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Original Article

Hepatoprotective Effect of Glycyrrhiza Glabra in Carbon Tetrachloride-Induced Model of Acute Liver Injury

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Abstract

Acute liver injury is a serious state of extensive damage of liver tissue caused by various reasons. Certain medicinal plants had been used to cure some liver diseases. The present study was done to evaluate the hepatoprotective effect of aqueous extract of *Glycyrrhiza glabra* roots in rabbit models with acute liver injury induced by Carbon tetrachloride at a dose of 1.25 ml/kg as a mixture with olive oil. Aqueous extract of *G. glabra* was administered in a dose of 2gm/kg/day orally for 7 days. Its protective effect was assessed via liver function tests and histopathological liver sections. Significant reduction in the hepatic enzymes levels, serum bilirubin and improvement of serum protein was found in animals treated with the extract as well as restoration of hepatocellular architecture, absence of necrosis with mild degree of fatty infiltration. Our results demonstrate that the aqueous extract of *G. glabra* had a significant effect in amiolerating liver functions as well as restoring hepatic tissue in acute liver diseases when it was given in a single dose per day of 2gm/kg body weight. Therefore the aqueous extract of *G. glabra* roots can be used for prevention and treatment of liver disorders.

Keywords: Glycyrrhizin, hepatocellular architecture, licorice, saponins

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Introduction

Acute liver injury (ALI) is a clinical condition, results from severe and extensive damage of the hepatocellular tissue with reduced cell mass and blood flow (Sebate et al., 2007). A significant alteration in the levels of various hepatic markers e.g serum alanine aminotansferase (SALT), serum aspartate aminotansferase (SAST), serum alkalanine phosphatase (SALP), total serum bilirubin (TSB) and total serum protein (TSP) had been took place in conformity with the extent of liver damage (Pattanayak et al., 2011). In the absence of effective liver-protective drugs in modern medicine, a number of medicinal plants in traditional medicine, like Glycyrrhiza glabra, have been used to cure and prevent some liver diseases (Subramoniam and Pushpangadan, 1999) but without a scientific clarification study. Therefore, it is interesting to evaluate its potential hepatoprotective effect in experimental rabbit model of acute liver injury induced by a hepatotoxic agent (carbon tetrachloride) (Basu, 2003). G. glabra, also known as licorice, is a herbaceous perennial, with pinnate leaves and purple to whitish blue flowers (Shibata, 2003). It is native to the Mediterranean and certain areas of Asia. The sweet-taste of licorice comes mainly from an aromatic ether compound called anethole. G. glabra contains (Obolentseva et al., 1999): flavonoides, glycosides, glycyrrhizin (the main active component, found in the root), glycyrrhizic acid (has a lowering effect of elevated liver transaminases levels) (Curreli et al., 2007), saponins which reduce the risk of coronary heart disease by increasing HDLconcentration (Balogun and Akinloye, 2012), glabrene which possesses estrogen-like activity (Tamir et al., 2001), starches (30%) and yellow coloring matter.

Materials and Methods

Chemicals

All chemicals used in the study were of analytical grade. CCl_4 was procured from Merck India Ltd., Mumbai, India. The kits for the estimation of SALT, SAST and TSP were from Dialab, Austria. Kits for TSB and ALP were from Biolabo SA, Maizy, France.

Plant Extraction

The licorice was purchased from the well-known herbal bureau (Al-Medina) in Baghdad and was identified and authenticated by Iraqi National Institute for Herbs. The roots of liocorice were cleaned, dried, and powdered with an electrical grinder, then passed through sieve no.40 to remove the debris. The sieved powder was stored in airtight container at room temperature. The aqueous extract was prepared by diluting one volume of well-grinded powder to ten volume of water at 80°C in a stoppered flask after shaking well. Then, it was allowed to stand for 10 minutes to be cold and filtered for laboratory purposes. The aqueous extract should be used within 12 hours (Al-Razzuqi et al., 2011).

Animals

Eighteen local domestic rabbits (750-900 g) were supplied by animal house of Baghdad College of Medicine. They were housed in separated cages which provided with a wide wire mesh floor and at a controlled temperature of 28°C with a 12-hour light/dark cycle. They were fed standard oxoid pellets and water *ad libitum*. The study was conducted according to the Animal Ethics Committee of the college of pharmacy, Al-Yarmouk University (Approval No. AEC/34/11/CPAYU).

Animals were randomly allocated to three groups of six animals each. They were given a single dialy dose of the following for seven days (at 9.00 a.m):

Group I (control) - received normal saline 3 ml p.o

Group II -received distilled water 3ml p.o

Group III - received aqueous extract of licorice as 2gm/kg p.o (Al-Jawad et al., 2009)

At 11.00 a.m of 8^{th} day, Groups II and III were given CCl_4 as 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1.25 ml/kg, p.o for induction of ALI. 3 hours later, blood samples were collected from marginal ear vein of the animals of all the groups for biochemical analysis of SALT, SAST, SALP, TSB and TSP using spectrophotometer method for comparison with the values of the samples collected before the induction (Corl and Ashwood, 1999). Then, all the animals were sacrificed under light

ether anesthesia to have liver specimens. The histopathological examination was carried to check for hepatocellular changes using polarised microscope after fixating the sections in 10% formalin for 48 hours and staining with hematoxylin and eosin dye (Putt and Fredrick, 1972).

Statistical Analysis

All the results were expressed as mean ± SEM. The difference among means had been analyzed by student's *t* test (Woolson, 1987) using SPSS version12. P values < 0.05 were considered to be statistically significant.

Results and Discussion

Administration of CCl_4 to the animals resulted in a marked increase in TSB, SAST, SALT, and SALP, while TSP decreased when compared with control group. Group III which was given the aqueous extract of *G. glabra* showed a reduction in the SALT, SAST, SALP, and TSB levels. It also reversed the depletion of total protein significantly when compared with Group II (group administered CCl_4) [Table 1].

Table 1: Effect of G. glabra extract on CCl4-induced hepatotoxicity in rabbits

Groups	SALT (U/L)	SAST (U/L)	SALP (U/L)	TSB (µmol/L)	TSP (g/dI)
I (control)	38.31 ± 1.71	41.09 ± 4.15	49.66 ± 2.53	55.91 ± 0.36	09.7 ± 0.36
II (CCl4 only)	140.3 ± 1.80*	173.8 ± 1.99*	291.73 ±7.99*	63.26 ± 0.49	07.3 ± 0.13*
III (G. glabra+ CCl4)	30.59 ± 1.27**	38.46 ± 2.79*	46.83 ± 0.59**	53.33 ± 0.66**	09.2 ± 0.41**

Values are mean ± SEM, No.=6; P<0.01*compared with Group I, P<0.05**compared with Group II, SALT- serum alanine aminotansferase, SAST- serum aspartate aminotansferase, SALP- alkalanine phosphatase, TSB- total serum bilirubin, TSP- total serum protein

Licorice had a strong effect in lowering SALT and SAST levels faster than its effect on the other parameters, especially on 4th post-induction day, with levels equal to 30.59 ± 1.27 U/L and 38.46 ± 2.79 U/L than 38.31 ± 1.71 U/L and 41.09 ± 4.15 U/L respectively.

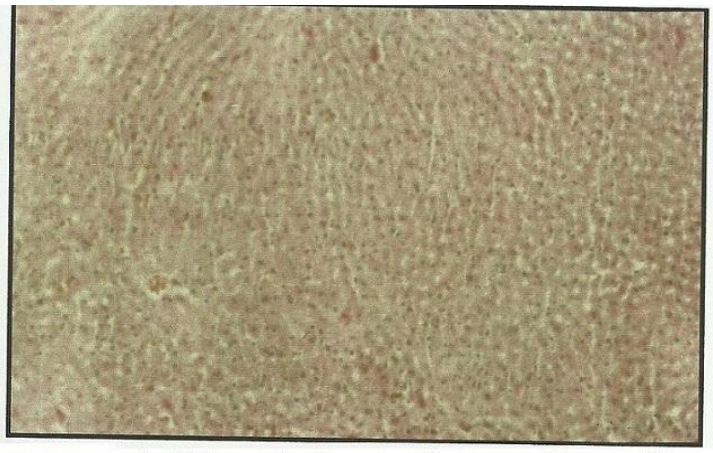
The histological studies of liver sections support the results obtained from serum enzyme assays which showed normal hepatic architecture in Group I [Figure 1], whereas liver sections of Group II showed total loss of hepatic architecture with massive fatty changes, congestion of sinusoids, intense necrosis, and infiltration of the lymphocytes around the central vein [Figure 2]. The histological architecture of liver sections of Group III showed a more or less normal lobular pattern with a mild degree of fatty change, cell necrosis, and lymphocyte infiltration indicating the protective effect of the plant extracts[Figure 3].

CCl_4 is a well-known compound used to induce hepatotoxicity in experimental animal models. It is biotransformed in the cytochrome P450 system to its metabolite " trichloromethyl free radical (CCl_3)", which in the presence of oxygen forms trichloromethyl peroxyl free radical (CCl_3O_2), that attacks lipids of endoplasmic reticulum eliciting lipid peroxidation with the leakage of liver enzymes like ALT, AST, and ALP in the blood, besides an increase in TB levels and decrease in total protein (Reckengel et al., 1989). Administration of aqueous extract of *G. glabra* to CCl_4–intoxicated animals showed significant hepatoprotective activity by restoring the hepatocellular activity. It has been reported that the high level of flavonoids like luteolin, rutin, and apigenin in *G.glabra* possess antioxidant properties (Hesham and Shgeru, 2002). The flavonoid compound, rutin, is particularly having free radical scavenging property so inhibits the lipid peroxidation (Khalid et al., 2002). Also, it is found that glycyrrhetinin acid (the active component of licorice) blocks the bioactivity of CCL4 by inhibiting the activity of P4502E1(the enzyme responsible for CCl_4 metabolism), thereby preventing the hepatoperoxidation (Jeong et al., 2002).

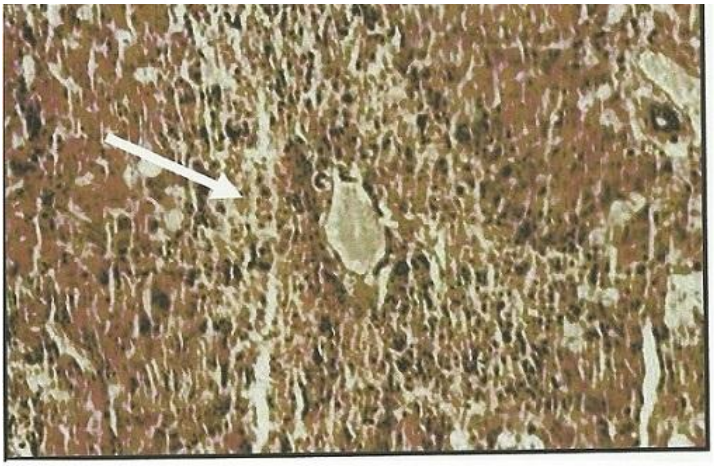
Conclusion

Our results demonstrate that the aqueous extract of *Glycyrrhiza glabra* showed a significant effect in amiolerating liver functions as well as

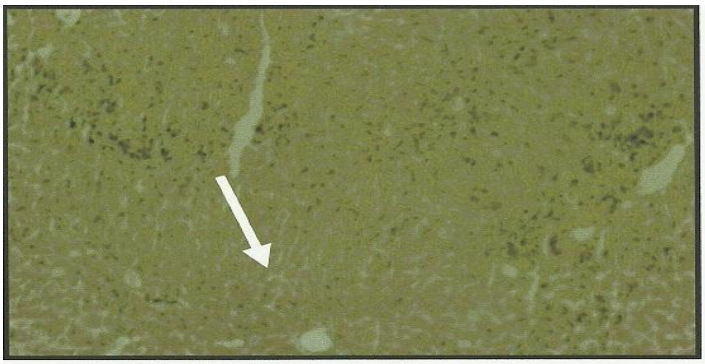
restoring hepatic tissue in acute liver diseases when the extract was administered in single dose per day of 2gm/kg body weight.



Figure(1): normal rabbit liver section shows normal hepatocyte architecture with normal lobular appearance (10X , H & E stain)



figure(2): Rabbit liver section after administration of CCl4 as a single oral dose , showing massive necrosis , fatty change lymphocyte infiltration and congestion (10X , H & E).



figure(3): Rabbit liver section treated with licorice extract showing no necrosis , very mild fatty change , very mild inflammatory infiltration and mild congestion (10X , H & E stain) .

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