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Research Article

In vivo antioxidant and hepatoprotective potential of *Glycyrrhiza glabra* extract on carbon tetra chloride (CCl_4) induced oxidative-stress mediated hepatotoxicity

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ABSTRACT

Objective: This study aimed to evaluate the antioxidant and hepatoprotective potential of *Glycyrrhiza glabra* hydromethanolic root extract against carbon tetra chloride (CCl_4) induced oxidative-stress mediated hepatotoxicity in liver tissue of *Swiss albino* mice.
Background: Medicinal plants play a vital role for the development of new drugs. *Glycyrrhiza glabra* is a widely used medicinal plant. It has many phyto-constituents and active components, which can be used for many diseases.
Methods: For the antioxidant and hepatoprotective study, measurement of GSH, CAT, LPO bio-markers in Liver tissue of *Swiss albino* mice were taken. The animals were divided in six different groups each having 4 mice. The requisite dose of CCl_4 was dissolved in appropriate solvent (1.5ml/kg body wt) and administrated as single i.p. dose per mice after 6 hr of last treatment of extract to the animals in each group. Mice were received orally administration of extract up to 7 days. Positive Control group received single i.p. injection of 1.5ml/kg body wt CCl_4 in 0.9% saline.
Results: The results suggest that, the crude extract of root of *G. glabra* at the doses of 300 and 600mg/kg body wt. expressed significant hepatoprotective potential against CCl_4 induced oxidative stress mediated hepatotoxicity in student 't' test ($p<0.05$) at dose dependent manner in the Liver tissue of *Swiss albino* mice. *G. glabra* root extract alone has not induced hepatotoxicity.
Conclusion: Based on this study, It may be concluded that *Glycyrrhiza glabra* root extract possess hepatoprotective potential in *Swiss albino* mice.

Keywords: Antioxidant, CCl_4, Catalase, Glutathione, *Glycyrrhiza glabra*, Hepatotoxicity, Intraperitoneal, Liver, Oxidative stress

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are triterpenoid, saponin, flavonoids, tannins, alkaloids, phenolic compounds.[1] In the traditional system of medicine, there are number of medicinal plants which are used in the treatment of liver disorders. Their extracts, fractions and active components exhibit marked hepatoprotective action, which has been related to their antioxidant properties.[2]

Reactive oxygen species (ROS) and free radicals contribute to cellular aging, possibly through the destabilization of membranes in cells. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Free radicals and ROS are highly reactive molecules involved in many physiological processes and human

diseases, such as, Cancer, Aging, Arthritis, Parkinson's syndrome, ischemia and Liver injury. The elevation of free radicals seen during the lever damages showing to enhanced production of free radicals and decreased scavenging potential of cells. The main characteristic of an antioxidant is its ability to trap free radicals. During evolution, the organisms have developed an antioxidant defence system to cope with oxidative stress. A variety of intrinsic antioxidants (Proteins, Reduced glutathione, Superoxide dismutase, Catalase and Peroxidase) are present in the organisms, which protect them from oxidative stress, thereby forming a first line of defence.[3]

Glycyrrhiza glabra Linn (Family: *Papilionaceae/ Fabaceae*) is an old age plant used in traditional medicine across the globe for its ethano-pharmacological value to cure varieties of ailments. *Glycyrrhiza glabra* is known as mulaithi in north India. The root and rhizome of *G. glabra* have been widely used in medicines for its unique and diverse pharmacological properties viz., antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, immuno-stimulant, anti-allergenic etc.[4,5] Traditionally the plant has been recommended as a prophylaxis for gastric and duodenal ulcers and dyspepsia as an anti-inflammatory agent during allergenic reactions.[6]

METHODS

Plant Collection and Identification

The root of *Glycyrrhiza glabra* were procured from Bhopal (Madhya Pradesh), India and authenticated by Botanist, Dr. Zia Ul Hasan (Voucher Specimen No: 441/BOT/Safia/13), Prof. & Head, Dept. of Botany, Safia Science College, Bhopal, Madhya Pradesh (India).

Chemicals

Carbon tetra chloride, Vitamin 'C', Phasphate Buffer Saline, 10% TCA, 1.0% TBA, n-butanol, H_2O_2 (0.2M), Potassium dichromate, Glacial Metaphosphoric acid, EDTA, NaCl, DTNB (5, 5'-Dithio-bis 2 Nitrobenzoic acid), Sodium citrate.

Preparation of Glycyrrhiza glabra Root Extract

The collected root were dried in shade and grinded with mechanical grinder. About 30g powder was filled in separating funnel with 50% methanol for 48hrs. The collected residues kept at 55-60°C in Boiling Water Bath to concentrate it and finally transfer into the Hot Air Oven to dry it. About 5.8gm powder of crude extract was obtained (Yield= 19%) and used for the further studies.

Experimental Animals

Random bred of male *Swiss albino* mice (7- 8 weeks old), weighing 23 ± 2 gm body wt. obtained from the animal colony of our Research Centre were used for the experiments. Experimental animals were handled according to the Institutional Legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. These animals were housed in polypropylene cages in the animal house at temperatures of 22 ± 1.5^0C and 12 hours light and dark cycle. The animals were provided with standard pallet diet (From Golden feed Ltd., New Delhi, India) and water *ad libitum.*

Experimental design

Assessment of Hepatoprotective Activity

The *In vivo* anti-oxidant activity test was performed biochemically method followed by Jain *et al.,* (2012).[7] The animals were divided in six different groups each having 4 mice. The requisite dose of CCl_4 was dissolved in appropriate solvent (1.5ml/kg body wt) and administrated as single i.p. dose per mice after 6 hr of last treatment of extract to the animals in each group. Mice were received orally administration of extract up to 7 days. Positive Control group received single i.p. injection of 1.5ml/kg body wt CCl_4 in 0.9% saline.

The *G. glabra* root extract was prepared in stock according to the toxicity dose was observed. The extract was administered in 2 different doses and one group i.e. standard was received Vitamin 'C' (Celin). The experimental groups are as follows:

Experimental Groups

- Group I (Vehicle alone): DDW daily *via* oral route.
- Group II (Positive control): Single i.p. of CCl_4 1.5ml/kg body weight.
- Group III (*G. glabra* extract alone): Orally administered 300mg/kg body wt. *G. glabra* extract once a day up to 7days.
- Group IV (*G. glabra* extract + Vitamin C): 100mg/kg bwt of Vitamin C was orally given (dissolved in DDW) up to 7 days and single i.p. of CCl_4 1.5ml/kg body wt. 6 hr after the last treatment.
- Group V (*G. glabra* extract + CCl_4): 300mg/kg bwt of *Glycyrrhiza glabra* hydromethanolic root extract up to 7days and single i.p. of CCl_4 1.5ml/kg body wt. 6 hr after the last treatment.
- Group VI (*G. glabra* extract+CCl_4): 600mg/kg bwt of *Glycyrrhiza glabra* hydromethanolic root extract up to 7days and single i.p. of CCl_4 1.5ml/kg body wt. 6 hr after the last treatment.

Biochemical characterization

Procedure for estimation of Oxidative Stress Markers

After 24 h of the last dose, all the animals were then sacrificed and liver tissues were collected for the

evaluation of *in vivo* antioxidant and other studies. For histopathological study, a portion of liver tissue from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique.[8]

Tissue sample preparation for LPO, GSH, and Catalase assay

1g of liver tissue was collected from each experimental mouse, washed in normal saline and soaked in filter paper. 10%w/v tissue homogenate was prepared by mincing and homogenizing the tissues in 0.1 M phosphate buffer (pH 7.4). After centrifugation at 1000 rpm for10 minutes, the clear supernatant was used for the estimation of non-enzymatic and enzymatic antioxidants.

Estimation of Lipid peroxides (LPO)

Lipid per-oxidation (LPO) was assayed according to the method of Ohkawa *et al.,* (1979).[9] The levels of lipid peroxides were expressed as nM of MDA/mg wet tissue using extinction co-efficient of 1.56 x105 M^{-1} cm^{-1}.

Estimation of Glutathione (GSH)

This method was performed as per the method was reported by Owens *et al.,* (1995).[10] The absorbance was taken at 412 nm using UV-Spectrophotometer. Level of Reduced Glutathione was expressed as µmol/gm.

Estimation of Catalase (CAT)

The Catalase was assayed by the method of Aebi, (1984).[11] The level of Catalase was expressed as µmole/min/mg of protein using extinction coefficient of 0.0436 Millimolar of hydrogen peroxide at 240 nm (cm^2/µmol).

Statistical analysis

The experimental results were expressed as Mean ± SEM. Data were assessed followed by student't'-test. P<0.05 was considered as statistically significant.

RESULTS

The present study was carried out to evaluate, Carbon tetrachloride (CCl_4) induced hepatotoxicity, for the study of *in vivo* antioxidant effect of *G. glabra* hydromethanolic root extract using GSH, LPO and CAT biochemical parameters. The results showed that, the mice which received *G. glabra* root extract at the doses of 300 and 600mg/kg bwt *via* oral rout up to 7days and single i.p. administration of CCl_4 6 hr after the last treatment, a reduction was observed in LPO level in Liver tissue of mice which was increased at higher level in CCl_4 control group. Treatment groups showed statistically significant when compared with the hepatotoxic control group and Vitamin 'C', which is a potent antioxidant. The hepatoprotection % in standard Vitamin 'C' group was found to be 76.82% and in *G. glabra* extract groups was 46.94% and 60.21%. In similar study, an enhancement was observed in GSH and CAT level in Liver tissue of mice which was decreased in hepatotoxic (CCl_4) control group. The treated groups showed statistically significant when compared with the hepatotoxic control and Vitamin 'C' group. The hepatoprotection % of GSH in standard Vitamin 'C' group and *G. glabra* extract groups were found to be 65.51%, 12.07% and 30.36% respectively. The hepatoprotection % of CAT in standard Vitamin 'C' group and *G. glabra* extract groups were found to be 70.65% and 21.52% and 35.46% respectively. Results showed treatment with the *G. glabra* hydromethanolic crude extract recovered the injured Liver and has antioxidant potential against CCl_4 intoxicated mice. Results are furnished in Table 1.

Table 1: The effect of *Glycyrrhiza glabra* hydro-methanolic root extract on the level of Glutathione (GSH), Catalase (CAT) and Lipid Peroxide (LPO) in liver homogenate of *Swiss albino* mice.

S. No.	Groups	Treatment Doses (mg/kg bwt)	Level of Biochemical Parameters in Mean ± SEM			Hepatoprotection %		
			LPO (MDA content in nmol/ mg)	GSH (µmol /gm)	CAT (µmol/min /mg)	LPO	GSH	CAT
1.	I (n=4)	Vehicle alone (DDW)	4.47±0.45	62.57±3.29	14.51±0.96	-	-	-
2.	II (n=4)	CCl_4 alone 1.5ml/kg bwt (Positive control)	33.47±3.25	47.32±3.50	2.89±0.32	-	-	-
3.	III (n=4)	CCl_4 1.5ml/kg bwt + Vitamin 'C' 100mg/kg bwt	11.19±0.22	57.31±4.18	11.10±1.67	76.65	65.51	70.65

4.	IV (n=4)	CCl_4 1.5ml/kg bwt + *G. glabra* root extract 300mg/kg bwt	19.86±1.82	49.16±5.77	5.39±1.16*	46.94	30.36	35.46
5.	V (n=4)	CCl_4 1.5ml/kg bwt + *G. glabra* root extract 600mg/kg bwt	16.01±4.90*	51.95±2.39*	7.01±0.43*	60.21	12.70	21.52
6.	VI (n=4)	*G. glabra* root extract alone 300mg/kg bwt	4.21±0.38	62.45±3.45	13.24±1.80	-	-	-

(*) denotes statistical significance as compared to hepatotoxic control at p<0.05 followed by Student "t" test.

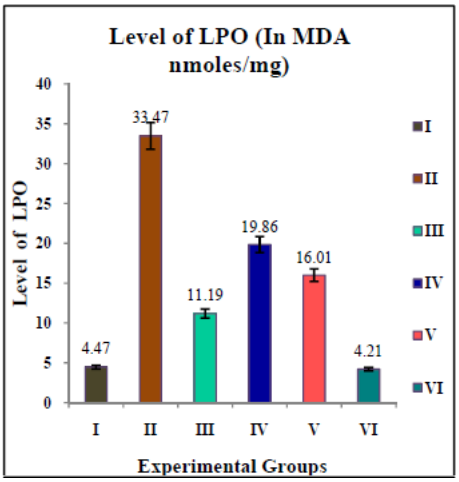

Figure 1: Showing effect of *G. glabra* root extract on LPO level in liver tissue.

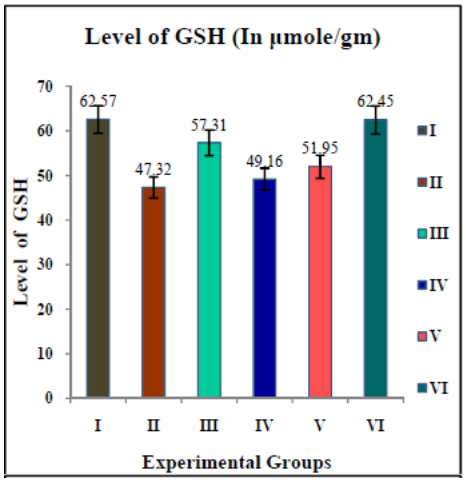

Figure 2: Showing effect of *G. glabra* root extract on GSH level of liver tissue.

Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein, whereas that of CCl_4 intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein and apoptosis. The treatment with Vitamin 'C' which is standard antioxidant returned the injured liver to quite normal. Treatment with the hydromethanolic root extract of *G. glabra* exhibited significant recovery at dose dependent manner.

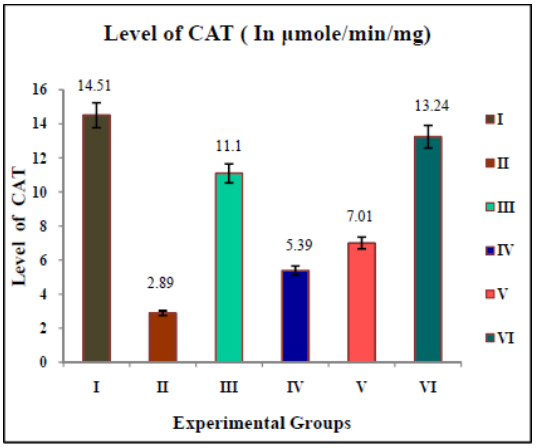

Figure 3: Showing effect of *G. glabra* root extract on CAT level in liver tissue.

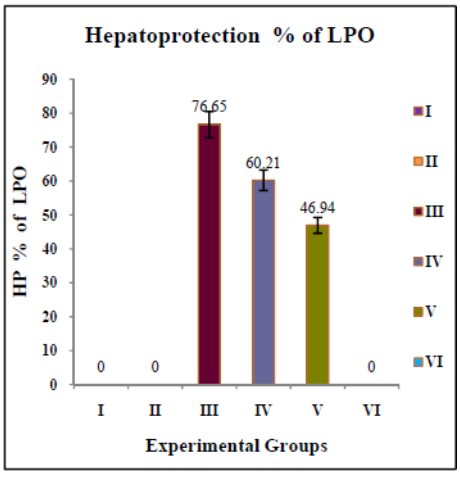

Figure 4: Showing hepatoprotective effect of *G. glabra* root extract on GSH level in liver tissue.

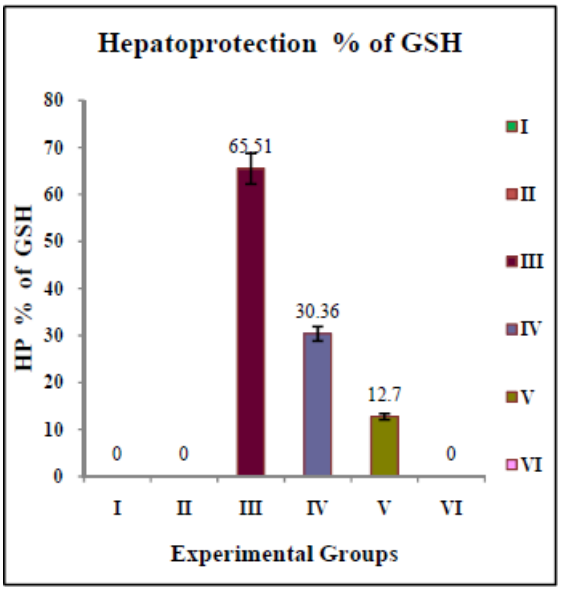

Figure 5: Showing hepatoprotective effect of *G. glabra* root extract on LPO level in liver tissue.

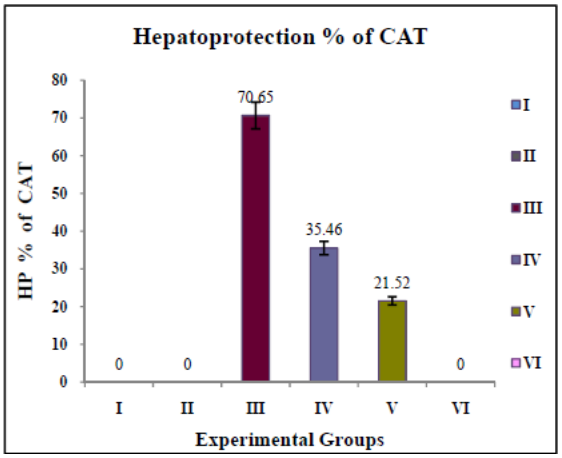

Figure 6: Showing hepatoprotective effect of *G. glabra* root extract on CAT level in Liver tissue.

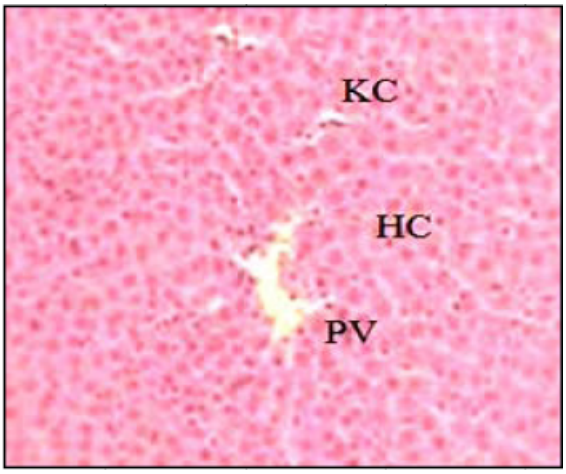

Figure 7: Micros view of liver tissue of normal mice.

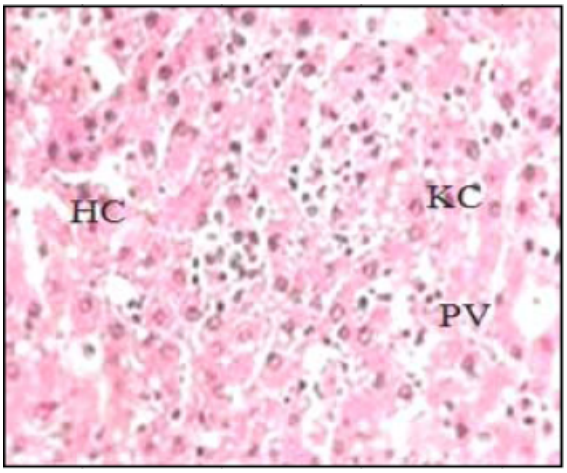

Figure 8: Micros view of liver tissue of CCl_4 induced hepatotoxic mice.

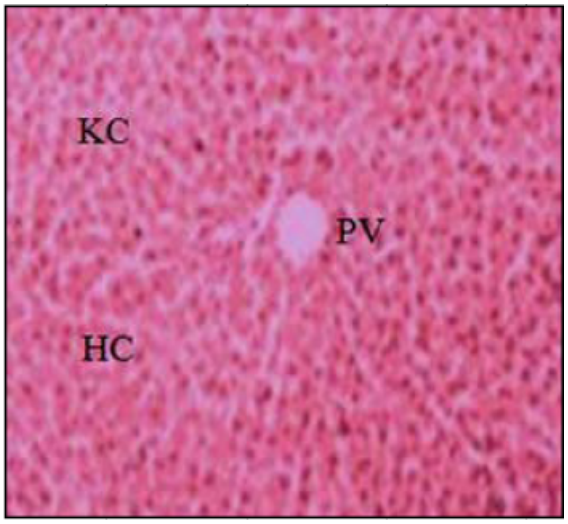

Figure 9: Micros view of liver tissue standard antioxidant Vitamin 'C'.

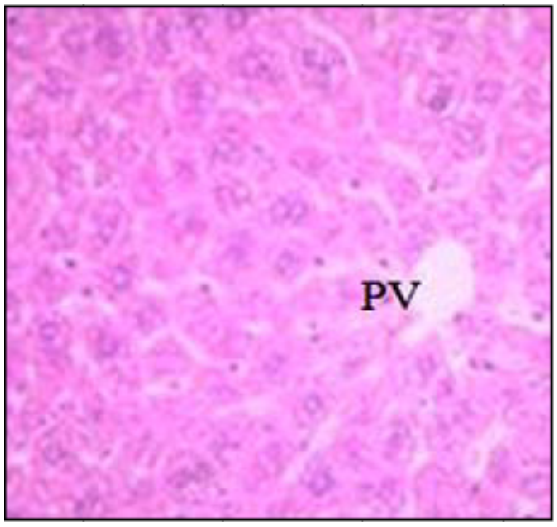

Figure 10: Micros view of liver tissue of hydromethanolic root extract of *G. glabra*.

DISCUSSION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction.[12] The major functions of liver are Carbohydrate, Protein, Fat metabolism, Detoxification, Secretion of bile and storage of Vitamins. When mice were treated with carbon tetrachloride it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidize in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid per-oxidation. This result in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury.[13] Vitamin C is an excellent source of electrons; therefore, it can donate electrons to free radicals such as hydroxyl and superoxide radicals to quench their reactivity.[14] Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, and anticancer activities. As per phytochemical demonstrations, *G. glabra* hydromethanolic root extract was found rich in flavonoids, saponins, terpenoids, glycosides, etc.[15] Literature reveals that, the carbonyl groups present in the flavonoids and phenolic compounds were responsible for antioxidant activity.[7] The presence of Flavonoids, saponins, terpenoids and many other phytoconstituents may be stimulates hepatoprotective activity of *G. glabra* crude extract. The protection of liver cells against toxic materials including drugs, lipid peroxidation, and free radical injury may decrease inflammation.[16]

CONCLUSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer, hepatic damage etc. In most of the developing countries, the incidence of viral hepatitis is more. So, the investigation for an efficient hepatoprotective drug from the natural resource is an urgent necessity. The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. CCl_4 is therefore a useful tool for the induction of hepatic damage in experimental animals. The ability of hepatoprotectivity of *G. glabra* hydromethanolic root extract to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effects. In conclusion, the results of the present study suggest that the *G. glabra* extract possess hepatoprotective activity against CCl_4 intoxication in *Swiss albino* mice. According to these results it may be concluded that *G. glabra* may be possess significant protective effect against hepatotoxicity induced by CCl_4 which may be attributed to the individual or combined action of phytoconstituents present in it. Further studies are however needed to isolate and characterize the active principles responsible for hepatoprotective activity.

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