

## Biochemical and Histopathological Studies on the Liver of Rats Administrated with Different Concentrations of Aqueous Extract of *Glycyrrhiza glabra*

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**Abstracts:** It had been demonstrated that *Glycyrrhiza glabra* extract has biological capabilities include detoxication, antioxidation and antiinfection properties, although there is little data about its probable side effects on the liver integrity. So, the effects of *G. glabra* administration on the liver's histology and some biochemical parameters were investigated in the rats. Thirty-two Sprague-Dawley male rats were randomly distributed into four groups (n=8). Experimental groups were injected intra-peritoneal with aqueous extract of *G. glabra* at 50, 100 and 200 mg/kg/day, respectively, for 30 consecutive days while control group was injected with tap water without any add-on material. At the end of the experiments, tissue and blood samples were taken for histological evaluation and serum biochemical parameters assay, separately. The obtained results showed a decrease in diameter of hepatocytes and hepatocytes nuclear diameter and also an increase of sinusoids volume ( $P<0.05$ ) in the liver of extract treated animals. Furthermore, hepatic necrosis, local mononuclear leukocyte infiltration and centrilobular hepatic congestion were observed in the liver of experimental animals. In addition, serum levels of ALP, AST and ALT showed a significant increase in 200 mg/kg extract received animals in comparison with control ( $P<0.05$ ). These findings showed that *G. glabra* could cause harmful impacts on the liver integrity, so caution should be paid to popular consumption of this plant. The present histological will also need to be confirmed using electron microscopy for exact ultra structural changes.

**Key words:** *Glycyrrhiza glabra* • Histology • Liver • Biochemistry • Rat

### INTRODUCTION

Liver is the key organ for metabolism of various xenobiotics and therapeutic agents which accumulate in various tissues, while the hepatocytes carry them to the bile for elimination [1]. Measuring the levels of various hepatic markers e.g serum alanine aminotransferase, serum aspartate aminotransferase, serum alkaline phosphatase, total serum bilirubin and total serum protein had been took place in conformity with the extent of liver damage [2]. It has been noted that *G. glabra* is an herbaceous perennial, with pinnate leaves and purple to whitish blue flowers [3] which has been used as a medicinal plant for thousands of years in the traditional medicine. In ancient Chinese medicine and during Roman times, *G. glabra* was also recommended to cure sterility in women [4, 5]. In addition, *G. glabra* has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis and also has been used as a laxative, antitussive and expectorant [6,7]. The screening of plant

extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases [8]. In the absence of effective liver-protective drugs in modern medicine, a number of medicinal plants in traditional medicine, like *G. glabra*, have been used to cure and prevent some liver disorders [9] but without a scientific clarification investigations. So, in the present work the effects of different concentrations of *G. glabra* on the liver histology and some biochemical parameters of rats were investigated.

### MATERIALS AND METHODS

**Experimental Animals:** The study was carried out for one month and to do the experiments, thirty-two Sprague-Dawley male rats were randomly distributed into four groups (n=8). The treatment groups were injected intra-peritoneal with aqueous extract of *G. glabra* at 50, 100 and 200 mg/kg/day, respectively, for 30 consecutive days. Control group was injected with tap water without any add-on material. The doses were determined on the

basis of a primary study. The animals of each group were housed in separate cages with sawdust bedding. Rats were housed in one stainless-steel cage under conventional conditions (temperature  $22 \pm 1^\circ\text{C}$ ; relative humidity  $50 \pm 10\%$ ; 12 : 12 h light-dark natural cycle) and had ad-lib access to drinking water and food. The animals were allowed to be acclimatized to the laboratory environment at least 6 days before commencement of testing. All procedures that involved animals were approved by the Veterinary Ethics Committee of the Faculty of Para-Veterinary Medicine of Liam University.

**Plant Extraction:** The *G. glabra* (Licorice) root was purchased from Emam-Reza medicinal plants market (Liam, Iran) and botanical identification was confirmed at the herbarium of Liam University (Exsiccateae number: 132-4-91). For extraction preparation, the roots of plant was washed with sterile water, dried in shade at room temperature for 3 weeks and ground in an electric mill to obtain particles smaller than 4 mm. This material was extracted by maceration in 70% methanol solution at  $50^\circ\text{C}$  for 2 hours. The extract was filtered through a Wattman  $\#1$  paper and evaporated to dryness in a rotary evaporator under reduced pressure. The dried material was stored under refrigeration at  $4-8^\circ\text{C}$  until its use [10].

**Blood Collection and Biochemical Analyses:** Twenty four hours after the last doses were administrated; the animals were anaesthetized with chloroform vapor, quickly brought out of the jar and sacrificed. Whole blood was collected into sterilized vials after direct cardiac puncture. Blood samples were allowed to clot at  $4^\circ\text{C}$  and centrifuged at 5000 rpm for 10 minutes. After separation of sera, they were put into sterile tubes to measure the biochemical parameters. Determinations of serum Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured by kinetic enzyme assays (Ziest Chemie, Iran).

**Histopathological Assessment for Liver Injury:** The histological specimens of the right lobe of liver were imprisoned overnight in 10% neutral buffered formalin to be fixed. Then the livers were mounted to allow  $5\text{-}\mu\text{m}$  sections. They were stained via haematoxylin and eosin (H&E). Sections were photographed directly using a stereo microscope in 400 high power fields with Microsoft system. The following criteria were used for registering the histological changes of the liver: (++++), a dominant change in all animals; (+++), a relatively common change in all animals; (++) , a change in all animals; (+), a change in a few animals.

**Statistical Analysis:** Data were analyzed using version 16 of SPSS software (SPSS Inc. Chicago, IL, USA). The results of enzymes levels were expressed as mean  $\pm$  SEM. Differences between means were analyzed using one-way ANOVA and then the means were compared with Duncan test. P values of 0.05 or less were taken as being statistically significant.

## RESULTS

**Effect of *G. Glabra* Administration on Biochemical Parameters:** The serum levels of ALP only showed a significant increase in the animals received plant extract at 200 mg/kg ( $P < 0.05$ ) (Fig. 1). Also, we observed a statistically elevation of serum levels of AST ( $P < 0.05$ ) in all treatment groups compared to control (Fig. 2). Similarly, ALT levels exhibit a considerable increase in all treatment groups compared to control (Fig. 3) ( $P < 0.05$ ).

**Effect of *G. Glabra* Administration on Histopathological Changes:** Liver histology results from control and plant extract administrated rats are illustrated in Fig 4. In the control group, the liver exhibited a normal architecture and pathological abnormalities were not seen. (Fig 4) (Table1). In the hepatic parenchyma of animals treated with different concentrations of *G. glabra* extract, a decrease in diameter of hepatocytes and hepatocytes nuclear diameter, increase of sinusoids volume, hepatic necrosis and centrilobular hepatic congestion were seen. Additionally, the liver of plant extract treated animals showed local mononuclear leukocyte infiltration (Fig 4) (Table1). These histological alterations were more evident in rats exposed to 200 mg/kg of plant extract. (Fig 4). (Table 1).

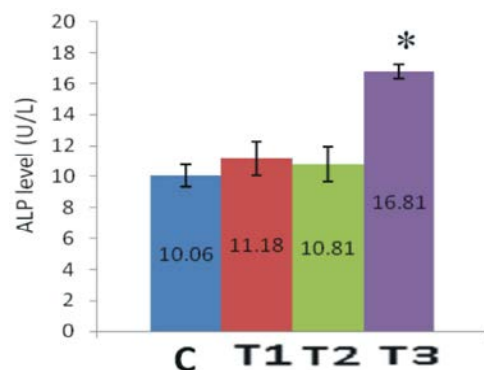


Fig 1: The ALP (U/L) levels in control, test group received 50mg/kg (T1), test group received 100mg/kg (T2) and test group received 200mg/kg (T3) aqueous extract of *G. glabra*. Each test group was compared with the control group. (\* =  $P < 0.05$ )

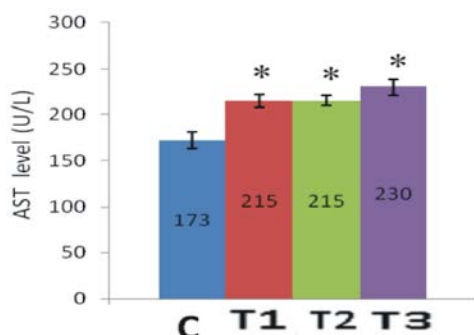


Fig. 2: The AST (U/L) levels in control, test group received 50mg/kg (T1), test group received 100mg/kg (T2) and test group received 200mg/kg (T3) aqueous extract of *G. glabra*. Each test group was compared with the control group. (\* =  $P < 0.05$ )

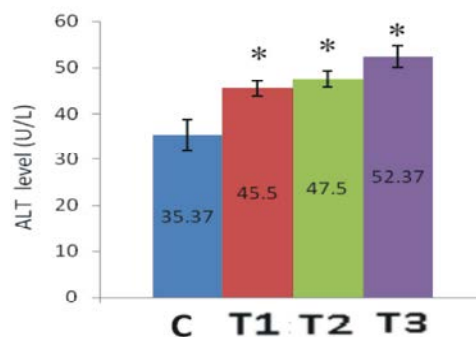


Fig 3: The ALT (U/L) levels in control, test group received 50mg/kg (T1), test group received 100mg/kg (T2) and test group received 200mg/kg (T3) aqueous extract of *G. glabra*. Each test group was compared with the control group. (\* =  $P < 0.05$ )

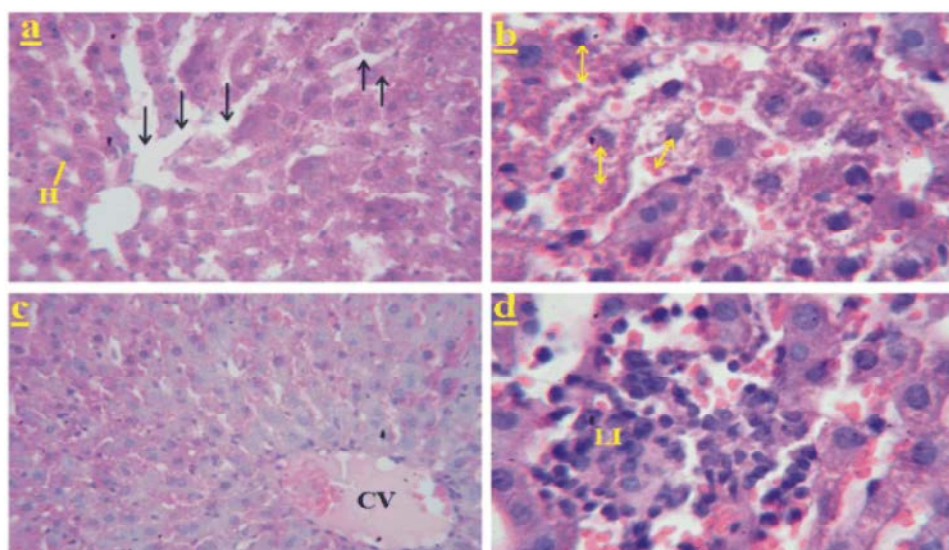


Fig 4 (a) : Liver transverse section of the rats treated with *G. glabra* extract at 50 mg/kg for 30 continuous days. The section shows lower diameter of hepatocytes (H) and hepatocyte nuclear diameter and also increase of sinusoids size (arrows) is obvious. (b): Liver transverse section of the rats treated with *G. glabra* extract at 100 mg/kg for 30 continuous days. In this section shows necrosis in the hepatocytes (double head arrows). (c): Liver transverse section of the rats treated with *G. glabra* extract at 200 mg/kg for 30 continuous days. In this section centrilobular hepatic congestion (CV) is observed. (d): Liver transverse section of the rats treated with *G. glabra* extract at 200 mg/kg for 30 continuous days. The microphotograph shows local mononuclear leukocyte infiltration (LI) in the liver parenchyma. (Haematoxylin and Eosine stain) (a,c:  $\times 400$  and b,d:  $\times 1000$ ).

Table 1: Summarized histological changes in the liver of rats treated with different concentrations of *G. glabra* extract, intra-peritoneally, during 30 days in comparison with control animals.

Finding/Groups	Control	<i>G. glabra</i> as 50 mg/kg/day	<i>G. glabra</i> as 100 mg/kg/day	<i>G. glabra</i> as 200 mg/kg/day
Decrease in diameter of hepatocytes	-	-	++	+++
Increase of sinusoids volume	-	+++	+++	++++
Hepatic necrosis	-	++	++++	+++
Centrilobular hepatic congestion	-	-	+++	++++
Local mononuclear leukocyte infiltration	-	+++	++	+++

## DISCUSSION

There are many of the therapeutic benefits of *G. glabra* in the traditional medicine literatures [3, 9, 10] without any scientific clarification data. On the other hands, it has been demonstrated that various herbal toxicity clearly represents a serious human health threat and is an important issue that to be tackled [11]. Also, there have been increasing reports on the adverse reactions associated with herbal consumption. For many of these adverse reactions, the underlying biochemical mechanisms are unknown, but bioactivities of herbal compounds to generate reactive intermediates have been implicated [11]. In this study, we observed a significant increase of ALT, AST and ALP levels as well as histopathological structural changes in all experimental groups treated with *G. glabra* extracts. These findings indicated the detrimental effects of *G. glabra* extracts administering in the rat. In this study, histological findings indicated a decrease in diameter of hepatocytes and hepatocytes nuclear diameter, while an increase in sinusoids size, focal necrosis centrilobular hepatic congestion and local mononuclear leukocyte infiltration in the liver of the rats exposed to *G. glabra*. Changes in hepatocyte tissue could be caused by metabolism of plant extract in the liver [12]. Any changes in size and shape of hepatocyte's nucleus could be considered as a sign of increased metabolic activity. Focal necrosis of the liver tissue observed in the experimental group could be driven from the animal's excessive activity to get rid of the toxicant from its body during the process of detoxification. Also, the liver incapability in regenerating new cells may lead to necrosis [1]. The elevated serum ALP activity in the present study could be due to *G. glabra* cytotoxicity [13]. Conversely in a study accomplished by Al-Razuqi and *et al.* (2012), it was demonstrated that when liver was damaged with CCl<sub>4</sub>; the levels of these enzymes decreased considerably [7]. This dissimilarity may be associated with different toxic agents and also different procedures for agent administering. There are no exact mechanism (s) of action for *G. glabra* hepatotoxicity in the literature. But it has been reported that *G. glabra* plant has a variety of complex chemical constituents including flavonoides, glycosides, glycyrrhizin, saponin, glabrene, starches and yellow coloring matter that could be responsible for its toxic properties [13,14].

## CONCLUSION

In conclusion, *G. glabra* could cause its harmful impacts on the liver integrity, so caution should be paid to popular consumption of this plant. The histological results of the present study will also need to be confirmed using electron microscopy for exact ultra structural changes.

## ACKNOWLEDGMENT

We would like to appreciate laboratory of Liam para veterinary faculty for technical support.

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