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The Protective Effects of Aqueous Extract of *Glycyrrhiza Glabra* Root Against Hepatic Dysfunction Induced by Thioacetamide in Male Rats

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ABSTRACT

Thioacetamide is a liver toxin that causes centrilobular necrosis. In this study, the protective effect of aqueous extract of Glycyrrhiza glabra root against hepatic dysfunction induced by thioacetamide in male rats was investigated.35 male rats were divided into 5 groups of 7 : control group; sham group: receiving a single dose of 150mg/kg thioacetamide intraperitoneally; experimental groups 1 and 2 and 3: they received the aqueous extract of Glycyrrhiza glabra root at the doses of 100,200,300mg/kg daily orally during 3 months respectively and then a single dose of thioacetamide at 150 mg/kg as intraperitoneal injection . The serum levels of SGOT, SGPT, ALP, GGT and LDH were measured. The mean levels of SGPT in the experimental groups 3 and 2 (194.50 ± 13.50) and (222.50± 10.37) showed a significant decrease compared to the group receiving thioacetamide (361± 10.01).The average concentrations of GGT in the experimental groups 3 and 2 (2.50±0.22) and (2.28 ± 0.28) compared to the group receiving thioacetamide (4.50± 0.28) significantly reduced.The mean levels of ALP, LDH and SGOT in all experimental groups did not show significant changes compared to the group receiving thioacetamide (P≤05).The results of this study showed that the aqueous extract of Glycyrrhiza glabra root had protective effects against the hepatic dysfunction induced by thioacetamide in male rats.

Keywords: *Glycyrrhiza glabra* root, thioacetamide, hepatic dysfunction, male rats

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INTRODUCTION

The liver has a crucial role in regulating a variety of physiological processes, including metabolism, secretion and storage .Furthermore, the detoxification of drugs and xenobiotics is done in the liver[1]. Thioacetamide is a compound containing thiono - sulfur used as fungicide, organic solvent and engine oil stabilizer. Thioacetamide is a liver toxin that induces hepatic necrosis by producing free radicals [2]. Thioacetamide induces centrilobular necrosis, hepatic cirrhosis, and hepatocellular carcinoma [3].

Using natural substances with plant origin for the treatment and protection of the liver has a long history in traditional medicine. Phenolic compounds and flavonoids are abundantly present in plants which have multiple biological activities, including the ability to neutralize free radicals and antioxidant activity. These compounds have received much attention in the treatment and protection against oxidative damages [4].

Glycyrrhiza glabra also known as licorice and sweetwood, belongs to the family of Leguminosae. Its root is highly effective in wound healing and liver and heart protection [5].The compounds isolated from *Glycyrrhiza glabra* root

include saponins, triterpenes, flavonoids, ascorbic acid, isoflavonoids , chalcones, liquiritigenin, glycyrrhizin, glycyrrhetinic acid, glycyrrhizic acid ,quercetin, phyto-sterols and coercin. The triterpenes present in *Glycyrrhiza glabra* root contain licorice acid, glycyrrol, glabrodile, isoglaborlide and glabradin. *Glycyrrhiza glabra* root has anti-ulcer, anti-inflammatory, anti-oxidative, anti-viral, anti-cancer, and liver protecting effects [6]. The aqueous extract of *Glycyrrhiza glabra* root(licorice) has a protective effect against the toxicity induced by cadmium.*Glycyrrhiza glabra* root(Licorice) and liquiritigenin prevent planned and unplanned cell death induced by treatment with cadmium alone or in combination with buthionine sulfoximine[7].

Studies have shown that several plant species are effective in the treatment of liver diseases with minimal side effects one of which is *Glycyrrhiza glabra* root. Liver enzymes are synthesized exclusively in the liver hepatocytes and measuring them is one of the ways to diagnose hepatic function. On the other hand, no studies have been done on the evaluation of blood biochemical factors in the groups pre-treated with aqueous extract of *Glycyrrhiza glabra* root within 3 months after liver toxicity. Regarding the anti-inflammatory and antioxidant effects of *Glycyrrhiza glabra* root and its long use in traditional medicine for the treatment of liver disorders, the protective effect of aqueous extract of *Glycyrrhiza glabra* root on hepatic dysfunction induced by thioacetamide in male rats was investigated in this study.

MATERIALS AND METHODS

Laboratory animals

This was an experimental study. Animals were collected from Fars Razi Vaccine and Serum Research Institute. All guidelines for ethical conduct in the care and use of laboratory animals developed by the Ministry of Health and Medical Education were observed in the present study.35 male Wistar rats weighing 200±10 grams and the age range from 2.5 to 3 months were randomly divided into 5 groups of 7 and were kept under a photoperiod of 12 hours of light and 12 hours of darkness and were maintained at 20 ° C and sufficient moisture in standard cages .They had free access to standard food and water.

Treatment

35 adult male rats were divided into 5 groups of 7 and after they spent a period of adaptation to the heat and humidity, they entered the experiment. The grouping of the animals was as follows: Control group which underwent no stress including no oral administration. Sham group: in this group the animals received thioacetamide at a dose of 150 mg/kg intraperitoneally once at the end of the 3 months period. Experimental group 1: animals in this group received 100 mg/kg of *Glycyrrhiza glabra* root aqueous extract orally for 3 months and a single intraperitoneal injection of thioacetamide at a dose of 150 mg/kg at the end of 3 months. The experimental group 2: animals in this group received 200 mg/kg of *Glycyrrhiza glabra* root aqueous extract daily orally for 3 months and a single intraperitoneal injection of thioacetamide at a dose of 150 mg/kg at the end of 3 months. The experimental group 3: animals in this group daily received 300 mg/kg of *Glycyrrhiza glabra* root aqueous extract orally for 3 months and a single intraperitoneal injection of thioacetamide at a dose of 150 mg/kg at the end of the 3 months period. The doses of thioacetamide(Stigma-Aldrich, Switzerland) and *Glycyrrhiza glabra* root aqueous extract were selected according to previous studies[8 , 9].

Biochemical study

As the effects of toxic thioacetamide usually become apparent about 2 days after injection, 48 hours after the last injection all animals were anesthetized with ether (Merck, Germany), and their blood samples were directly taken from the heart.

The obtained blood samples were kept under laboratory conditions for 20 minutes and then were centrifuged at 5000 rounds per minute for 15 minutes (Hettich, Germany).The levels of lactate dehydrogenase (LDH) and serum glutamic pyruvic transaminase (*SGPT*) were measured by DGKC method, and based on the following reactions:

$$\text{L - Alanine} + \propto -\text{ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L} - \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L} - \text{Lactate} + \text{NAD}^+$$

The evaluation of serum glutamic oxaloacetic transaminase *AST* (SGOT) was done by IFCC method based on the following reactions:

$$L-Aspartate+\propto -ketoglutarate \xrightarrow{AST} L-Glutamate+Oxaloacetate$$

$$Oxalacetate+NADH+H^{+} \xrightarrow{MDH} L\text{ - }Malate+NAD^{+}$$

For the measurement of alkaline phosphatase (ALP) the method of P-Nitrophenyl phosphate AMP was used in which ALP reacted on the colorless 4-nitrophenyl phosphate substrate changed it to yellow 4-nitrophenol. The changes in optical density is proportional to the activity of the alkaline phosphatase enzyme.

$$P\text{ - }Nitrophenylphosphate+H2O \xrightarrow{ALP} P\text{ - }Nitrophenol+Inorganic\,phosphate$$

SGOT, SGPT, ALP enzymes were measured by an auto analyzer device model 3000 BT- (Biotechnical Co., Italy) .
The measurement of gamma-glutamyl transferase (GGT) was done by enzymatic method (Diasys, Germany).Gamma-glutamyl transferase (GGT) was determined following the Szasz method,in which the substrate L-γ-glutamyl-3-carboxy-4-nitroanilide in the presence of glycylglycine was converted by GGT in the sample to 5-amino-2-nitrobenzoate which could be measured at 405 nm.The amount of 5-amino-2-nitro-benzoate was proportional to the activity of GGT which could be measured by photometry. [10 , 11].

Statistical analysis

SPSS software (version18, chicago, IL, USA) was used for data analysis. The ANOVA test was performed on the data. Tukey test was used to evaluate the significant differences of the data (Tukey-HSD) and all the differences were considered as significant at $P \le 0.05$.The plasma concentration of ALP, SGPT, SGOT,GGT and LDH were presented as $mean \pm SE$.

Preparation of *Glycyrrhiza glabra* root aqueous extract

Glycyrrhiza glabra root was collected from the farms in the vicinity of Kazerun in early summer and confirmed by the botanical biology department of Azad University of Shiraz and kept at the herbarium code of P06204001A .Then the roots were washed and dried at the ambient room temperature and then poured in an electric grinder .The fine powder obtained in this way was taken to a laboratory for extraction. 1000 gr of *Glycyrrhiza glabra* root powder was poured in 15 liters of distilled water and was boiled for 30 minutes and then centrifuged for 10 minutes at the rate of 8000 r/m. Then it was filtered in order to remove the cellulose fibers. The obtained mixture was then heated in an oven (Finetech, Korea) at 40 °C so its water vaporized and a thick syrup was obtained .The resulting mixture was dried in such a way that it kept only 23% of its initial weight. Next, it was poured into different amounts of distilled water in order to make various concentrations of the extract [8].

RESULTS

In this study the mean serum concentrations of Alkaline phosphatase (ALP),lactate dehydrogenase(LDH),Serum glutamic-oxaloacetic transaminase(SGOT), Serum glutamic-pyruvic transaminase(SGPT),Gamma-glutamyl transpeptidase(GGT), in experimental, control and thioacetamide groups were compared and statistical analysis was performed . Results were presented in tables. Tukey's test was done to check the statistical analysis and $p \le .05$ was considered as significant.

The average concentration of serum SGOT in the group receiving thioacetamide showed a significant increase compared to the control group. The average concentration of serum SGOT in all experimental groups which received aqueous extract of *Glycyrrhiza glabra* root and thioacetamide decreased compared to the group receiving thioacetamide but it was not significant. The average concentration of serum SGOT only in the experimental group receiving 200mg/kg of aqueous extract of *Glycyrrhiza glabra* root and thioacetamide showed a significant increase compared to the control group (Table 1).The average concentration of SGPT in the group receiving thioacetamide showed a significant increase compared to the control group. SGPT levels in the experimental groups which received aqueous extract of *Glycyrrhiza glabra* root at the doses of 200, and 300 mg/kg and thioacetamide showed a significant decrease compared to the group receiving thioacetamide(Table 1).The average concentration of ALP in all experimental group, control and sham control groups and also the thioacetamide group showed no significant changes(Table1). The average concentration of serum GGT in thioacetamide group showed a significant increase in comparison to the control group. The average concentration of serum GGT in the experimental groups which received 200,300 mg/kg of aqueous extract of *Glycyrrhiza glabra* root and thioacetamide significantly decreased

compared to the thioacetamide group(Table 1).The average concentration of serum LDH in the group receiving thioacetamide showed a significant increase compared to the control group.The average concentration of serum LDH in the experimental groups which received 100,300 mg/kg of aqueous extract of *Glycyrrhiza glabra* root and thioacetamide decreased compared to the group receiving thioacetamide ,but it was not significant. The average concentration of serum LDH in the experimental group received the aqueous extract of *Glycyrrhiza glabra* root at the doses of 100 and 200 mg/kg and thioacetamide showed a significant increase compared to the control group (Table 2) .

Table 1: The effect of different doses of aqueous extract of *Glycyrrhiza glabra* root on serum biochemical parameters in male rats with thioacetamide induced toxicity

All groups	SGOT **(U/L)**	SGPT **(U/L)**	ALP **(IU/L)**	GGT **(U/L)**	LDH **(U/L)**
control	83.66±1.17	189.60±10.60	1456.66±54.66	2±0.26	554.28±6.58
Thioacetamide(TAA)	187.50±1.44[a]	361±10.01[a]	1173±70.15	4.50±0.28[a]	849±0.57[a]
100mg/kg G.G+TAA	132.66±14.29	286.75±14.14	1369.33±58.73	3.75±0.25	829.66±52.41[c]
200mg/kg G.G+TAA	177.16±9.25[c]	222.50±10.37[b]	1182.66±63.77	2.28±0.28[b]	869.60±29.51[c]
300mg/kg G.G+TAA	122±8.28	194.50±13.50[b]	1267±84.96	2.50±0.22[b]	711.83±44.03

Letter a represents a significant difference in the groups receiving thioacetamide with the control group at P <0.05; letter b represents a significant difference between the group receiving thioacetamide with experimental groups at P <0.05; letter c represents a significant difference between the control group and the experimental groups at P <0.05.

DISCUSSION

The results of this study showed that the values of SGPT, SGOT, ALP,GGT and LDH in groups treated with thioacetamide increased significantly compared to the control group. The average concentration of SGPT in the experimental groups which received 200 and 300 mg/kg of *Glycyrrhiza glabra* root aqueous extract and thioacetamide showed a significant decrease compared to the group receiving thioacetamide.The mean serum GGT in the experimental groups receiving 200,300 mg/kg of aqueous extract of *Glycyrrhiza glabra* root and thioacetamide significantly decreased (P<0.05).This means that the extract had protective effects on liver cells against damage caused by thioacetamide .

Quercetin is one of the compounds present in *Glycyrrhiza glabra* root. Padama and colleagues in a study in 2012 found that quercetin modified lindan induced oxidative stress in wistar rats' kidney and liver. The consumption of quercetin led to reduced levels of enzymes in the liver and improved symptoms of renal failure [12].Kimura and colleagues (2008) found that the glycyrrhizin found in *Glycyrrhiza glabra* root accelerated the liver regeneration and quickly decreased the activity of serum transaminase in mice which had lost 70% of their livers[13].Nakagawa et al. in their study in 2010 found that the flavonoid oil in *Glycyrrhiza g*labra in rats with heptic carcinogenesis had inhibitory effect on the activity of glutathione- s - transferase positive foci. *Glycyrrhiza glabra* flavonoid oil with a concentration of 600 mg/kg had inhibitory effects on hepatic carcinogenesis [14] .Using a combination of martine, gycyrrhizin could decrease the deaths from high doses of acetaminophen and could alleviate the hepatotoxicity induced by carbon tetrachloride [15].

In the study done by Hasan et al. in 2015 it was shown that 18-B-glycyrrhetinic acid improved liver toxicity induced by 2- acetylaminofluorence in wistar rats. These effects were applied by the correction of oxidative stress, inflammation and increased cell proliferation[16].The study conducted by Maatoog and colleagues in 2010 showed that bioactive microbial metabolites from glycyrrhetinic acid had protective effects against the peroxidation induced by ascorbic acid / Fecl3 in normal mice liver homogenate, induction of nitric oxide production in macrophages of rats and in vivo hepato protection against hepatotoxicity induced by CCL4 in albino mice [17].Hosseini and colleagues in 2014 showed that *Glycyrrhiza glabra* extract had protective effects against toxicity induced by doxorubicin h9c2 cells which were applied through reducing the oxidative stress and inhibiting apoptosis[18].In the study done by Orazizadeh and colleagues in 2014 glycyrrhizic acid was shown to have protective effects against liver toxicity induced by nanoparticles of titanium dioxide in rats [19]. Khorsandi et al. in 2015 showed that glycyrrhizic acid corrected the lipid peroxidation induced by titanium dioxide in rat's liver[20].The study of Tsai et al. in 2013 indicated that glycyrrhizin reduced the acute liver damage related to total parenteral nutrition in rats through decreasing endoplasmic reticulum and reactive nitrogen [21]. Hsiang et al. in 2015 showed that glycyrrhizin, silymarin and urosodeoxycholic acid could regulate the expression of genes associated with cell death and oxidative stress in HePG2 cells. In addition, these liver protective effects might be due to NF-Kb reduction [22].

In the study done by Gaur et al. in 2010 it was shown that liqirtigenin derivatives had protective impacts against liver toxicity induced by D-galactosamine-lipopolysaccharide by reducing the increased levels of SGOT, SGPT, ALKP, TG, NO and LDH [23].

Wang and his colleagues in 2015 showed that quercetin improved the liver damage induced by tripterygium glycosides possibly through reducing oxidative stress and its anti-inflammatory properties [24]. In the study done by Chen et al. in 2013 it was shown that glycyrrhizic acid had liver protective effects in mice by NrF2 dependent upregulation[25].The study of Park et al. in 2015 showed that liquirtigenin had protective effects against the liver damage induced by tacrine (an oral AChE inhibitor) exerted through the inhibition of GSK3-beta[26]. Glycyrrhizic acid available in *Glycyrrhiza glabra* root modified t-BPH-induced cell death in rat liver cells .GA protective effect against cell death was resulted from preventing glutathione loss ROS formation and inhibiting the mitochondrial membrane depolarization [27].The combination of silybum marianum (silymarin) and *Glycyrrhiza glabra* (Glycyrrhizin) in different doses has protective effect against oxidative stress in the liver [28].The saponins from the root of *glycyrrhiza inflata* has protective impacts on hepatic cells of rats intoxicated by D-galactosamine through reducing the levels of ALT and AST liver enzymes [29]. *Glycyrrhiza glabra* root extract and its active ingredients including liquirtigenin, glycyrrhizic acid inhibit the increase of pre-inflammatory cytokinin such as tumor necrosis factor-alpha (TNF-α) , interleukin-1 B and interleukin-6 in the mice's liver treated with t-BPH. *Glycyrrhiza glabra* root extract and its bioactive components improve liver oxidative damage and inflammatory diseases [30].

In general the results of this study are in line with the results of other studies.It seems that the oral administration of aqueous extract of *Glycyrrhiza glabra* root has protective effect on thioacetamide induced liver toxicity by neutralizing free radicals, stimulating the activity of antioxidant enzymes, and reducing the production of inflammatory cytokinin . However, further research is necessary to identify the active ingredients in *Glycyrrhiza glabra* root extract (which may be responsible for its antitoxic activity). As no similar study on the protective effects of aqueous extract of *Glycyrrhiza glabra* root on hepatic enzymes and histological changes could be found, it was not possible do a comparative study in this respect. Anyhow, more studies should be conducted to examine the hepatic antioxidant enzymes and molecular changes inducing apoptosis so that the effects of this plant on healing liver toxicity can be determined with higher certainty .

The results showed that oral administration of aqueous extract of *Glycyrrhiza glabra* root has positive protective effects against thioacetamide-induced liver toxicity. If confirmed by further research, adding aqueous extract of *Glycyrrhiza glabra* root to the diet of people with hepatic dysfunction can be beneficial.

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