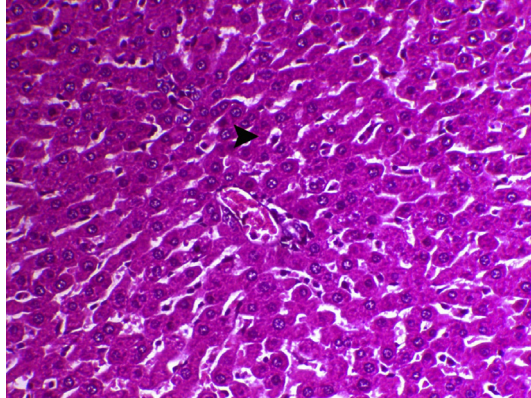
**Fig. 7.** Graphical representation of real-time quantitative PCR analysis of the expression of CAT gene in liver tissues on oral administration of LE 400 mg/kg body weight in albino rats fed on basal diet and CCL4-induced chronic hepatitis.



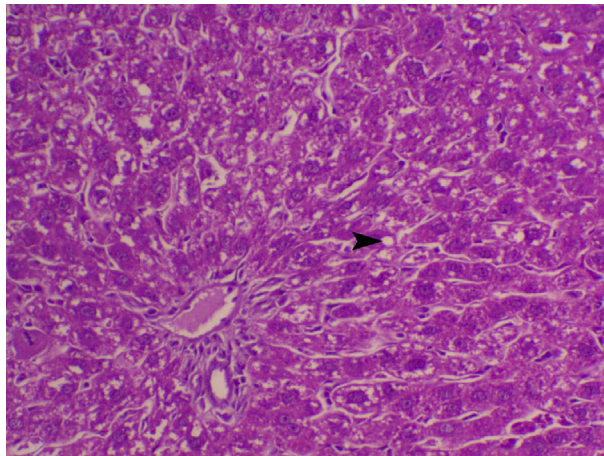
**Fig. 8.** Graphical representation of real-time quantitative PCR analysis of the expression of CAT gene in liver tissues on oral administration of 400 mg/kg body weight in albino rats fed on basal diet and CCL4- induced chronic hepatitis.

the elevation of lipid peroxides and serum aspartate aminotransferase, alanine aminotransferase and lactic dehydrogenase, while the glutathione concentration in liver tissue remained at control level. Glycyrrhizin suppressed liver damage by acting as a hydroxyl-radical. Pre-treatment with glycyrrhizin also reduced the morphological damage as assessed by electron microscopy.

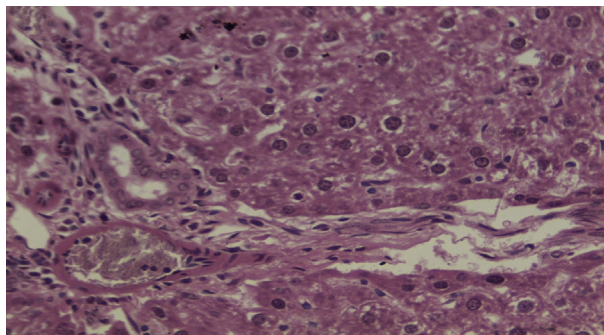




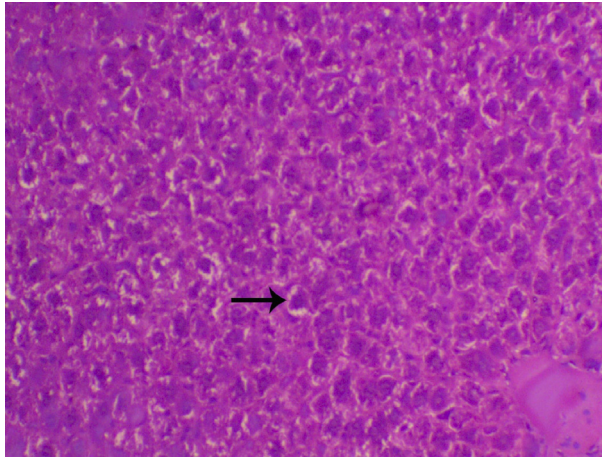
**Fig. 9.** Liver of normal control rat showing normal hepatocytes around the portal area (arrowhead), H&E, X200.



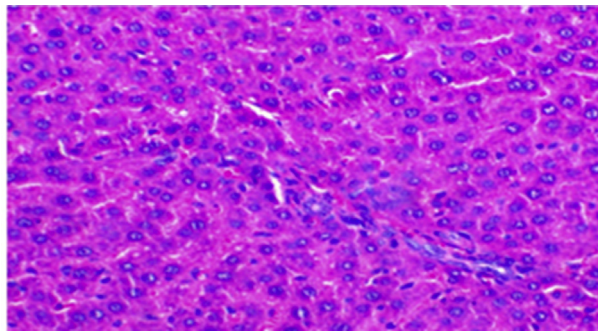
**Fig. 10.** Liver of CCL4-treated rat showing marked vacuolation of hepatocytes within the periportal area mostly of fatty degeneration type (arrowhead), H&E, X200.



**Fig. 11.** liver of CCL4-treated rat showing degenerative changes with increased number of fibroblast and slight fibrosis (H&E X400).



**Fig. 12.** Liver of CCL4 rats protected with high dose of liquorice extract showing mild vacuolation of hepatocytes (arrow), H&E, X200.



**Fig. 13.** Liver of CCL4 rats protected with silymarin showing normal liver structures, H&E, X200.

Furthermore [14] showed that oral administration of glycyrrhizin can significantly improve liver function. In their study 7.5 mg glycyrrhizin in a capsule had been given orally twice a day for 30 days for acute hepatitis and for 90 days for chronic hepatitis.

Licorice constituents possess significant antioxidant and hepatoprotective properties. Glycyrrhizin and glabridin inhibit the generation of reactive oxygen species (ROS) by neutrophils at the site of inflammation. In vitro studies have demonstrated licorice isoflavones, hispaglabridin A and B, inhibit mitochondrial lipid peroxidation in rat liver cells. Other research indicates glycyrrhizin lowers lipid peroxide values in animal models of liver injury caused by ischemia reperfusion. Licorice constituents also exhibit hepatoprotective activity by lowering serum liver enzyme levels and improving tissue pathology in hepatitis patients [15].

High content of phenolic component in ethanolic extract of Liquorice is responsible for its powerful antioxidant activity by means of significant free radical scavenging, hydrogen-donating, metal ion chelating, anti-lipid peroxidative and reducing abilities [16]. Liquorice flavonoids have exceptionally strong antioxidant activity. Antioxidant activity of liquorice flavonoids was found to be over 100 times stronger than that of antioxidant activity of vitamin E. A dose of 2.58 mg/ml liquorice flavonoids can scavenge more free radicals than 258 mg/ml of vitamin E. Flavonoids from liquorice are currently the strongest natural antioxidants known. Thus, liquorice extract can be efficiently used to formulate cosmetic products for the protection of skin and hair against oxidative damage [17].

## 5. Conclusion

In our study, CCl<sub>4</sub> produce hepatic tissue damage and oxidative stress similar to those seen in human chronic hepatitis evidenced by markedly elevation of liver function parameter and toxic product resulted from peroxidation of biological membrane polyunsaturated fatty acids (MDA). Also, our results demonstrate the hepatoprotective effects of liquorice extract against chronic hepatitis as well as silymarin through antiinflammatory and antioxidants mechanism.

## Conflict of Interest

None declared.

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