Original Research Article

An Assessment of Hepatoprotective Activity of Gl*ycyrrhiza glabra* Root Extract against Hepatic Injured Rodent Model

Abstract

Hepatotoxicity, or liver damage, is caused by hepatotoxins, which can originate from chemicals, dietary supplements, prescription drugs, and medicinal plants. Nowadays, medicinal and aromatic plants are excellent resources for creating novel drugs and curing both psychological and physical illnesses. Given this, licorice, a perennial herb, rich in properties of potential health advantages have been investigated with CCl_4 induced hepatic injury on the rodent model to assess its hepatoprotective potential. The study results demonstrated an altered hepatic condition with reduced CCl_4 concentration in the liver. The study results show a significant ($p<0.05$) decrease only at high dosages in SGPT, creatinine level, and total body cholesterol whereas, for low and medium doses, non-significant alleviations were noticed in a dose-dependent manner when compared with the negative control group. Besides, SGOT, urea, LDL, and triglyceride levels were observed with a dose-dependent and non-significant decrease compared to the negative control group. Consequently, more research is required to properly use licorice (*Glycyrrhiza glabra*) as a less hazardous and more effective substitute for synthetic medications in the field of hepatotoxic therapy, given that it has been found to have potential hepatoprotective properties against hepatotoxicity in this study.

Keywords: *Glycyrrhiza glabra,* hepatotoxins, hepatoprotective potential, medicinal plants, synthetic medications

Introduction:

Hepatotoxins, which can originate from chemicals, dietary supplements, prescription medications, and medicinal plants, are the cause of hepatotoxicity, or liver injury. It may result in liver failure in extreme circumstances and decreased liver function. [1] According to statistical studies, liver illness (cirrhosis, viral hepatitis, and liver cancer) is responsible for about two million fatalities each year and 4% of all deaths globally (1 out of every 25 deaths). [2] Similarly, unfinished statistics indicate that over a thousand medications have the potential to induce varying degrees of liver damage. According to estimates, the incidence of liver disease in Western nations ranges from 1 to 20 per 100,000 people. [10]

Detoxification, antioxidant, anti-inflammatory, and hepatocyte membrane protection are the main pharmacologic effects of hepatoprotective medications. [5] Silymarin, a well-known hepatoprotective medication, functions via antioxidant, antiviral, immunomodulatory, antiproliferative, and antifibrotic pathways. [3] Curcumin, another hepatoprotective medication, reduces hepatic steatosis by inhibiting the inflammatory enzyme NF-kB. [4] Furthermore, polyene phosphatidylcholine (PPC), a major bioactive component of significant phospholipids, is essential for maintaining the hepatocyte membrane's fluidity and activity. [6] Moreover, the thiol-containing tripeptide glutathione (GSH) is composed of L-glutamate, cysteine, and glycine. In the human body, glutathione (GSH) is an essential antioxidant that eliminates free radicals and inhibits dangerous electrophilic xenobiotics. [7,8,9] Although these medications have hepatoprotective properties, their long-term usage can have adverse effects such as gastrointestinal symptoms like nausea and vomiting, abdominal pain or diarrhea, allergic reaction, elevated blood pressure, and low potassium levels. [10]

Aromatic and medicinal plants are great sources for developing new medications and healing physical and mental ailments. Since medicinal drugs derived from medicinal plants exert numerous pharmacological and physiological actions inside live cells due to their numerous constituents and offer options for genetic modification, they may be free of side effects. In contrast, drug metabolites derived from synthetic drugs have fewer therapeutic benefits and adverse side effects. [11,15] Previous studies have shown that hepatoprotective effects can be achieved in the treatment of hepatotoxicity with *Aloe vera* (Aloaceae), *Murraya koenigii (L.) Spreng*, *Telfairia occidentalis* leaves, *Ocimum lamiifolium* leaves, *Crassocephalum vitellinum* leaves medicinal plants. [12,17]

A root extract from a perennial herb, *Glycyrrhiza glabra* from the Fabaceae family of Plantae kingdom, also known as licorice, which is native to South-Western and Central Asia as well as the Mediterranean region, was used in this investigation using a hepatic injury rodent model. [13] Additionally, glycyrrhizic acids (GAs), flavones, liquiritins (LQ), and liquiritigenins (LG) are the main physiologically active ingredients of licorice. Furthermore, a growing body of research indicates that licorice possesses anti-inflammatory, antioxidant, anti-cancer, anti-microbial, antiviral and anti-tumor properties including effects against respiratory diseases. [14,16]

This research aims to investigate the possible hepatoprotective impact in an experimental rat model which would be generated by *Glycyrrhiza glabra (G. glabra)*, CCl_4 as well as their possible hepatotoxic consequences. In this quest, the study itself would be more evident to provide details regarding its effectiveness, safety, affordability, and the availability of safer medication.

The objective of this study is to determine the precise and efficacious hepatoprotective properties of Glycyrrhiza glabra (G. glabra) with negligible or no toxicity. Furthermore, experiments on the pre-clinical and clinical efficacy of the medicinal plant in the human body are required. In addition, more research must be conducted in conjunction with the extraction of the substance from the plant in order to accurately identify the therapeutic component exhibiting hepatoprotective activity and to report a novel finding in the disease management system.

Material and Methods:

Plant Collection and Extract Preparation

Glycyrrhiza glabra fruits were gathered from the Dhaka local market. The Department of Pharmacy of the University of Dhaka acknowledged the content. *Glycyrrhiza glabra's* wet fruit was air-dried and roughly crushed. After that, the fruit powder was extracted in 50% ethanol for 15 days. Three-day intervals were used to filter the extract. In a rotating evaporator, the extracted material was dried under low pressure and temperature. Ultimately, the necessary pharmacological testing was performed on the crude residue.

Drugs and Chemicals

The well-known hepatotoxic chemical carbon tetrachloride (CCl_4) was acquired from a company named Sigma, located in the United States. Livasil 140 mg, a common anti-oxidant drug, was acquired from Incepta Pharmaceuticals Ltd.

Experimental Animal Procurement, Nursing, and Grouping

From Jahangirnagar University in Savar, Dhaka, a total of 100 male rats, weighing between 100 and 120 grams, were obtained. They were all kept at the University of Dhaka's Institute of Nutrition & Food Science (INFS) in a climate-controlled environment with a 12-hour light/dark cycle, temperature of 25±3°C, and relative humidity of 55±5%. They were allowed to drink clean water and fed a standard diet. Before the adaptation investigation, every animal was kept in such a habitat for a minimum of one week. Every experimental procedure complied with the Institutional Animals Ethics Committee's (IEAC) guidelines.

Animal Model Sample Size Detection

Initially, 100 rats weighing between 100 and 120 grams were purchased, and were randomly split into 10 groups of ten rats each. Ten rats were selected at random from ten distinct groups of 100 rats. During mating season, however, we kept a close eye on each rat daily. Our study included both positive and negative control groups.

Dose Selection and Route of Administration for Respective Study

A typical chemical agent used in lab settings to investigate a variety of liver ailments, both acute and chronic, is carbon tetrachloride (CCl_4). The CYP2E1 isozyme-produced CCl_4 metabolite known as trichloromethyl free radical (CCL_3) reacts with proteins and lipids in cells to produce trichloromethyl peroxy radical, which damages lipids on the endoplasmic reticulum membrane more quickly than trichloromethyl free radical and results in lobular necrosis and lipid peroxidation. In all animal groups except the typical control group, a single oral administration of CCl_4 combined with olive oil as a vehicle in a 1:1 ratio (3 ml/kg of rat body weight) resulted in liver damage. Animals with hepatic injuries received P. guajava extracts as a post-treatment. The extract was taken orally in different doses.

Evaluation of Hepato–Protective Activity

Nine groups were formed and the experiments ran for a total of 28 days. First off, there is no medicine in the negative control group. *G. glabra* is utilized as a medicinal plant in different doses (300,600,1200) to evaluate its potential for hepatoprotective function. Treatment groups 4,5, and 6 were compared to the positive control group using one-way ANOVA. The second group was the positive control, or disease control, where we frequently induced CCl4. Group three, on the other hand, is used to assess whether the market medicine is providing the right treatment and whether the environment is appropriate. Moreover, group 7,8,9 ascertains whether any adverse effects exist.

All the rats were chosen randomly and equally split into nine groups for this experiment (Table 1).

Table 1: Application on Treatment Efficacy

Group Number	Group Specifications	Treatment Spicies	Dose Treatment Species (ml/kg)	Abbreviation of Groups
1	Negative Control	Physiological saline	10 ml/kg	N
2	CCl_4 Control	N/A	N/A	A
3	CCl_4 + Silymarin	Silymarin	10	$A+S_{10}$
4	CCl_4 + *G. glabra*	*G. glabra*	300	$A+GG_{300}$
5	CCl_4 + *G. glabra*	*G. glabra*	600	$A+GG_{600}$
6	CCl_4 + *G. glabra*	*G. glabra*	1200	$A+GG_{1200}$
7	*G. glabra*	*G. glabra*	300	GG_{300}
8	*G. glabra*	*G. glabra*	600	GG_{600}
9	*G. glabra*	*G. glabra*	1200	GG_{1200}

We conducted a 7-day pilot research in 9 distinct groups, gradually delivering the medication at different doses of 300, 600, 1200 mg/kg body weight, and found that only a high dose of1200mg/kg exhibited significant therapeutic effect. However, our major trial, which is four times longer than our pilot study, runs for 28 days. Therefore, after being monitored for 28 days, there's a good chance that the *G. glabra* treatment will show potential therapeutic activity at lesser doses as well. Because of this, we utilized low, medium, and high dosages (300, 600, and 12,000 mg/kg) in the seven-day pilot trial.

Statistical analysis:

With regard to numerical parameters, all of our findings (raw data) were documented and examined on a broadsheet utilizing the MS Excel program. Descriptive statistics were applied to the collected data, and the results were presented as mean SD. We interpreted inter-group heterogenicity in terms of many biological parameters using the "One-way Anova test" feature of the SPSS 16 software to assess statistical significance. The statistical significance of the occurrences is established by the fact that the 'p' value was less than 0.05 ($p<0.05$).

Results and Discussions:

Table 2: Rats' liver function tests (SGPT and SGOT) following medication and *Glycyrrhiza glabra* extract administration.

Group Number	Abbreviation of Groups	SGPT	SGOT
1	CCl_4 Control	37.21±4.56	41.21±3.47
2	CCl_4 + Silymarin	109.22±9.24	116.23±11.43
3	CCl_4 + *G. glabra*	58.34±8.24	54.48±7.44
4	CCl_4 + *G. glabra*	106.52±8.63	112.24±10.46
5	CCl_4 + *G. glabra*	103.21±8.89	107.51±9.43
6	*G. glabra*	*****99.31±7.46	101.23±8.62
7	*G. glabra*	40.22±6.23	38.63±6.41
8	*G. glabra*	36.15±5.23	40.42±5.93
9	CCl_4 Control	36.93±3.71	37.31±4.27

It was found that there was a decrease in the levels of SGPT and SGOT in all three groups (low, medium, and high) in dose dependent manner. *Glycyrrhiza glabra* extract only causes significant reduction ($p<0.05$) in high dose in case of SGPT; low and medium doses resulted in non-significant ($p>0.05$) decreases. Similarly, the SGOT levels also decline in non-significant ($p>0.05$) approach for low and medium dose. However, high dose exhibits significant reduction in elevated SGPT Level with potential hepatoprotective actitviy.

Table 3: Kidney functioning tests (Creatinine and Urea) of rat after administration of drug and extract of *Glycyrrhiza glabra*.

Group Number	Abbreviation of Groups	Creatinine	Urea
1	CCl_4 Control	0.54±0.24	38.26±2..64

2	CCl_4 + Silymarin	2.94±0.79	109.34±9.32
3	CCl_4 + *G. glabra*	1.37±0.48	55.50±5.36
4	CCl_4 + *G. glabra*	2.73±0.67	107.23±6.37
5	CCl_4 + *G. glabra*	*2.13±0.94	104.93±5.82
6	*G. glabra*	**1.69±0.82	102.91±8.81
7	*G. glabra*	0.73±0.38	36.24±3.24
8	*G. glabra*	0.88±0.46	37.47±5.16
9	CCl_4 Control	0.83±0.39	35.86±4.26

Creatinine levels dropped in a manner that was statistically significant ($p<0.05$) both for medium and high dosages giving maximum therapeutic activity. Moreover, creatinine levels dropped in the case of low dose, although the drop was not statistically significant ($p>0.05$). However, all three doses—low, medium, and high—showed a non-significant ($p>0.05$) drop in urea level.

Table 4: Rat lipid profile (total cholesterol, HDL, LDL, and triglycerides) following medication administration and *Glycyrrhiza glabra* extract.

Group Number	Abbreviations of Groups	Total Cholesterol	HDL	LDL	Triglyceride
1	CCl_4 Control	113.24±6.36	86.47±3.31	41.29±4.67	54.24±5.56
2	CCl_4 + Silymarin	194.72±8.46	41.46±5.82	152.29±14.22	118.28±11.29
3	CCl_4 + *G. glabra*	149.25±3.89	70.43±7.18	79.24±10.93	73.82±7.88

4	CCl_4 + *G. glabra*	191.46±4.82	42.61±4.68	150.73±12.41	114.24±10.50
5	CCl_4 + *G. glabra*	187.63±6.81	45.30±2.99	146.24±9.81	109.23±6.26
6	*G. glabra*	*****183.40±4.80	45.96±4.73	139.66±7.28	107.61±5.58
7	*G. glabra*	109.30±5.29	87.24±5.50	42.38±5.20	53.20±7.20
8	*G. glabra*	113.60±5.31	83.29±3.64	40.32±2.86	51.61±6.18
9	CCl_4 Control	110.57±4.64	85.94±3.84	45.67±4.23	52.27±6.52

It was observed that in comparison to the negative control group, there was a significant ($p>0.05$) drop in total cholesterol level only in high doses that demonstrates most significant hepatoprotective activity whereas for low and medium dosages, there was a non-significant ($p>0.05$) drop. In the case of total LDL and triglyceride, the levels declined but non-significantly ($p>0.05$) in dose-dependent manner for all low, medium and high doses. However, the HDL level increased for all three (high, medium and low) doses but in a non-significant ($p>0.05$) form. When compared to the negative control group, LDL levels dropped significantly ($p<0.05$) at high dosages demonstrating maximum therapeutic effect and non-significantly ($p>0.05$) at low and medium doses.

The total outcome demonstrates that only at high doses (1200 mg/kg) does the therapy exhibit the most significant hepatoprotective efficacy. One possible explanation for this result could be that the medicinal plant naturally has insufficient amounts of therapeutic compounds. Furthermore, the season and geographical region in which the plant was grown may have contributed to the plant's lack of therapeutic compounds, which lowers the plant's therapeutic activity. The choice of ethanol as the extraction medium could be another factor. Given their respective hydrophilicity and hydrophobicity, hexane or water would have been a better option as a solvent in this instance. Therefore, by altering the solvent system, extracting the plant with sufficient therapeutic

compounds found in specific geographical regions and extracting the plant during the right season can result in higher hepatoprotective effect of the plant in all dosages.

Conclusion:

To conclude, this study demonstrated that the ethanolic extract of *Glycyrrhiza glabra* had hepatoprotective potential and was crucial for restoring an altered hepatic condition. As *Glycyrrhiza glabra* has the potential to be a hepatoprotective agent, making it a viable natural substitute for hepatotoxic medication more further study is required to verify its safety and effectiveness. This calls for more research to be conducted in conjunction with the extraction of the substance from the plant in order to accurately identify the therapeutic component exhibiting hepatoprotective activity and to report a novel finding in the disease management system.

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