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Original Article

Evaluation of Protective Effect of *Glycyrrhiza glabra* L. Extract on Isoniazid-Rifampicin Induced Hepatocellular Damage in Rats

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ABSTRACT

Context: Haphazard use of drugs is one of the key reasons for progression of liver diseases. Drugs such as paracetamol, isoniazid, rifampicin etc. cause hepatotoxicity. There is currently no single synthetic drug which is effective for the treatment of such conditions. Drugs from natural sources have been used by humans since before written records. Thus, plants serve to be an important source to explore hepatoprotectives. **Objective:** The current study was designed to assess the hepatoprotective activity of *Glycyrrhiza glabra* extract. **Materials and Methods:** *Glycyrrhiza glabra roots* were dried in shade, powdered and extracted with ethanol and phytochemical screening was performed. The extract phenolic and flavonoids contents were estimated. Hepatoprotective studies were performed using isoniazid- rifampicin induced hepatotoxicity in rats. **Results:** Results of the phytochemical studies demonstrated that the extract was rich in flavonoids, glycosides and polyphenolics. The extract also demonstrated excellent hepatoprotective activity against isoniazid- rifampicin induced hepatotoxicity in rats. **Discussion and Conclusion:** Results of study demonstrate that ethanol extract of *Glycyrrhiza glabra* is potent source of phytochemicals that are responsible to demonstrate hepatoprotective activity.

Key words: Liver, Liquorice, Antitubercular, Hepatotoxicity, AST, ALT, ALP.

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INTRODUCTION

The liver is the largest organ present in the body and constitutes about 2-3% of the body weight. The liver is considered as metabolic organ. It plays an essential role for maintaining homeostasis in the body.[1] A variety of vital processes including vitamins storage, energy generation, bile production, carbohydrate metabolism and many more are performed by the liver. The liver participates in and plays a major role in the metabolism of proteins, carbohydrates and lipids. It also aids in the biotransformation of xenobiotics.[2] The blood approaching liver through the portal vein carries a number of toxic substances. As a result, liver gets exposed to various toxins and harmful drug metabolites. Isoniazid and rifampicin are two of the important anti-tubercular drugs utilized in combination in the management of tuberculosis to fight the resistant *Mycobacterium* spp.[3] However, the metabolites from these drugs cause damage to the liver. Therefore, their continual use for more than four to six months leads to hepatotoxicity. In India, the predominance of anti-tubercular drugs induced-hepatotoxicity is nearly 11%, which makes anti-tubercular therapy hazardous and thus confines the use of these drugs in the management of tuberculosis.[4,5] The general consequences of such type of hepatotoxicity include increase in AST, ALT and ALP levels. There is a significant increase in lipid per-oxidation and bilirubin clearance. The general symptoms of hepatotoxicity include abdominal pain, nausea, urine discoloration, pyrexia and anorexia.[6] Oxidative stress seems to be the major mechanism behind isoniazid-rifampicin induced hepatotoxicity.[6]

Medicines from plants are good sources of pharmaceuticals. Many modern medicines find their origin from the structural modification of naturally occurring phytoconstituents. *Liquorice* (*Glycyrrhiza glabra* L.; Family: *Fabaceae*) is a well-recognized plant in Ayurveda. The stolon and roots of the plants are used in medicine. The plant is rich in glycyrrhizin, glycyrrhizic acid and glycyrrhetinic acid.[7] *G. glabra* is well recognized for anti-diabetic, spasmolytic, laxative, anti-ulcer, anti-inflammatory and anti-depressive effects.[8] *Liquorice* has also been studied for its protective effect against carbon tetrachloride induced epatocellular toxicity.[9] The present work aimed to determine the hepatoprotective effect of *Glycyrrhiza glabra* on isoniazid-rifampicin induced hepatocellular damage in rats.

MATERIALS AND METHODS

Preparation of plant extract

The root of *Glycyrrhiza glabra* were collect from local market of Jabalpur, India. It was authenticated by Dr. A.B. Tiwari, Department of Plant Physiology and Taxonomy, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. The plant root was ground to a coarse powder. The powdered material was first extracted with petroleum ether (for defatting) and then with ethanol (95 %). The yield (% w/w) of extract was found to be 7.5%. Phytochemical tests were performed on prepared extract by standard methods.[10]

Animals

Wistar rats (180-200 g) of either sex were housed in polypropylene cages and maintained under standardized conditions (12 h light/dark cycles, 28±2°C). Animals were provided with standard pellet food and had free access to drinking water. All the animal study protocols were duly approved by the institutional animal ethics committee.

Selection of dose, animal group and dosing

Aqueous extract of *G. glabra* at 100, 200 and 400 mg/kg body weight were used in this study.[11] Animals were divided into six groups with six animals in each group.

Group, I Normal saline (2 ml/kg)

Group II Rifampicin (50mg/kg) and isoniazid (100mg/kg)

Group III Rifampicin (50mg/kg) and isoniazid (100mg/kg) + silymarin

Group IV Rifampicin (50mg/kg) and isoniazid (100mg/kg) + *G. glabra* extract (100mg/kg)

Group V Rifampicin (50mg/kg) and isoniazid (100mg/kg) + *G. glabra* extract (200mg/kg)

Group VI Rifampicin (50mg/kg) and isoniazid (100mg/kg) + *G. glabra* extract (400mg/kg)

On the 21th day, blood was collected for the estimation of biochemical parameters. Animals were subsequently sacrificed under ether anesthesia. The liver was collected, washed and used for histopathological studies.

Biochemical analysis and Preparation of Liver Homogenate

Blood samples were collected and centrifuged for 10 min at 7000 rpm using a micro-centrifuge to separate the serum. The levels of serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic-pyruvic transaminase (SGPT/ALT) serum alkaline phosphatase (SALP) were estimated using commercial kits (Span Diagnostics, Surat, India). The liver (10 % w/v) homogenate was prepared in phosphate buffer (0.1 M, pH 7.4 having 0.15 M KCl) using the homogenizer. The homogenate was centrifuged at 3000 rpm for 15 min and the clear cell-free supernatant obtained was used for the study.

Antioxidant studies

Superoxide dismutase assay

Superoxide Dismutase (SOD) activity in liver homogenate was determined according to the method of Minami and Yoshikawa.[12] The method was based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by Nitro Blue Tetrazolium (NBT) (Central Drug House Mumbai, India; analytical grade) formazan color development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development). The liver homogenate was centrifuged to 105,000 for 15 mins at 4°C. To 0.25 ml of supernatant, 0.5 ml of tris cacodylic buffer, 0.1 ml of 16% Triton x- 100 (Central Drug House Mumbai, India; analytical grade) and 0.25 ml NBT were added. The reaction was started by the addition of 0.01 ml diluted pyrogallol (Central Drug House Mumbai, India; analytical grade). Incubation was maintained for 5 mins at 37°C. The reaction was stopped by the addition of 0.3 ml of 2M formic acid (Central Drug House Mumbai, India; analytical grade). The formazan color developed was determined spectrophotometrically at wavelength 430nm. Enzymatic activity was expressed as µg/gm of tissue.

Catalase activity

The catalase activity was measured according to method of Sinha.[13] A 0.1ml volume of liver homogenate was mixed with 1.0 ml of 0.01M phosphate buffer (pH 7.4) and incubated with 0.4 ml of 0.2M H_2O_2 (Central Drug House Mumbai, India; analytical grade) at 37°C accurately for 1.0 min and reaction was stopped with 2.0 ml of 5% potassium dichromate (Central Drug House Mumbai, India, analytical grade) (1:3 with glacial acetic acid). The samples were then incubated in boiling water for 15 min. The tubes were centrifuged at 5000 rpm for 15 min and the supernatant was used to quantify the amount of H_2O_2 to calculate catalase activity at 570 nm. One unit represents 1.0µmole of H_2O_2 consumed/min/mg protein

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out by using one-way ANOVA, followed by Dennett's' test and $p<0.01$, $p<0.001$ was considered significant.

RESULTS

AST, ALT and ALP Determination

In the present study, administration of *G. glabra* extract to the laboratory animals resulted in a significant restoration of antioxidant enzyme levels. With regard to AST, administration of *Glycyrrhiza glabra* extract in the dose of 100, 200 and 400 mg/ kg caused a significant ($p<0.001$) decrease as compared to Group II animals (toxic control) (Figure 1). Likewise, for ALT (Figure 2) and ALP (Figure 3), there was a highly significant ($p<0.001$) restoration of antioxidant enzyme levels compared to Group II untreated control animals. Administration of silymarin also resulted in a significant decrease in AST, ALT and ALP levels.

SOD and aatalase activity levels

The administration of isoniazid and rifampicin to the test animals caused a decrease in levels of SOD (Figure 4). However, treatment with *G. glabra* extract (100, 200 and 400 mg/ kg) resulted in a significant ($p<0.001$) increase in SOD levels when compared to toxic control group (Group II). Correspondingly, the administration of *G. glabra* extract (100, 200 and

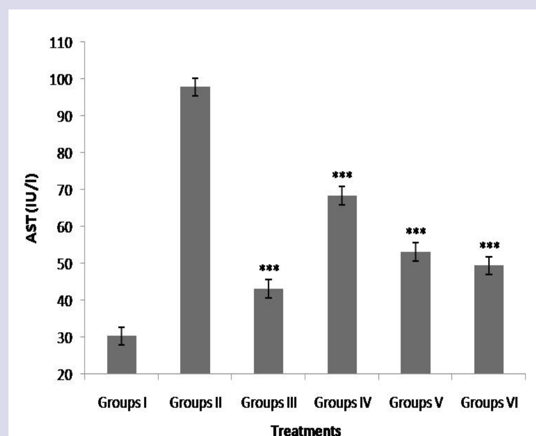

Figure 1: Effect of *Glycyrrhiza glabra* extract administration on AST levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at *$P<0.05$; **$P<0.01$; ***$P<0.001$.

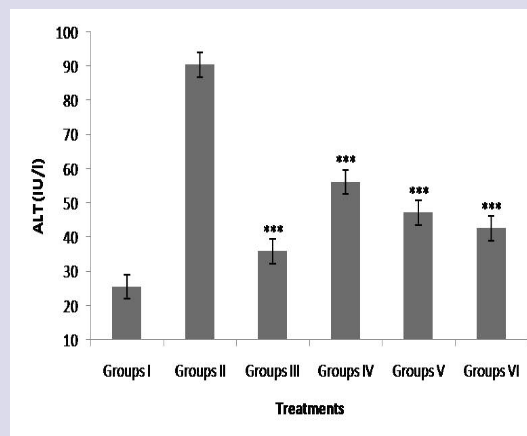

Figure 2: Effect of *Glycyrrhiza glabra* extract administration on ALT levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at *$P<0.05$; **$P<0.01$; ***$P<0.001$.

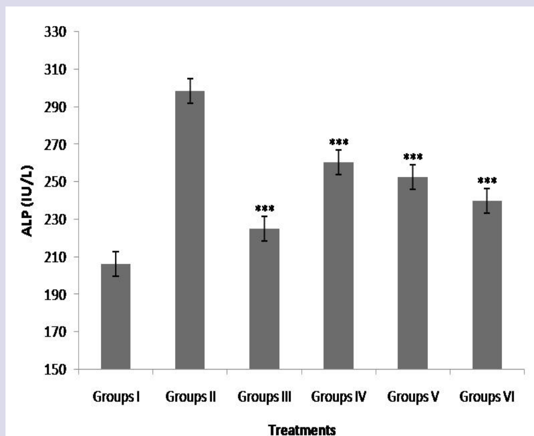

Figure 3: Effect of *Glycyrrhiza glabra* extract administration on ALP levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at *$P< 0.05$; **$P< 0.01$; ***$P< 0.001$.

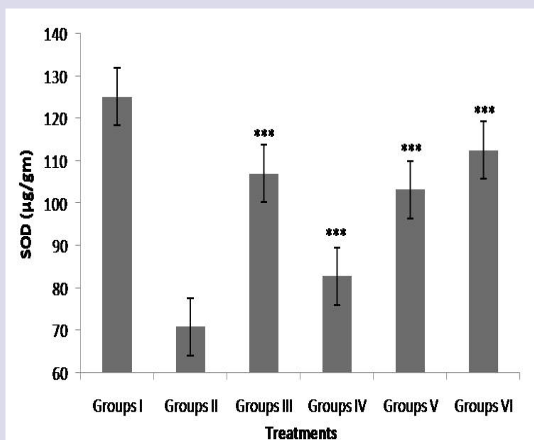

Figure 4: Effect of *Glycyrrhiza glabra* extract administration on SOD levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at *$P< 0.05$; **$P< 0.01$; ***$P< 0.001$.

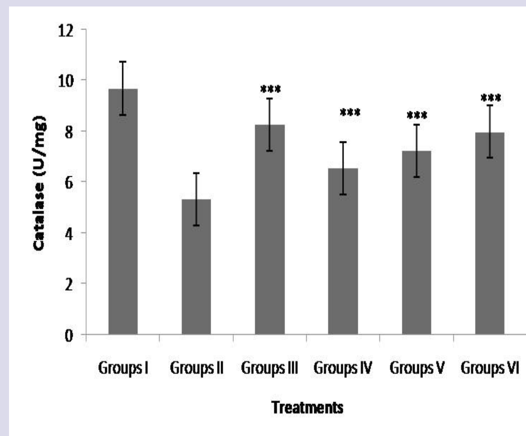

Figure 5: Effect of *Glycyrrhiza glabra* extract administration on catalase levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at *$P< 0.05$; **$P< 0.01$; ***$P< 0.001$.

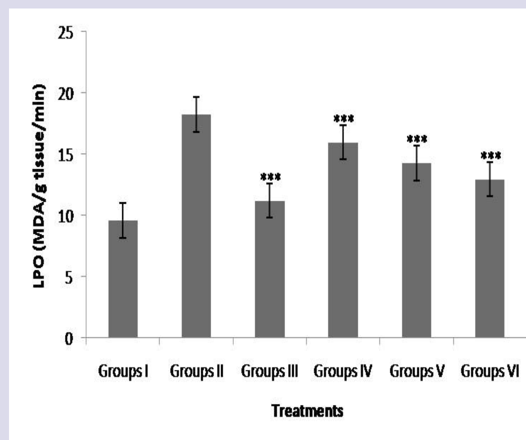

Figure 6: Effect of *Glycyrrhiza glabra* extract administration on LPO levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at *$P< 0.05$; **$P< 0.01$; ***$P< 0.001$.

400 mg/ kg) in the test animals resulted in significant ($p<0.001$) increases in the levels of catalase activity (Figure 5). The administration of silymarin also resulted in the restoration of SOD and catalase levels.

LPO and bilirubin levels

The administration of isoniazid and rifampicin to animals caused an increase in lipid peroxidation due to oxidative stress (Figure 6). However, administration of *G. glabra* extract (100, 200 and 400 mg/ kg) resulted in a decrease in lipid peroxidation. The levels of bilirubin were also restored due to resveratrol (Group IV-VI) administration (Figure 7).

DISCUSSION

The present study was undertaken to evaluate the protective effect of *G. glabra* extract over isoniazid-rifampicin induced hepatotoxicity in rats. In the present study, aqueous extract of *Glycyrrhiza glabra* was administered at doses of 100, 200 and 400 mg/ kg to experimental animals. Administration of isoniazid and rifampicin to rats caused a significant increase in serum AST, ALT and ALP levels. There was also alteration in the levels of SOD and catalase.

ALT and AST are the key enzymes which aid the transfer of α-amino groups from alanine and aspartate to the α-keto group of ketoglutaric acid and produce pyruvic acid and oxalacetic acid respectively. These metabolites are intermediates in the Krebs cycle. The free radicals pro-

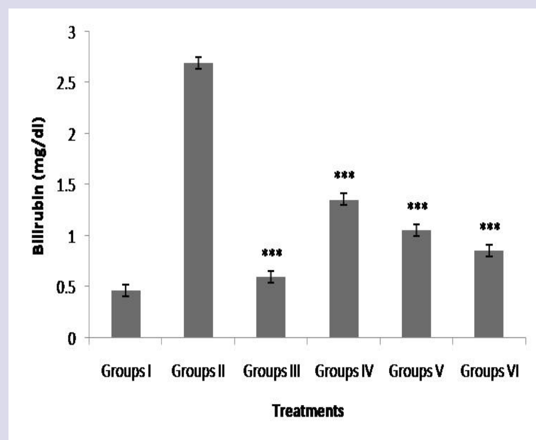

Figure 7: Effect of *Glycyrrhiza glabra* extract administration on bilirubin levels in Isoniazid-Rifampicin treated rats. Results are given as mean ± SEM of six animals in each group. Group II compared with rest of the treated groups.
Significance at **P*< 0.05; ***P*< 0.01; ****P*< 0.001.

duced as a result of metabolism of xenobiotics are responsible for damage of hepatocyte membrane and thus lead to cell death. Such type of damage results in the release of AST and ALT into the blood stream (which are naturally present in the hepatocytes). These elevated levels of ALT, AST and ALP indicate hepatocellular damage.[14,15] In the present study, there was an increase in the levels of AST, ALT and ALP due to isoniazid-rifampicin treatment. Conversely, administration of *G. glabra* extract resulted in a significant diminution in the levels of these enzymes in the blood stream. Superoxide dismutase and catalase are antioxidant enzymes which are decreased during oxidative stress. The administration of *G. glabra* extract resulted in the significant restoration of activity for these enzymes, which in turn indicates the antioxidant potential of *G. glabra* extract.[16] Lipid breakdown from the cell membrane demonstrates the harm induced to the cell membrane. The breakdown of phospholipids and cholesterol along with the release of lipids leads to lipid peroxidation. In the present study, administration of *G. glabra* extract to experimental animals resulted in a significant decreases in lipid peroxidation along with restoration of bilirubin. The present study therefore affirms the hepatoprotective effect of *G. glabra*.

CONCLUSION

The outcomes of the present study demonstrate that *Glycyrrhiza glabra* extract may exert beneficial effects against isoniazid-rifampicin induced hepatocellular damage in rats. The administration of *G. glabra* extract resulted in decreased ALT, AST and ALP activities. Furthermore, there was restoration of SOD and catalase activity. A decrease in lipid peroxidation due to *G. glabra* extract was also observed. The present study asserts the protective role of *G. glabra* extract against isoniazid-rifampicin induced hepatotoxicity in rats. However, further investigation on protein expression, cytokine profiling and imaging studies are necessary to elucidate the precise mechanism of action.

CONFLICT OF INTEREST

The authors declares no conflicts of interest.

ABBREVIATIONS

ALT: Alanine aminotransferase; **AST:** Aspartate aminotransferase; **ALP:** Alkaline Phosphatase; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **LPO:** Lipid Peroxidation.

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PICTORIAL ABSTRACT

SUMMARY

The administration of *G. glabra* extract re-sulted in decreased ALT, AST and ALP activities. Furthermore, there was restoration of SOD and catalase activity. A decrease in lipid peroxidation due to *G. glabra* extract was also observed. The present study asserts the protective role of *G. glabra* extract against isoniazid-rifampicin induced hepatotoxicity in rats.

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