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Histo-morphological assessments of the kidney and liver after *Glycyrrhiza glabra* extract administration in the rat

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ABSTRACT

Glycyrrhiza glabra L. (family:Fabaceae:Leguminosae) is one of the important medicinal plant, commonly called as liquorice. Thirty-two Sprague-Dawley male rats were randomly distributed into four groups (n=8). Experimental groups were injected intra-peritoneally 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 distilled water without any add-on material. At the end of the experiments, renal and hepatic tissue samples were taken and histo-morphological changes of the kidney and liver were examined using light microscope. In the rats administrated with G. glabra extract at different concentrations, the kidney showed massive congestion changes and a few necrosis of the nuclei in the epithelial cells of renal tubules. In addition, mild interstitial lymphoplasmacytic nephritis was seen in the kidney of the plant administrated groups. We observed dilation of urinary space in the renal glomerulus as well as dilation of various urinary tubules lumen in the kidney of the plant administrated groups. Hepatic structural changes included the decrease in diameter of hepatocytes, an increase of sinusoids volume ($P<0.05$), congestion and necrosis of hepatocytes in the liver of extract treated animals. In conclusion, however there are many documents exhibiting detoxification, antioxidation, and antiinfection properties of G. glabra, we observed some of the likely side effects of G. glabra on the kidney and liver structure in rats. So caution should be paid to popular consumption of this plant.

Key words: *Glycyrrhiza glabra*, histology, kidney, liver, rat

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INTRODUCTION

There is growing interest in the use of herbs to aid in the maintenance of health. Licorice (*Glycyrrhiza glabra* L.) is a well-known medicinal herb that grows in various parts of the world. It is one of the oldest and widely used herbs from the ancient medical history of Ayurveda, both as a medicine and also as a flavoring to disguise the unpleasant flavor of other medications [1]. In the traditional system of medicine, the roots and rhizomes of *G. glabra* (Family: *Pappilionaceae/ Fabaceae*) have been employed clinically for centuries for their antiinflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities [2]. Liquorice has been shown to have great antioxidant, free radical scavenging [3] and anticonvulsant activities [4]. It has been shown to decrease circulating levels of testosterone in men [5, 6]. There are many useful compound in licorice root such as, glycyrrhizin (saponin- like glycoside -50 time sweeter than sugar) and its aglycone, glycyrrhetinic acid which are clinically used for hyperlipidemia [7]. Licorice flavonoid constituents mainly include flavones, flavonals, isoflavones, chalcones, bihydroflavones and bihydrochalcones. It had been demonstrated that *G. glabra* extract has biological capabilities

include detoxication, antioxidation, and antiinfection properties, although there is little data about its probable side effects on the kidney and liver integrities. So, in this work we studied the effect of different concentrations of aqueous extract of *G.glabra* on the histo-morphological aspects of the kidney and liver of rats.

MATERIALS AND METHODS

2.1. Experimental Animals

The study was carried out for one month and for carrying out the experiments, thirty-two Sprague-Dawley male rats were randomly distributed into four groups (n=8). The treatment groups were injected intra-peritoneally with aqueous extract of *G. glabra* at 50, 100 and 200 mg/kg/day, respectively, for 30 consecutive days. Control group was injected with distilled 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 ± 1 ºC; relative humidity 50 ± 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 Ilam University.

2.2. Plant Extraction

The *G. glabra* (Licorice) root was purchased from Emam-Reza medicinal plants market (Ilam, Iran) and botanical identification was confirmed at the herbarium of Ilam University (Exsiccatae 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 ˚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 ˚C until its use.

2.3. Histo-morphological assessment for kidney and liver injuries

At the end of the experiments, the rats were anesthetized, the abdomen was opened, kidneys and livers were removed. Then, length, width and weight of the right kidney and also the weight of the liver were measured. The histological specimens of the cranial pole of the right kidney as well as the right lobe of liver were imprisoned overnight in 10% neutral buffered formalin to be fixed. Then the specimens were mounted to allow 5-µm sections. They were stained via hematoxylin and eosin (H&E). Sections were photographed directly using a stereo microscope in 400 high power fields with Microsoft system. For exact description of structural changes in kidney and liver tissues, a histometrical analyze was performed. For this purpose, in any of images, the diameters of renal corpuscle, lumen of proximal convoluted tubules and the height of epithelium of proximal convoluted tubules and also the diameters of hepatocytes together with hepatic sinusoids size were measured by Motic 2001 image analyzer software and their results were statistically analyzed.

2.4. Statistical analysis:

Data were analyzed using version 16 of SPSS software (SPSS Inc., Chicago, IL, USA).The results of quantitative parameters of kidney and liver were expressed as mean ± 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

The results of the present study showed significant decrease in the length, width and weight of the kidney as well as the weight of liver. Histometrical results of kidney included decrease of diameter of renal corpuscle, increase of diameter of lumen of proximal convoluted tubules, decrease of height of epithelium of proximal convoluted tubules (Fig 1) (Table 1). In the hepatic parenchyma of animals treated with different concentrations of *G. glabra* extract, a decrease in diameter of hepatocytes, an increase of sinusoids volume ($P<0.05$), hepatic necrosis, and centrilobular hepatic congestion were seen. (Fig 2) (Table 1). These histological alterations were more evident in rats exposed to 200 mg/kg of plant extract.

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

Morphometric parameters /Groups	Control	*G. glabra* as 50 mg/kg/day	*G. glabra* as 100 mg/kg/day	*G. glabra* as 200 mg/kg/day
Length of kidney(mm)	8.7 ±0.03	6.4 ±0.07	3.5 ±0.02	4.5 ±0.08*
Width of kidney(mm)	4.3±0.02	4.4 ±0.09	4.3 ±0.06	4.1 ±0.03
Weight of kidney(g)	1.9±0.028	1.1 ±0.01	0.9 ±0.04	1.02 ±0.06*
Weight of liver(g)	`8.4±0.05	2.6 ±0.04	2.8 ±0.02	2.7 ±0.06*
Diameters of renal corpuscle(µm)	879.9±4.98	353.2 ±5.34	247.4 ±1.45	116.3 ±2.08*
Diameters of lumen of proximal convoluted tubules (µm)	`5.5±0.03	6.3 ±0.02	6.5 ±0.01	5.5 ±0.08
The height of epithelium of proximal convoluted tubules (µm)	38.4 ±0.02	37.9 ±0.04	38.2 ±0.08	37.6 ±0.04
Diameter of hepatocytes (µm)	9.8±0.05	3.7 ±0.02	4.67 ±0.04	3.28 ±0.01*
Area of sinusoids (μm^2)	437.3±6.78	851.2 ±2.55	938.5 ±6.96	1059.6 ±8.68*

** Significant between rows as: P<0.05*

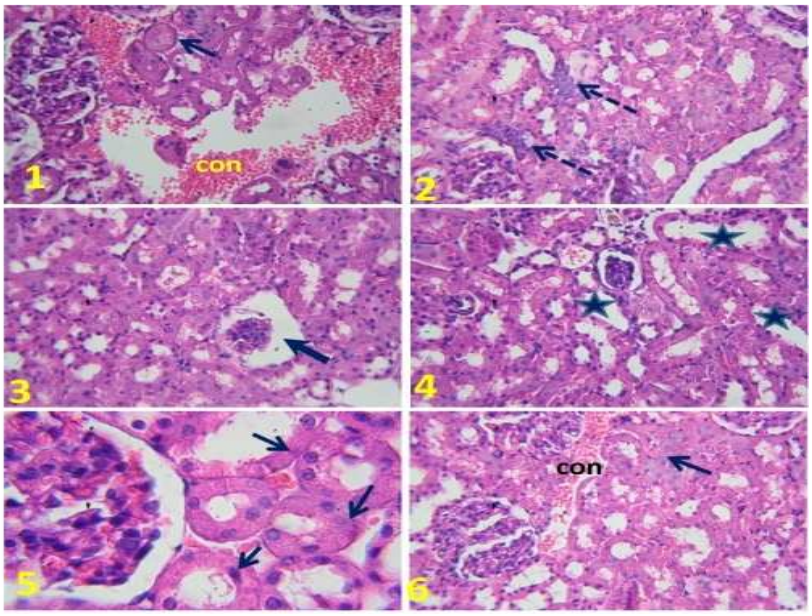

Figure 1. (1): Kidney transverse section of rats treated with *G. glabra* plant extract at 200 mg/kg showing massive congestion (Con) and tubular epithelium necrosis (arrow) in the renal tissue. (2): Kidney transverse sections of the rats treated with *G. glabra* plant extract at 100 mg/kg. The section shows interstitial lymphoplasmacytic nephritis (arrows) in the parenchyma of kidney. (3): Kidney transverse sections of the rats treated with *G. glabra* plant extract at 100 mg/kg. The section shows dilation of urinary space in the renal glomerulus (arrow). (4): Kidney transverse sections of the rats treated with *G. glabra* plant extract at 50 mg/kg. The section shows dilation of various urinary tubules lumen (stars). (5): Kidney transverse section of rats treated with *G. glabra* plant extract at 50 mg/kg showing tubular epithelium necrosis (arrow) in the renal tissue. (6): Kidney transverse sections of the rats treated with *G. glabra* plant extract at 100 mg/kg. The section shows congestion (Con) and tubular epithelium necrosis (arrow) in the renal parenchyma. (Haematoxylin and Eosine stain) (1-6: × 400, 5: × 1000).

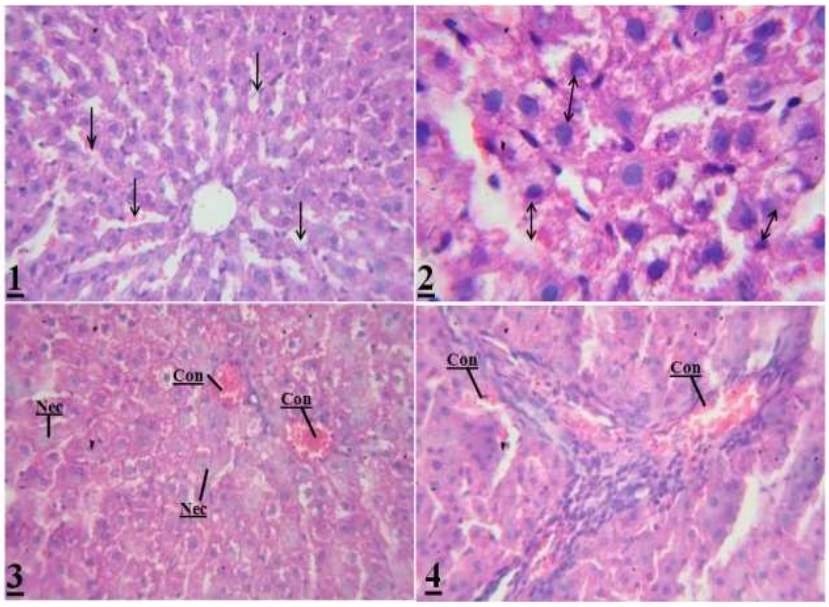

Figure 2. (1): Liver transverse section of rats treated with *G. glabra* plant extract at 100 mg/kg showing increase of sinusoids size (arrows) in the hepatic tissue. (2): liver transverse sections of the rats treated with *G. glabra* plant extract at 200 mg/kg. The section shows decrease of diameter of hepatocytes (double head arrows) in the parenchyma of liver. (3): Liver transverse sections of the rats treated with *G. glabra* plant extract at 50 mg/kg. The section shows centrilobular hepatic congestion (Con) and also hepatocytes necrosis (Nec). (4): Liver transverse sections of the rats treated with *G. glabra* plant extract at 200 mg/kg. The section shows hepatic congestion in the portal region of liver (Con). (Haematoxylin and Eosine stain) (1-4: × 400).

DISCUSSION

Estimation of renal histological changes has provided useful information on the health status of the kidneys [8]. In addition, 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 [9]. There are many of the therapeutic benefits of *G. glabra* in the traditional medicine literatures [10,11,12] 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 [13]. 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 [13]. In this study, we observed significant histo-morphological toxic changes in the kidney and liver after *G. glabra* administration. These findings indicated the detrimental effects of *G. glabra* extracts administering in the rat. A finding of the present study was atrophic alteration in the renal corpuscle under light microscope, which is in accordance with Tootian et al.'s study [14] and it is supported by significant decrease in the length, width and weight of kidney as gross anatomical assay. In this study, histological findings indicated a decrease in diameter of hepatocytes, while an increase in sinusoids size, focal necrosis and centrilobular hepatic congestion 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 [15]. 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 [9]. After oral administration, glycyrrhizin is metabolized to glycyrrhetinic acid by intestinal bacteria which contain β-D-glucuronidase [16]. Furthermore, intravenously administered glycyrrhizin is metabolized in the liver by lysosomal β-D-glucuronidase to 3- mono-glucuronide glycyrrhetinic acid. This metabolite is excreted with bile into the intestine, where it is metabolized by bacteria into glycyrrhetinic acid, which can be reabsorbed [17].Glabridin showed an antinephritis effect in the mouse glomerular disease model [18]. Also, glycyrrhizin could ameliorate renal defects in gentamicininduced acute renal failure in rats [19].In contrast, our study indicated, mild interstitial lymphoplasmacytic nephritis was seen in the kidney of the plant administrated groups. Previous works indicated that mice were treated with licorice root extract for 30 or 90 days at 0, 8 and 25 % of the diet, the treated mice showed a range of treatment related clinical signs

including, poor weight gain with 30 % mortality in rats dosed liquorice root at 25 % of the diet and Lesions were noted in the liver,spleen and thymus [20]. Also in our research, massive congestion changes and a few necroses of the nuclei in the epithelial cells of renal tubules were observed (Fig.1). In another study, the toxicity of licorice extract was shown in the liver of Black molly fish [21]. We observed dilation of urinary space in the renal glomerulus as well as dilation of various urinary tubules lumen in the kidney of the plant administrated groups (Fig.1) this changes may be in relation with the hypertension caused by decreased 11B-HSD2 activity. This enzyme is responsible for the renal conversion of cortisol to cortisone. Thus, licorice leads to activation of renal mineralocorticoid receptors by cortisol, resulting in a state of apparent mineralo-corticoid excess and therefore large amounts of licorice may result in severe hypertension, hypokalemia and other signs of mineralocorticoid excess [22] and also inhibitory effects of 18β-glycyrrhetinic acid (one of the ingredients of *Glycyrrhiza glabra*) on gap junction channels of arteriolar smooth muscle, endothelial cells, renal pelvis, ureter and mesenteric small arteries were studied [23] that probably cause dysfunction of urinary tract smooth muscle and consequently give back and retort the urea up to pelvis and kidney.

In conclusion, our finding indicates *G. glabra* extract has ability to induce several structural changes in the kidney and liver of rats. So caution should be paid to popular consumption of this plant.

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