

Protective activity of *Glycyrrhiza glabra* Linn. on carbon tetrachloride-induced peroxidative damage

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ABSTRACT

Objective: To evaluate the potential efficacy of *Glycyrrhiza glabra* Linn. (Fabaceae) in protecting tissues from peroxidative damage in CCl₄-intoxicated rats.

Material and Methods: Peroxidative hepatic damage in rats was studied by assessing parameters such as thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), superoxidedismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px) and glutathione (GSH) in liver and kidneys. The effect of co-administration of *G. glabra* on the above parameters and histopathological findings of the liver in experimental animals was studied.

Results: The increased lipid peroxide formation in the tissues of CCl₄-treated rats was significantly inhibited by *G. glabra*. The observed decreased antioxidant enzyme activities of SOD, CAT, GSH-Px, GST, and antioxidant concentration of glutathione were nearly normalized by *G. glabra* treatment. Carbon tetrachloride-induced damage produces alteration in the antioxidant status of the tissues, which is manifested by abnormal histopathology. *G. glabra* restored all these changes.

Conclusion: *Glycyrrhiza glabra* is a potential antioxidant and attenuates the hepatotoxic effect of CCl₄.

KEY WORDS: Lipid peroxidation, hepatotoxicity, antioxidants

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Introduction

The liver is an organ of paramount importance, which plays an essential role in the metabolism of foreign compounds entering the body. Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment. In addition, human beings consume a lot of synthetic drugs during diseased conditions which are alien to body organs. All these compounds produce a variety of toxic manifestations.¹ Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety.

India is well known for a plethora of medicinal plants. The medicinal use of many plants (as hepatoprotectants) like *Andrographis paniculata*, *Azadirachta indica*, *Cassia fistula*, *Elephantopus scaber*, *Hibiscus rosasinensis*, *Phyllanthus debilis*,

Picrorrhiza kurroa has been reported in the literature.²⁻³

Glycyrrhiza glabra Linn. of the family Fabaceae is a tall perennial undershrub. Its underground stems and roots are used medicinally. Its hypocholesterolaemic and hypoglycemic activities have been reported.⁴ It is known in the traditional system of medicine for its use in liver diseases. It is a major component of many antihepatotoxic polyherbal formulations.⁵ Isoflavan derivatives glabridin, hisplaglabridin A, hisplaglabridin B and 4' O-methyl glabridin have been isolated from *G. glabra*. These chemicals were reported to provide protection against oxidative stress.⁶ The biochemical damage produced by active oxygen species and free radicals has emerged as a fundamental pathway of liver injury. Despite the use of *G. glabra* in liver disorders, no systematic studies on its active oxygen scavenging properties have been reported. In this communication, we present the antiperoxidative effect of *G. glabra* on CCl₄-induced oxidative damage in rats, supported by histopathological evidence.

Material and Methods

Male albino rats of Sprague-Dawley strain, weighing between 120 g to 150 g were used for the experiment. They were housed in polypropylene cages under standard conditions (23±2°C, humidity 60-70%, 12 h light/dark cycles) and given standard pellet diet (M/s Hindustan Lever Ltd, Mumbai, India). Water was given *ad libitum*. The animals were divided into 3 groups of 6 rats each. Group I served as pair-fed control which received the normal feed. Groups II and III received a dose of 0.3 ml CCl₄ in liquid paraffin (3:1, v/v) per 100 g body weight subcutaneously twice a week for a period of two months. Group III rats, in addition to CCl₄ received a dose of 1000 mg/kg body weight/ day of *G. glabra* root powder mixed with the feed for two months. The concentration of the powder in the feed was adjusted to the amount of food consumed. The dose of the medicinal plant was ascertained by a pilot study over a range of dosages varying from 500 mg/kg, body weight to 1500 mg/kg, body weight/day. Over the range of dosages studied, the plant did not show any toxicity.

Glycyrrhiza glabra roots were collected from the crude drug market, Pala, Kottayam district, Kerala. They were dried overnight at 45°C and powdered. This preparation was used for the experiment.

At the end of the experimental period, the animals were killed by decapitation. The liver and kidney were dissected out immediately and transferred into ice-cold physiological saline for various biochemical estimations.

Sections of liver tissues were collected in 10% formol saline for proper fixation. Slices of fixed tissues were processed, embedded in paraffin, sectioned to a thickness of 5 mm, mounted on glass slides, and stained with hematoxylin and eosin for histopathological evaluation.

A 10% tissue (liver and kidney) homogenate was prepared using tris-HCl buffer (0.1M; pH 7.5) and used for the analysis. Lipid peroxidation was assessed in terms of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD).^{7,8} Changes in the antioxidant status were determined by estimating the activities of catalase, superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione (GSH) and glutathione peroxidase (GSH-Px) in the liver and kidney.⁹⁻¹³ The protein content of the tissues was estimated by the method of Lowry *et al.*¹⁴ The results expressed as percent hepatoprotective activity (H) were calculated by the formula,

$H = [1 - (HC - N) / (C - N)] \times 100$ where HC, C and N are the parameters measured in herbal preparation + CCl₄ treated rats, CCl₄ treated rats and normal (pair-fed) control rats, respectively.

Statistical analysis

The results are presented as the mean ± SEM. One-way analysis of variance (ANOVA) followed by the Bonferroni test were applied for statistical analysis with the level of significance set at $P < 0.05$.

Results

Feeding CCl₄ to rats for two months resulted in significant loss of body weight. Treatment with the medicinal plants along with CCl₄ prevented the loss in body weight.

There was a significant increase in the concentrations of TBARS and CD during CCl₄ treatment as compared with the pair-fed control. Administration of *G. glabra* together with CCl₄ resulted in significant decrease of TBARS and CD in the liver and kidney compared with the corresponding CCl₄-intoxicated group (Table 1).

Table 1

Effect of *G. glabra* on the level of TBARS, CD, GSH and the activities of SOD, CAT, and GSH-Px in the liver and kidney

Parameter	Tissue	Group I Pair-fed control	Group II CCl ₄ -treated	Group III CCl ₄ + <i>G. glabra</i>	% Hepato protection	F df=2,15	P
TBARS	Liver	1.32 ± 0.03	1.6 ± 0.66*	1.34 ± 0.06 [®]	92.86	6.13	<0.05
	Kidney	1.27 ± 0.06	1.72 ± 0.06*	1.32 ± 0.06 [®]	88.89	5.2	<0.05
CD	Liver	63.28 ± 1.55	81.50 ± 2.82*	65.70 ± 2.96 [®]	86.72	5.21	<0.05
	Kidney	18.20 ± 0.45	22.02 ± 0.76*	18.72 ± 0.84 [®]	86.39	4.1	<0.05
SOD [®]	Liver	5.62 ± 0.14	4.39 ± 0.15*	5.53 ± 0.25 [®]	92.68	5.2	<0.05
	Kidney	5.48 ± 0.14	4.41 ± 0.15*	5.31 ± 0.24 [®]	84.11	5.9	<0.05
CAT [®]	Liver	212.52 ± 5.31	101.72 ± 3.53*	199.17 ± 8.96 [®]	86.65	4.8	<0.05
	Kidney	214.35 ± 5.33	181.23 ± 6.34*	210.06 ± 9.46 [®]	87.05	5.2	<0.05
GST	Liver	0.32 ± 0.01	0.24 ± 0.01*	0.31 ± 0.01 [®]	87.5	5.9	<0.05
	Kidney	0.32 ± 0.01	0.19 ± 0.01*	0.32 ± 0.01 [®]	81.23	6.1	<0.05
GSH-Px [®]	Liver	9.71 ± 0.24	6.51 ± 0.23*	9.45 ± 0.41 [®]	91.88	6.3	<0.05
	Kidney	7.81 ± 0.20	5.19 ± 0.18*	7.60 ± 0.34 [®]	91.98	5.9	<0.05
GSH	Liver	148.70 ± 3.81	110.93 ± 4.04*	143.31 ± 6.47 [®]	85.73	5.73	<0.05
	Kidney	132.56 ± 3.31	109.76 ± 3.89*	129.15 ± 5.89 [®]	85.09	4.2	<0.05

* $P < 0.05$ as compared to Group I.

[®] $P < 0.05$ as compared to Group II.

Values are mean±SEM of 6 animals in each group. Values expressed as mM/100 g tissues for TBARS and CD, units/mg protein for SOD, CAT, GST, GSH-Px and mg/100 g tissue for GSH.

[®]One unit of enzyme activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in 1 min.

[®]mmol of H₂O₂ consumed per minute.

[®]mg of glutathione consumed per minute.

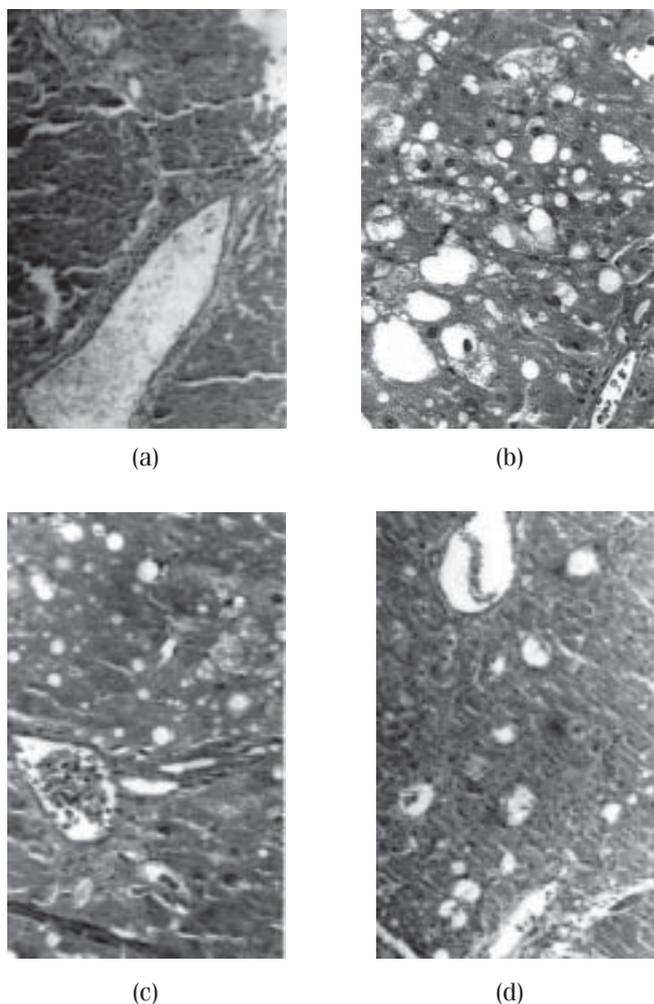


Figure 1: Photomicrographs of liver sections taken from a: Normal control rat showing central vein and portal triad surrounded by normal hepatocytes (H&E, x100); b: CCl₄ liquid paraffin treated rats showing portal triad with moderate fibrosis, inflammation and hepatocytes with fatty change and ballooning degeneration (H&E, x200); c: CCl₄ liquid paraffin treated rat showing dilated central vein (H&E, x200); d: CCl₄ liquid paraffin and *G. glabra* treated rat showing central vein and portal triad with normal hepatic cells (H&E, x100).

The activities of SOD, catalase, GST and GSH-Px in the tissues studied were significantly decreased in the CCl₄-treated rats compared with pair-fed control. There was also a decrease in the content of GSH in the tissues of Group II rats. Administration of *G. glabra* along with CCl₄ restored the activities of the above antioxidant enzymes and the level of glutathione to near normal compared to the corresponding CCl₄ administered rats.

Histopathological studies (compared to controls) demonstrated fatty change and ballooning degeneration of hepatocytes induced by CCl₄-liquid paraffin. The liver also showed distorted architecture with nodule formation, distorted central vein and the portal triad showed fibrous portal expansion with moderate fibrosis and moderate inflammation (Figures 1, a-c). Administration of the root powder of *G. glabra* at a dose of 1000 mg/kg, body weight exhibited significant im-

provement (Figure 1d).

As a part of the pilot study, we also evaluated the effect of *G. glabra* on normal rats. Over the range of dosages studied, the plant did not show any alteration of the antioxidant defense and on liver function tests.

Discussion

The reactive metabolites such as trichloromethyl (CCl₃) and trichloromethyl peroxy (CCl₃OO) radicals emanated from CCl₄ initiate peroxidation of membrane unsaturated fatty acids. This lipid peroxidation of membrane seriously impairs its function and produces liver injury.

The antioxidant enzymes SOD, catalase and peroxidases constitute a mutually supportive team of defense against reactive oxygen species (ROS).¹⁵⁻¹⁶ The decrease in the activity of SOD in the liver and kidney of CCl₄-treated rats may be due to the increased lipid peroxidation or inactivation of the enzyme by cross-linking with malondialdehyde. This will cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. GST binds to lipophilic compounds and acts as an enzyme for GSH conjugation reactions.³ The decrease in the activity of GST during CCl₄ toxicity may be due to the decreased availability of GSH and suggests a total inhibition of drug metabolism during CCl₄-intoxication.

Depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH consumption.³ The medicinal herb-treated rats restored the changes in the activity of the antioxidant enzymes and the level of glutathione.

Hepatotoxins develop hypoxic conditions which can damage the perivenular zone of the hepatic acinus. The highest expression of Cytochrome P450 2E1 (CYP2E1) in the perivenular region produces oxy-radicals that contribute to the injury. Moreover, hepatocytes in the perivenular area contain less antioxidant factors and antioxidant enzymes.¹⁷ Thus, while the lipid peroxidation mediated by oxy radicals is likely to be the highest in the perivenular area, the detoxifying capacity of the hepatocytes here is reduced, therefore, the production may exceed the detoxification in the perivenular area.

In short, CCl₄-induced damage produces alteration in the antioxidant status of the tissues, which is manifested as an abnormal histopathology. *G. glabra* restored all these changes. So, it can be concluded that the herb is a potential antioxidant and attenuates the hepatotoxic effect of CCl₄ by acting as an *in vivo* antioxidant and thereby inhibiting the initiation and promotion of lipid peroxidation or by an accelerated scavenging of free radicals and their products by conjugation with GSH aided by GST.

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