



RESEARCH ARTICLE

BIOCHEMICAL AND GENETICAL EVALUATION OF AMELIORATIVE ROLE OF LIQUORICE EXTRACT ON EXPERIMENTALLY INDUCED HEPATITIS

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ABSTRACT

Liver acts as a cornerstone in most biotransformation process. CCl<sub>4</sub> is one of the most common chemicals producing liver injury. Liquorice is a medicinal plant widely consumed in Egypt as a juice. In our study we aimed to estimate the hepatoprotective effect of liquorice crude extract against CCl<sub>4</sub>-induced hepatitis in rats and to clarify its underlying molecular mechanism through its effect on inflammatory biomarkers and antioxidant system. To achieve our objective a sixty male albinorats were divided into 6 groups, normal control group, hepatitis group, hepatitis group treated with low conc. liquorice extract, hepatitis group treated with high conc. liquorice extract, normal group with low conc. liquorice extract and normal group with high conc. liquorice extract. The outcome of the study exhibits a representative association between exposure to CCl<sub>4</sub> and alteration of liver function parameters and hematological profile and elucidate the hepatoprotective effect of liquorice extract through its anti-inflammatory and antioxidant properties.

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INTRODUCTION

Chronic liver diseases are a principle cause of mortality and morbidity among developing countries (Lozano et al., 2012). Hepatitis is an inflammation of the liver caused by viruses, toxic substances or other infections. This condition can be self-limiting or can be developed to fibrosis, cirrhosis or liver cancer (Hernandez-Gea and Friedman, 2011). CCl<sub>4</sub> is one of the xenobiotics that have been noted to induce acute and chronic tissue injuries. It has been widely used to study hepatotoxicity in experimental models by triggering lipid peroxidation, thus causing injuries to hepatic tissues. Liver is particularly susceptible to oxidative damage due to the direct release of CCl<sub>4</sub> metabolites and cytokines, which leads to inflammatory response (Murugesan et al., 2009) Herbal plants are extensively utilized in developing countries and many people depend on it as a source of treatment. One of these medicinal plants is liquorice (Glycyrrhizaglabra) belonged to family Leguminosae.

It contains a series of pharmacologically active compounds which traditionally used as antioxidants, anti-inflammatory, anti-estrogenic, Antiplatelet, Antimicrobial and hepatoprotective agent (Yu et al., 2015). The main objective of our work was to evaluate the hepatoprotective effect of liquorice crude extract against CCl<sub>4</sub>-induced hepatitis in rats and to elucidate its underlying molecular mechanism through its effect on liver function, inflammatory biomarkers and antioxidant system.

MATERIALS AND METHODS

**Preparation of Medicinal plant crude 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 liter of water/organic (distilled water : absolute ethanol 70:30 v/v). Maceration continued for 72 hours in refrigerator with intermittent shaking.

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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 (Attokaran, 2017). The obtained liquorice extract was administrated orally at a high concentration dose of 400 mg/kg body weight and a low concentration dose of 200 mg/kg body weight (Nakagawa, Kishida et al., 2004).

**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 (Alhassan, et al. 2009).

**Laboratory animals:** Sixty 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%.

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

**GROUP I (control group):** Rats were fed on normal diet and received 0.5ml corn oil/ rat/ i.p /twice weekly and 1ml saline solution/ rat/ orally/ daily for 8 weeks of the experiment.

**GROUP II (Hepatitis group):** Rats were fed on normal diet and received 0.5ml CCL<sub>4</sub>/ rat/ i.p/twice weekly and 1ml saline solution / rat/ orally/ daily for 8 weeks of the experiment.

**GROUP III (Low conc. LE treated group):** Rats were fed on normal diet and received 0.5ml CCL<sub>4</sub>/ rat/ i.p/ twice weekly and low concentrated dose 1ml LE/ rat/ orally/ daily for 8 weeks of the experiment which is equivalent to a dosage rate of 200 mg/kg body weight of liquorice; kept as positive treated group

**GROUP IV (high conc. LE treated group):** Rats were fed on normal diet and received 0.5ml CCL<sub>4</sub>/ rat / i.p/ twice weekly and high concentrated dose 1ml 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; kept as positive treated group.

**GROUP V(control group with low conc. LE):** Rats were fed on normal diet and received 0.5ml corn oil/ rat/ i.p/ twice weekly and low concentrated dose 1ml LE/ rat/ orally/ daily for

8 weeks of the experiment which is equivalent to a dosage rate of 200 mg/kg body weight of liquorice.

**GROUP VI (control group with high conc. LE):** Rats were fed on normal diet and received 0.5ml corn oil/ rat/ i.p/ twice weekly and high concentrated dose 1ml 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.

### Sampling

**Blood sampling for biochemical and hematological analysis:** Blood for serum was collected after 4 and 8 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 minutes 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 aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma glutamyl transferase (γGT). 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).

### Liver Tissue samples for biochemical analysis (liver tissue)

**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 minutes 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).

### 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-α (TNF-α), Interlukin-10, Interleukine-1-β (IL-1-β), Necrosis factor KAPPA-β (NF-KB)

**Statistical analysis:** All values were expressed as mean ± standard error (SE). All statistical analyses were performed using SPSS (version 19). Statistical differences among the experimental groups were assessed by ANOVA. Duncan's test was used as a follow-up test and significance was defined at p<0.05.

## RESULTS

Our results showed that the mean value of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Gamma glutamyltransferase ( $\gamma$ GT), A/G ratio and (Total, Direct and Indirect bilirubin) were significantly increased in hepatitis group in comparison with normal control group while the mean value of serum total protein (TP), Albumin and Globulin were significantly decreased in hepatitis group in comparison with normal control group. The mean value of serum  $\gamma$ GT, ALP, ALT and AST were significantly decreased in high conc. Liquorice extract treated group in comparison with hepatitis group while the mean value of serum TP, Albumin, Globulin and A/G were significantly increased in high conc. Liquorice extract treated group in comparison with hepatitis group. Related to hematological profile, our results showed that, the mean value of plasma WBC's, Hb% and Granulocytic count levels was significantly increased in hepatitis group in comparison with normal control group while the mean value of plasma RBC's, Hematocrit, RDW and Lymphocytic count levels was significantly decreased in hepatitis group in comparison with normal control group.

The mean value of plasma WBC's, Granulocytic count and RDW levels were significantly decreased in high conc. Liquorice extract treated group in comparison with hepatitis group while the mean value of plasma RBC's, Hb%, Hematocrit and Lymphocytic count levels were significantly increased in high conc. Liquorice extract treated group in comparison with hepatitis group. Reactive oxygen species parameters revealed a significantly increase in serum MDA and significantly decrease in serum GSH in hepatitis group compared to normal control groups and shown a significantly decreased with serum MDA and a significantly increased with serum GSH in Liquorice extract treated groups comparing to hepatitis groups. The molecular results showed a significantly up regulated with IL-1 $\beta$ , TNF- $\alpha$ , NFKB and COX-2 gene expression levels ( $P \leq 0.05$ ) and a significantly down regulation with IL-10, GPX, SOD and CAT in rats liver tissues in hepatitis group compare to normal control group. 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 treated groups comparing to hepatitis groups.

## DISCUSSION

Liver diseases are the most dangerous illness, they may be varying from acute or chronic hepatitis (inflammatory liver disease), hepatosis (non-inflammatory disease) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases can be caused by many chemical agents like chemotherapeutics, antibiotics, peroxidated oil, aflatoxin, carbon tetrachloride, acetaminophen, chlorinated hydrocarbons and some antibiotics. Also, excess consumption of alcohol, infections and autoimmune disorders (Kumar et al., 2011). Medicinal plants have an important role as they not only keep the health and vitality of human beings but also used in treatment of many diseases like liver disorders with leading to no toxicity (Madhuri and Pandey, 2009). Our data was in accordance with (Xiao-Hui, et al. 2007) who revealed that, CCl<sub>4</sub> injection in experimental models are accompanied by increasing the serum level of ALT, AST,  $\gamma$ -GT and ALP due to

hepatocytes enzyme leakage as a result of change membrane integrity and hepatocyte injuries. In addition, (Recknagel et al., 1989) reported that, carbon tetrachloride induce hepatotoxicity by formation of active intermediate metabolites like trichloromethyl radicals (CCl<sub>3</sub><sup>+</sup>) that is metabolized by cytochrome P<sub>450</sub> in the hepatic tissue. The extent of hepatic damage is usually associated with increased level of cytoplasmic enzymes (ALT, AST and ALP), thereby leads to leak out of large quantities enzymes into the blood stream. This was accompanied by centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver (Shankar et al., 2008). Furthermore (Ilavarasan et al., 2003) revealed that, the basis of CCl<sub>4</sub> hepatotoxicity refer to trichloromethyl and trichloromethyl peroxy radicals which produced from hepatic cytochrome P<sub>450</sub> biotransformation. A trichloromethyl radical forms covalent bond with lipids and proteins which can be interact with oxygen to form a trichloromethylperoxy radical, or remove hydrogen atoms to form chloroform. Since reactive oxygen species are a cornerstone in CCl<sub>4</sub>-induced hepatotoxicity, it seems that antioxidant compounds may have a hepatoprotective effect (Hsiao et al., 2003).

Also, (Ashafa et al., 2012) noted that, exposure to chemical agents at toxic levels results in changes in blood parameters that are leads to hematological disorders like anemia which is characterized by low hemoglobin content; neutropenia which occurs in cases of reduced production of white blood cells or increased utilization and destruction, or both. Moreover, (Gutyj et al., 2017) found that, in conditions of intoxication by carbon tetrachloride, the physiological level of hematological indicators was disrupted. This is indicated by the reduction in the amount of erythrocytes, hemoglobin content, and concentration of hemoglobin per erythrocyte, increase in the amount of leucocytes, mass of hemoglobin per erythrocyte. Also, suppression of protein synthesis function of the liver. The levels of total protein and albumin fraction were below the normal physiological level. High indicators were observed in the levels of creatinine, urea and total bilirubin. Recently, (Hamed et al., 2018) noted that, injection of a sole dose of CCl<sub>4</sub> caused a significant reduction of RBC, HB, PLT, HCT counts by 29%, 44%, 14% and 38% respectively. In addition, MCV and MCH levels showed a weak reduction (7% and 7.6% respectively), whereas MCHC levels revealed a significant increase (7%) in comparison to control group. Antioxidant systems represent the first line protection against oxidative tissue-damage by converting reactive free radicals such as hydrogen peroxide and other organic hydroperoxides to non-toxic products (Halliwell, 1990).

Our obtained results was in agreement with (Kyle et al., 1987) who reported that, administration of CCl<sub>4</sub> in experimental models leads to increase MDA, GPx, SOD, GR, GST levels which reflect oxidative damage in the liver tissue. In addition (Huo et al., 2011) showed that, hepatic tissues was extensively protected against CCl<sub>4</sub> induced liver damage in prior treatment with liquorice extract which approved by decreased serum ALP, AST and ALT activities and increased total protein, albumin and globulin levels. Furthermore (Wang and Han 1993) reported that, the main components of liquorice root are triterpene, saponins, glycyrrhizin/glycyrrhizic acid and glycyrrhetic acid. Glycyrrhizic acid exhibits a number of pharmacological activities including detoxification, antioxidation, anti-infection, anti-inflammatory and is used in hepatoprotective formulations.

Table 1: Effect of LE treatment on the bio-statistical analysis.

Parameters	Measurement points	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
ALT	4th week	65.00 ± 12.17	118.67 ± 1.73	96.67 <sup>a</sup> ± 11.20	58.0 ± 1.53	62.67 ± 4.06	77.33 <sup>ab</sup> ± 8.51
	8th week	90.33 ± 1.76	111.33 ± 11.14	85.33 <sup>ab</sup> ± 2.40	100.0 ± 6.43	96.67 ± 8.11	102.67 ± 7.51
AST	4th week	116.67 ± 21.84	239.33 ± 7.51	261.33 ± 29.06	252.0 ± 15.01	145.33 ± 14.17	132.67 ± 5.70
	8th week	101.33 ± 1.76	196.0 ± 11.14	238.0 ± 23.58	256.0 ± 11.14	224.0 ± 18.58	259.33 ± 7.86
Total Protein	4th week	8.10 ± 0.36	5.80 ± 0.25	4.70 ± 0.12	5.90 ± 0.50	5.33 ± 0.64	6.42 ± 0.12
	8th week	7.50 ± 0.09	5.10 ± 0.15	5.90 ± 0.12	6.40 ± 0.00	6.1 ± 0.20	6.60 ± 0.24
Serum albumin	4th week	3.55 ± 0.19	3.00 ± 0.23	3.1 ± 0.15	3.19 ± 0.11	3.0 ± 0.17	3.36 ± 0.28
	8th week	3.40 ± 0.10	3.10 ± 0.17	3.5 ± 0.07	3.4 ± 0.10	3.42 ± 0.25	3.65 ± 0.11
Serum Globulin	4th week	4.55 ± 0.21	2.26 ± 0.21	2.00 ± 0.47	2.71 ± 0.21	2.33 ± 0.46	3.04 ± 0.40
	8th week	4.5 ± 0.12	2.04 ± 0.12	2.53 ± 0.41	2.42 ± 0.18	2.68 ± 0.17	2.95 ± 0.18
A/G Ratio	4th week	0.78 ± 0.17	1.56 ± 0.25	1.5 ± 0.13	1.2 ± 0.15	1.3 ± 0.16	1.16 ± 0.28
	8th week	0.66 ± 0.07	1.5 ± 0.13	1.33 ± 0.11	1.64 ± 0.20	1.27 ± 0.18	1.23 ± 0.25
ALP	4th week	434.667 ± 12.13	781.333 ± 7.06	710.67 ± 181.20	465.33 ± 8.82	459.33 ± 25.62	468.67 ± 1.76
	8th week	553.33 ± 74.05	899.333 ± 114.78	609.33 ± 79.0	566.67 ± 125.86	640.67 ± 119.92	728.67 ± 199.52
γGT	4th week	2.20 ± 0.67	3.3 ± 0.58	2.0 ± 0.58	2.66 ± 0.67	2.5 ± 0.88	2.9 ± 0.33
	8th week	2.90 ± 0.88	4.3 ± 0.58	2.5 ± 0.88	3.0 ± 0.58	3.1 ± 0.67	3.11 ± 0.58
Total Bilirubin	4th week	0.21 ± 0.01	0.33 ± 0.01	0.20 ± 0.03	0.122 ± 0.00	0.13 ± 0.00	0.21 ± 0.04
	8th week	0.24 ± 0.01	0.37 ± 0.01	0.23 ± 0.03	0.20 ± 0.03	0.23 ± 0.03	0.22 ± 0.08
Direct Bilirubin	4th week	0.11 ± 0.01	0.19 ± 0.01	0.14 ± 0.03	0.11 ± 0.00	0.11 ± 0.00	0.12 ± 0.04
	8th week	0.13 ± 0.01	0.21 ± 0.01	0.15 ± 0.03	0.14 ± 0.03	0.15 ± 0.03	0.14 ± 0.08
Indirect Bilirubin	4th week	0.10 ± 0.01	0.21 ± 0.01	0.12 ± 0.05	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.03
	8th week	0.11 ± 0.03	0.23 ± 0.01	0.13 ± 0.01	0.12 ± 0.05	0.12 ± 0.05	0.15 ± 0.05

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

Group 1: normal control Group 2: hepatitis group Group 3: hepatitis + low conc. Liquorice extract Group 4: hepatitis + high conc. Liquorice extract  
Group 5: control + low conc. Liquorice extract Group 6: control + high conc. Liquorice extract

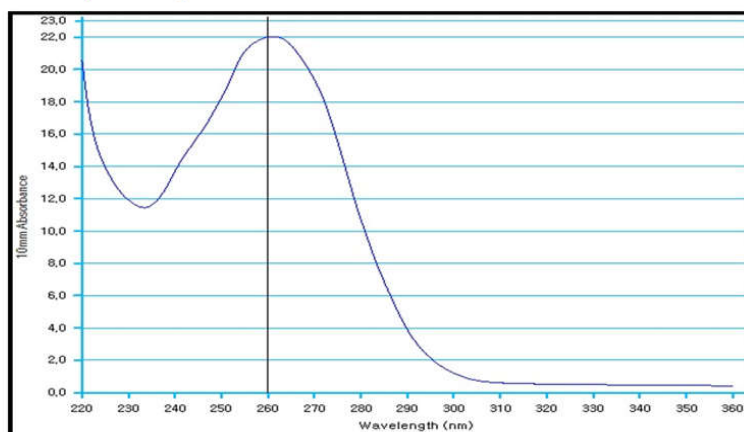
Table 2: Effect of LE treatment on the bio-statistical analysis.

Parameters	Measurement points	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
plasma WBC's	4th week	12.87 ± 2.63	11.9 ± 1.37	11.93 ± 1.66	12.87 ± 0.56	10.9 ± 0.38	12.2 ± 0.50
	8th week	7.5 ± 0.12	10.0 ± 0.86	8.05 ± 0.61	8.57 ± 0.58	8.17 ± 0.47	9.0 ± 0.81
plasma RBC's	4th week	2.03 ± 0.15	2.09 ± 0.11	3.74 ± 0.19	4.31 ± 0.41	4.11 ± 0.24	4.5 ± 0.42
	8th week	3.29 ± 0.29	2.83 ± 0.25	3.16 ± 0.31	2.75 ± 0.26	2.87 ± 0.40	3.9 ± 0.35
(Hb%)	4th week	9.2 ± 0.50	9.17 ± 0.19	9.47 ± 0.38	9.57 ± 0.19	9.63 ± 0.09	9.7 ± 0.32
	8th week	0.90 ± 0.23	9.03 ± 0.15	9.37 ± 0.35	9.57 ± 0.13	9.03 ± 0.20	9.17 ± 0.49
(HCT)	4th week	2.03 ± 0.15	2.09 ± 0.11	3.74 ± 0.19	4.31 ± 0.41	4.11 ± 0.24	4.5 ± 0.42
	8th week	3.29 ± 0.29	2.83 ± 0.25	3.16 ± 0.31	2.75 ± 0.26	2.87 ± 0.40	3.9 ± 0.50
lymphocytic count	4th week	3.35 ± 0.80	2.55 ± 0.11	3.62 ± 0.57	3.10 ± 0.19	2.43 ± 0.16	2.43 ± 0.09
	8th week	2.75 ± 0.40	2.29 ± 0.20	2.49 ± 0.25	2.46 ± 0.09	2.24 ± 0.18	2.34 ± 0.31
Granulocytic Count	4th week	8.26 ± 1.70	8.09 ± 1.03	7.17 ± 1.05	8.22 ± 0.36	7.33 ± 0.49	7.89 ± 0.42
	8th week	3.69 ± 0.57	6.42 ± 0.49	5.24 ± 0.29	5.32 ± 0.61	5.00 ± 0.37	5.63 ± 0.52
(RDW)	4th week	79.88 ± 10.98	47.78 ± 22.84	73.0 ± 1.00	71.0 ± 0.00	65.0 ± 1.00	67.0 ± 0.00
	8th week	66.0 ± 2.08	53.33 ± 8.25	54.67 ± 13.78	39.0 ± 2.65	50.33 ± 6.98	67.0 ± 0.00
(MDA)	4th week	33.67 ± 0.88	65.47 ± 0.88	49.30 ± 0.35	44.17 ± 1.13	35.27 ± 1.19	33.17 ± 0.44
	8th week	33.17 ± 0.84	70.3 ± 1.18	43.47 ± 1.01	41.33 ± 1.20	34.33 ± 1.07	32.37 ± 0.84
(GSH)	4th week	33.73 ± 0.91	10.4 ± 0.30	23.08 ± 0.57	26.40 ± 0.83	30.23 ± 0.64	33.51 ± 0.86
	8th week	35.0 ± 0.58	9.51 ± 0.25	25.0 ± 0.58	28.0 ± 0.58	32.0 ± 0.58	34.17 ± 0.44

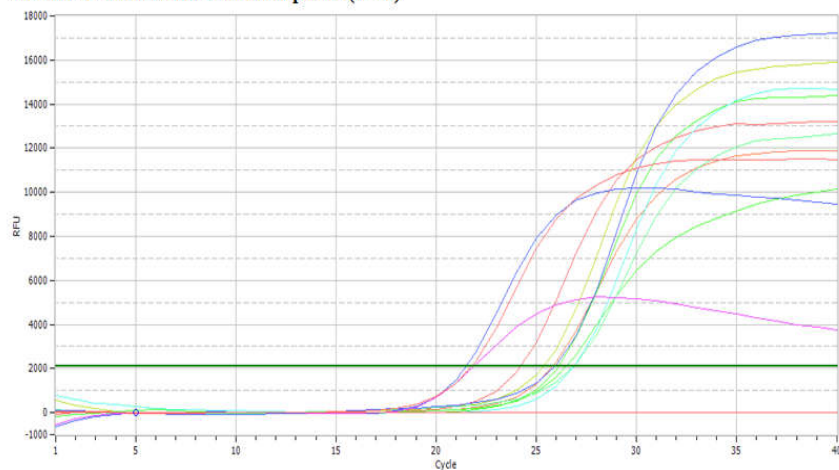
Data are presented as (Mean ± S.E). S.E = Standard Error

Group 1: normal control Group 2: hepatitis group Group 3: hepatitis + low conc. Liquorice extract Group 4: hepatitis + high conc. Liquorice extract  
Group 5: control + low conc. Liquorice extract Group 6: control + high conc. Liquorice extract

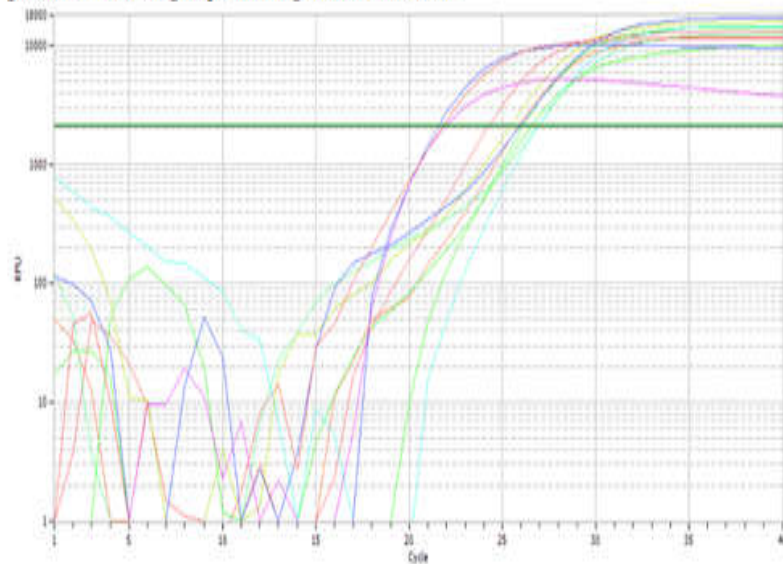
Figure 1: Nanodrop curve showing concentration and purity of extracted RNA from a representative sample which is 1970 ng/μl. In this curve the upper top presents at 260 and the bottom at 230 which indicates the presence of pure RNA



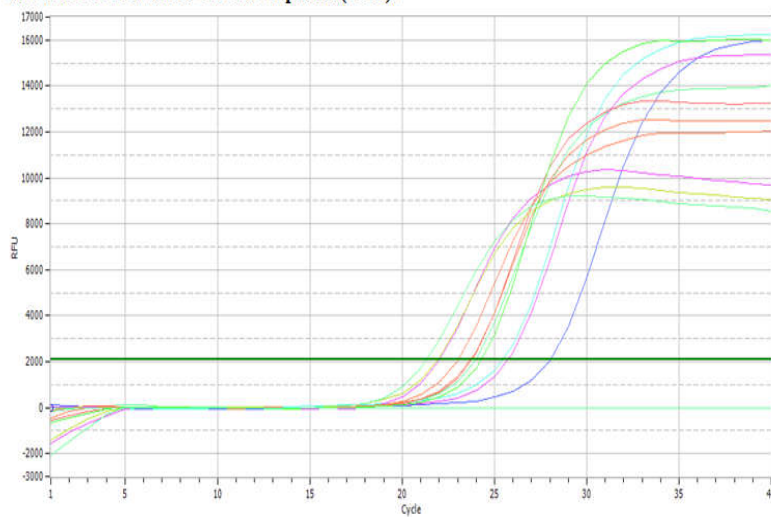
**Figure 2:** Graphical presentation of real-time quantitative PCR analysis of the expression of IL-10 gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



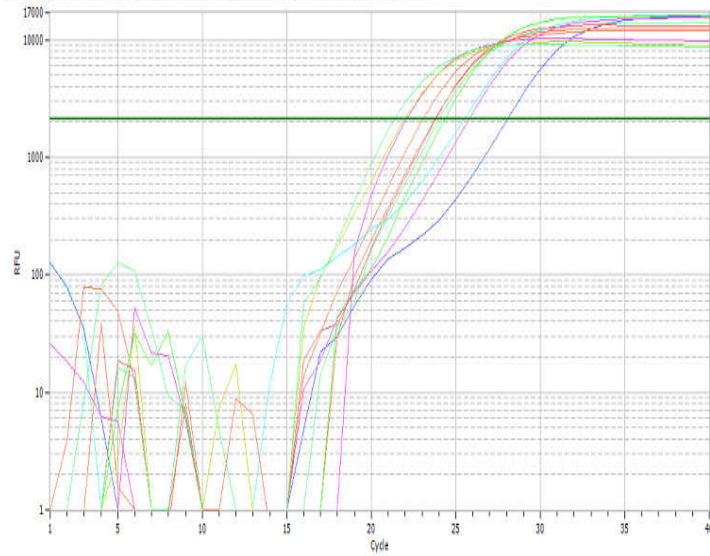
**Figure 3:** The linear (upper) and log (lower) amplification curves representing the Ct values of IL-10 gene in different rat groups following treatment as follow:



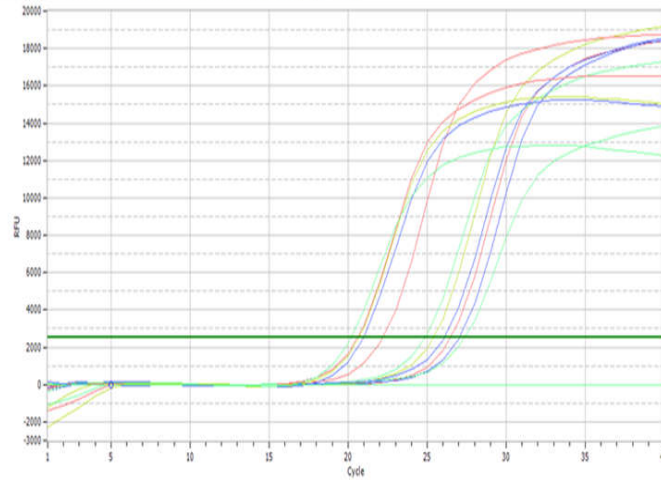
**Figure 4:** Graphical presentation of real-time quantitative PCR analysis of the expression of IL-1 $\beta$  gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



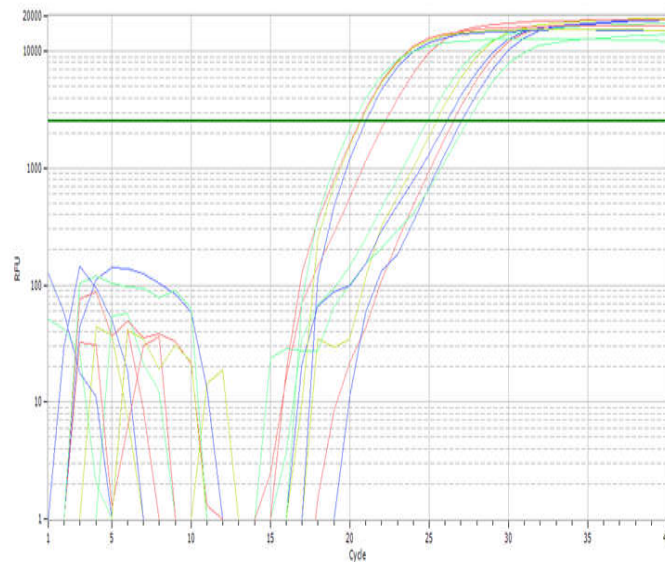
**Figure 5 :** The linear (upper) and log (lower) amplification curves representing the Ct values of IL-1 $\beta$  gene in different rat groups following treatment as follow:



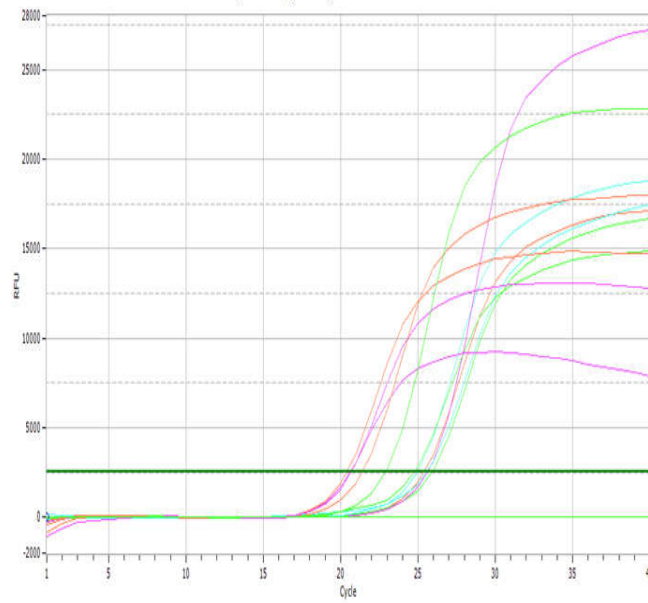
**Figure 6:** Graphical presentation of real-time quantitative PCR analysis of the expression of TNF- $\alpha$  gene in liver tissues on oral administration of 1 ml of both low and high concentrations of liquorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



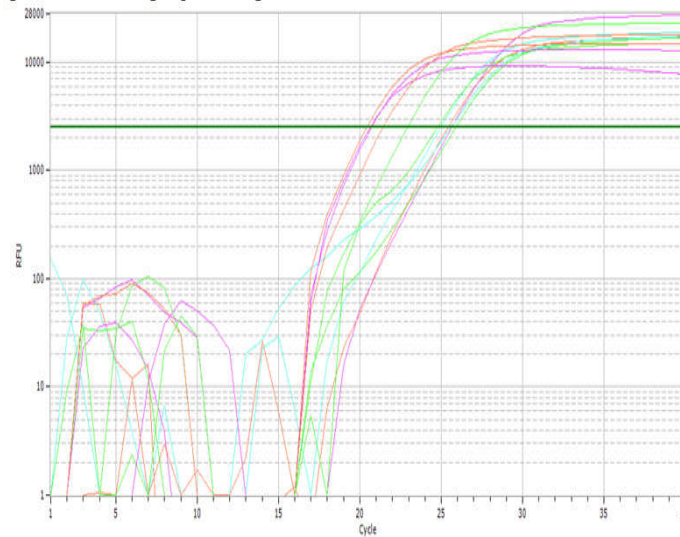
**Figure 7:** The linear (upper) and log (lower) amplification curves representing the Ct values of TNF- $\alpha$  gene in different rat groups following treatment as follow:



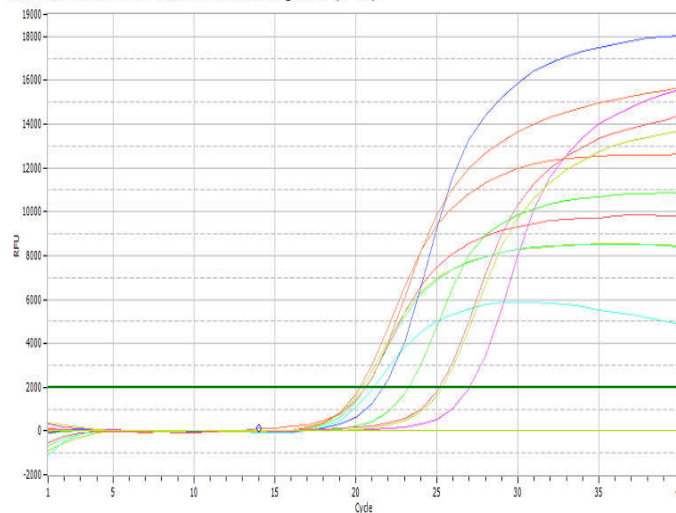
**Figure 8:** Graphical presentation of real-time quantitative PCR analysis of the expression of NFKB gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



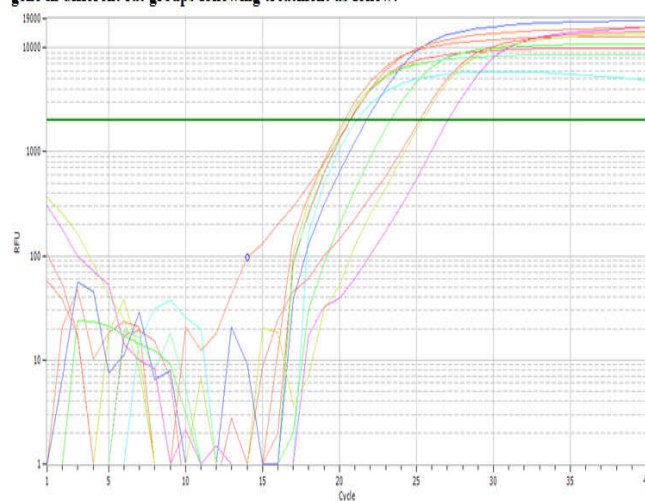
**Figure 9:** The linear (upper) and log (lower) amplification curves representing the Ct values of NFKB gene in different rat groups following treatment as follow:



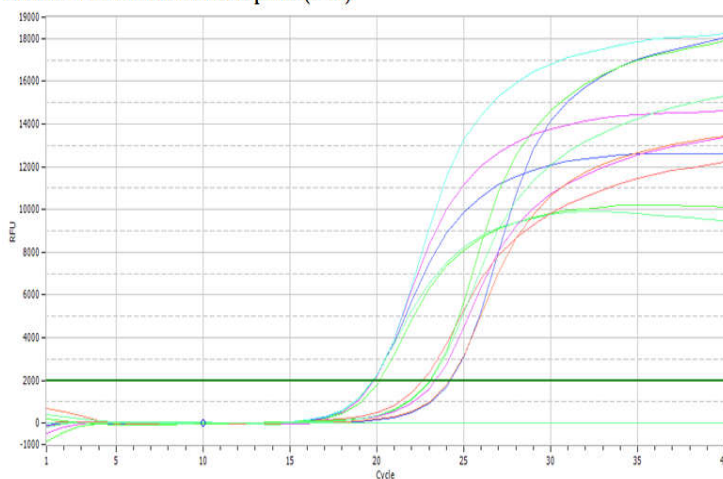
**Figure 10:** Graphical presentation of real-time quantitative PCR analysis of the expression of COX-2 gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



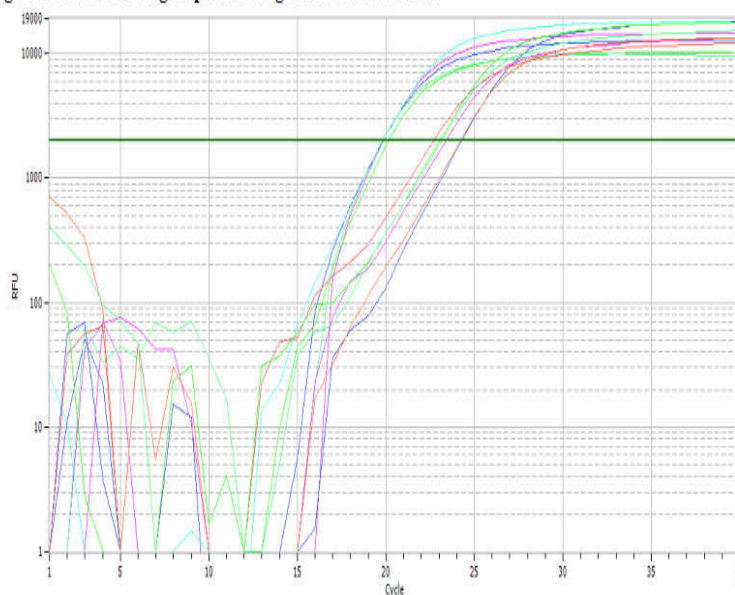
**Figure 11:** The linear (upper) and log (lower) amplification curves representing the Ct values of COX-2 gene in different rat groups following treatment as follow:



**Figure 12:** Graphical presentation of real-time quantitative PCR analysis of the expression of GPX gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).

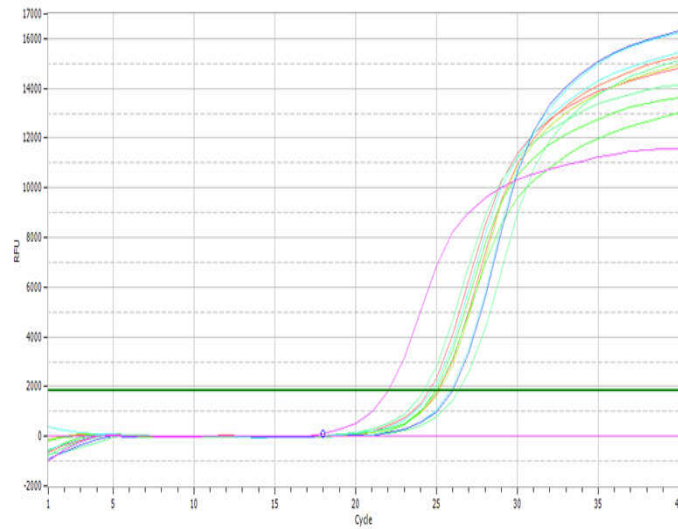


**Figure 13:** The linear (upper) and log (lower) amplification curves representing the Ct values of GPX gene in different rat groups following treatment as follow:

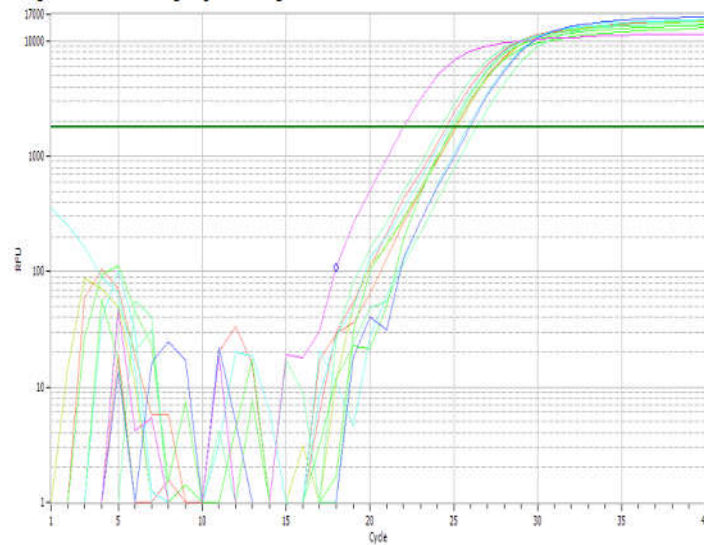




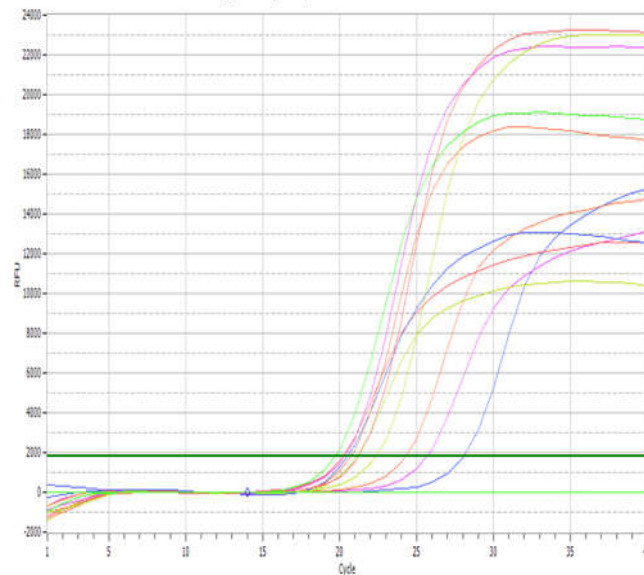
**Figure 14:** Graphical presentation of real-time quantitative PCR analysis of the expression of SOD gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



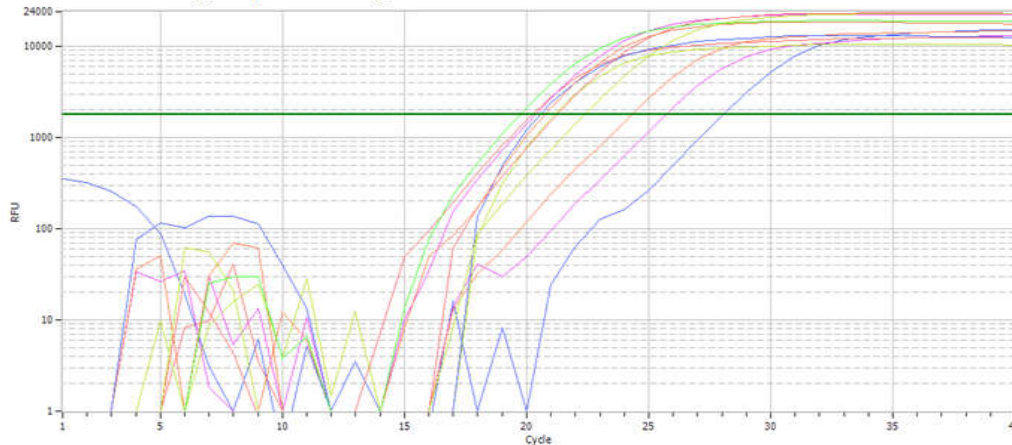
**Figure 15:** The linear (upper) and log (lower) amplification curves representing the Ct values of SOD gene in different rat groups following treatment as follow:



**Figure 16:** Graphical presentation of real-time quantitative PCR analysis of the expression of CAT gene in liver tissues on oral administration of 1 ml of both low and high concentrations of licorice extract solution (equivalent to 200 mg/kg body weight and 400 mg/kg body weight) in albino rats fed on basal diet and CCL4-induced chronic hepatitis (n=10).



**Figure 17: The linear (upper) and log (lower) amplification curves representing the Ct values of CAT gene in different rat groups following treatment as follow:**



Pretreatment with Glycyrrhizic acid has been reported to show protective action against carbon tetrachloride  $\text{CCl}_4$  induced liver injury in rats. (Tsuruoka et al., 2009) reported that, glycyrrhizin in liquorice extract decrease AST and ALT, inhibited iNOS mRNA expression and protein, cell infiltration and the degeneration of hepatocytes. Also, glycyrrhizin antagonizes  $\text{CCl}_4$  induced hepatotoxicity by removing free radical toxic properties and inducing heme oxygenase-1 and down regulating proinflammatory mediators. Our results agreed with (Yu et al., 2015) who reported that, the expression levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6) was inhibited by using liquorice extract in the livers of t-BHP-treated mice models due to three main bioactive components glycyrrhizic acid, liquiritin and liquiritigenin. Thus it could be applied for the treatment of several inflammatory disorders like oxidative tissue damage.

In addition (Chen et al., 2014) illustrated that, tumor necrosis factor-alpha (TNF- $\alpha$ ) is a critical inflammatory maker in the case of damaged hepatocytes. Nuclear factor-kappa B (NF- $\kappa$ B) reporter gene assay in TNF- $\alpha$  induced HepG2 was used as a screening platform. Results showed that TNF- $\alpha$  enhanced NF- $\kappa$ B activity was significantly decreased by glycyrrhetic acid in a concentration dependent manner in the NF- $\kappa$ B reporter gene assay. These findings suggest that glycyrrhetic acid may provide hepatoprotection against chronic liver inflammation through decreasing NF- $\kappa$ B activation to relief the inflammation.

## Conclusion

In conclusion, our results elucidate the hepatotoxic effect of  $\text{CCl}_4$  which accompany by increased level liver function parameter and producing harmful effect on hematological profile. Also, our results clarify the protective effects of liquorice extract versus  $\text{CCl}_4$  induced hepatitis. These findings augment the use of liquorice extract in folk medicine for the protection of liver damage.

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