INVESTIGATION OF HEPATOPROTECTIVE POTENTIAL ON GLYCYRRHIZA GLABRA LINNN

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Abstract— The present study investigates the hepatoprotective potential of *Glycyrrhiza glabra* Linn. (commonly known as licorice), a plant widely recognized for its therapeutic properties in traditional medicine. The study aims to evaluate its ability to protect the liver from damage induced by oxidative stress and hepatotoxins. Extracts of *Glycyrrhiza glabra* were prepared and subjected to phytochemical analysis, which revealed the presence of bioactive compounds such as glycyrrhizin, flavonoids, and saponins, known for their antioxidant and anti-inflammatory properties. Histopathological analysis further confirmed the protective effects, showing improved liver architecture in treated groups compared to the toxin-induced control group. In vitro studies on hepatic cell lines supported these findings, highlighting the role of *Glycyrrhiza glabra* in reducing cellular damage through modulation of oxidative stress pathways.

Keywords- Hepatoprotective, Histopathology, Anti-inflammatory

I. INTRODUCTION

Indian traditional medicine is based on various systems including Ayurveda, Siddha, and Unani. The evaluation of these drugs is primarily based on phytochemical, pharmacological, and allied approaches including various instrumental techniques such as chromatography, microscopy, and others. These traditional systems of Indian medicine are each unique but there is a common thread in their fundamental principles and practices. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different healthcare systems, the evaluation of the rich heritage of traditional medicine is essential.

The liver is the most important drug-eliminating organ in the body, and is capable of both metabolic transformation and biliary excretion. The liver is a highly specialized and heterogeneous organ, and its structure and attendant heterogeneities must be considered for a more quantitative expression of drug metabolism (metabolite formation) and subsequent metabolism of the metabolites. The smallest functional unit of the liver is known as the acinus, which consists of a terminal portal venule, hepatic arteriole, a bile duct, lymph vessels and nerves. A zonal relationship exists between the cells constituting the acinus and the blood supply.

The hepatocytes situated close to the portal space are first supplied with fresh blood (rich in oxygen and nutrients), and zonation is contingent upon the oxygen tension at the locale.

With a steady input of oxygen entering the organ, a concentration profile in space is created, declining from inlet to outlet. Zone 1 (or periportal region) is closest to the entry of the hepatic artery and portal vein or the portal triad and is having the highest oxygen tension. Zone 3 (perihepatic venous or pericentral region) is near the exit, where the oxygen tension is lowest.

An overlapping zone 2 (or midzonal) region exists, where the oxygen tension is intermediate.

The different acinar region are specialized in mediating various biochemical/physiological processes. However the shape of profile can be altered as zone 2 cells can be recruited to behave more like zone 1 or 3 cells, depending on flow and oxygen utilization. The sinusoids are surrounded by single plates of hepatocytes of similar lengths and lined by endothelial cells containing sieve plates with open fenestrae. There is also a freely accessible disse space (a functional extracellular interstitial space that allows for equilibriative exchange).

II. HEPATOCYTE

Hepatic parenchymal cells (hepatocytes) contain a well-developed organelle substructure. Mitochondria, which constitute approximately 18 % of the liver cell volume, are the sites of oxidative phosphorylation and energy production. They contain enzymes involved in the citric acid cycle and beta-oxidation of fatty acid. The rough endoplasmic reticulum is the site of synthesis of many protein, including albumin, coagulation

factors, enzymes (e.g. glucose-6-phosphatase), and triglycerides.

The smooth endoplasmic reticulum contains microsomes that are involved with bilirubin conjugation, detoxification (cytochrome P450 dependent isoenzymes), steroid synthesis, cholesterol synthesis, and bile acid synthesis. The enzymes in this system, including gamaglutamyl-transferase, are induced by many drugs and inhibited by others. This is the site of drug metabolism where many important drug interactions occur during the multi-drug therapy. Peroxisomes are found near the smooth endoplasmic reticulum and contain oxidases that utilize molecular oxygen to oxidize a variety of substrates, leading to the production of hydrogen peroxide along with catalase, which decomposes the peroxide. Peroxisomes also catalyze the betaoxidation of fatty acids and about 5 to 20 % of ethanol metabolism also occurs in peroxisomes. Lysosomes are dense organelles that contain hydrolytic enzymes that act as scavengers. Deposition of iron, bile pigment and copper occurs in the lysosomes. The Golgi apparatus lies near the canaliculus and is involved with the secretion of various substances, including bile acids and albumin (Tolman and Rej, 1999).

However, during the hepatic injury or damage the homeostasis of enzymatic functions and other cellular organellae is disturbed resulting in the functional and anatomical aberrations of the liver.

2.1 Acetaminophen induced hepatotoxicity

Acetaminophen (paracetamol) was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. It was shown that repletion of GSH prevented the toxicity. This finding led to the development of the currently used antidote N-acetylcysteine. The reactive metabolite was subsequently identified to be N-acetyl-p-benzoquinone imine (NAPQI). Although covalent binding has been shown to be an excellent correlate of toxicity, a number of other events have been shown to occur and are likely important in the initiation and repair of toxicity.

Recent data have shown that nitrated tyrosine residues as well as acetaminophen adducts occur in the necrotic cells following toxic doses of acetaminophen. Nitrotyrosine was postulated to be mediated by peroxynitrite, a reactive nitrogen species formed by the very rapid reaction of superoxide and nitric oxide (NO). Peroxynitrite is normally detoxified by GSH, which is depleted in acetaminophen toxicity. NO synthesis (serum nitrate plus nitrite) was dramatically increased following acetaminophen. In inducible nitric oxide synthase knockout mice, acetaminophen did not increase NO synthesis or tyrosine nitration; however, histological evidence indicated no difference in toxicity. Acetaminophen did not cause hepatic lipid peroxidation in wildtype mice but did cause lipid peroxidation in iNOS knockout mice. These data suggest that NO may play a role in controlling lipid peroxidation and that reactive nitrogen/oxygen species may be important in toxicity. The source of the superoxide has not been identified, butour recent finding that NADPH oxidase knockout mice were equally sensitive to acetaminophen and had equal nitration of tyrosine suggests that the superoxide is not from the activation of Kupffer cells.

It was postulated that NAPQImediated mitochondrial injury may be the source of the superoxide. In addition, the significance of cytokines and chemokines in the development of toxicity and repair processes has been demonstrated by several recent studies. IL-1β is increased early in acetaminophen toxicity and may be important in iNOS induction. Other cytokines, such as IL-10, macrophage inhibitory protein-2 (MIP-2), and monocyte chemo attractant protein-1 (MCP-1), appear to be involved in hepatocyte repair and the regulation of proinflammatory cytokines (James et al., 2003). Figure 1: Schematic representation depicting the role of metabolism in acetaminophen toxicity

III. MATERIAL AND METHODS

Chemicals

All chemicals used in the study were of analytical grade. CCl4 was procured from Merck India Ltd., Mumbai, India. The kits for the estimation of SALT, SAST and TSP were from Dialab, Austria. Kits for TSB and ALP were from Biolabo SA, Maizy, France.

Plant Extraction

The licorice was purchased and roots of liocorice were cleaned, dried, and powdered with an electrical grinder, then passed through sieve no.40 to remove the debris. The sieved powder was stored in airtight container at room temperature. The aqueous extract was prepared by diluting one volume of wellgrinded powder to ten volume of water at 80°C in a stoppered flask after shaking well. Then, it was allowed to stand for 10 minutes to be cold and filtered for laboratory purposes. The aqueous extract should be used within 12 hours (AlRazzuqi et al., 2011).

Animals

Wistar albino rats (150-200g) used in studies was procured from C.L.Baid metha college of pharmacy, Chennai-97 The animals were fed with standard

pellet diet (Hindustan lever Ltd. Bangalore) and water adlibitium.All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee afterAll chemicals used in the study were of analytical grade. CCl4 was procured from Merck India Ltd., Mumbai, India. The kits for the estimation of SALT, SAST and TSP were from Dialab, Austria. Kits for TSB and ALP were from Biolabo SA, Maizy, France.

- Group I (control) - received normal saline 3 ml p.o
- Group II -received distilled water 3ml p.o
- Group III - received aqueous extract of licorice as 2gm/kg p.o (Al-Jawad et al., 2009)

3.1 Phytochemical Screening Method

Mayer's test

A pinch of dried extracts was taken and 2 ml of dilute hydrochloric acid was added, mixed filtered. To the filtrate, one or two drops of Mayer's reagent were added.

Formation of pale yellow precipitate indicates the presence of alkaloids.

Dragendorff's test

A pinch of dried extracts was taken treated with 2ml of 2% Acetic acid, mixed thoroughly and filtered. To the filtrate 2 drops of Dragendorff's reagent was added.

Formation of orange-brown precipitate indicates the presence of alkaloids.

Hager's test

A pinch of dried extracts was taken and treated with drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids.

Wagner's test

A pinch of dried extracts was taken and treated with drops of Wagner's reagent. Formation of brown precipitate indicates the presence of alkaloids

3.2 Tests for Glycosides

Anthrone test

A pinch of extracts was taken in a watch glass and 2 drops of alcohol was added to extract. An equal quantity of anthrone was added and mixed thoroughly and dried. Then one drop of concentrated sulphuric acid was added, separated in a thin film with a glass rod in a watch glass. and heated over the water bath. Formation of dark green color indicates the presence of glycosides.

Test for anthra quinone glycosides

Borntrager's test

A pinch of the extracts was boiled with dilute sulphuric acid, filtered while hot and filtrate was extracted with solvent like benzene. It was shaken well and the organic layer was separated and to this equal volume of dilute ammonia was added. Rose pink colour in ammonia layer indicates the presence of anthraquinone glycoside.

Test for cardiac glycosides

Legal's test

The extracts were hydrolysed for few hours in a water bath. The hydrolysate was added with 2ml of pyridine, sodium nitropruside solution and was made alkaline with sodium hydroxide solution. Orange colour shows the presence of cardiac glycoside.

IV. HEPATOTOXIC PARAMETER

Experimental procedure:

The rats were weighed after the adaptation period and marked with serial numbers and divided randomly into 5 groups, 5 rats each, and then the doses were calculated according to individual body weights.

Blood samples:

Blood was obtained by puncturing retro orbital plexus (Poole, 1989), under anesthesia using Halothane and heparinized capillary tubes. Blood drops were collected, gently, serum was separated by centrifugation (2500 rpm for 15 min), and EDTA was used as an anticoagulant for hematological parameters. Samples were collected before and after dosing with the tested plants extracts at day 0, 5 and at day 10.

Determination of acute toxicity (LD50):

•14 days single dose oral acute toxicity and gross behavioral study Number of animals required: 6 rats (male)

•Number of groups: 2 groups (3 animals each group)

•Dose levels: 4000 mg/kg body weight of the animals.

•Study duration: 14 day

Preparation of dose: Ethanolic extract of glycyrrhiza glabra leaves was suspended in 3% CMC, to prepare a dose of 4000 mg/kg body weight of animal, and administered 1ml/100gm body weight of the animal.

V. PROCEDURE

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female animal (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with ethanolic extract of glycyrrhiza glabra (4000 mg/kg) orally. Individually animals were observed for 4

hours to see any clinical symptoms, any change in behaviour or mortality. 6 hours post dosing again body weights recorded. Form the next day onwards, each day for 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

Flow Chart:

OECD Guidelines: Test Procedure Starting Dose of 300 mg/kg B.W. START

⇩

300 mg/ kg / 3 animals

0 - 1 (None of animals died) 2000 mg/ kg

⇩

2 – 3 (None of animals died)

⇩

3000 mg/kg (none of animals died)

⇩

4000 mg/ kg / 3 animals

⇩

None of animals died

⇩

LD50 cut off 4000 mg/ kg B.W.

Chemicals all the chemicals and solvents were of analytical grade. Silymarin was obtained from silybon, Micro Labs, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India. Male Albino rats weighing between 150-200 gm used in the experiment were kept in animal house under standard environmental conditions and had free access to feed and waterad libitum. The animals were fasted for 16 hours before experiment but allowed free access to water.

VI. HISTOPATHOLOGY

Blood sample were collected intodryclean bottlesand allowed to clot for 30 min at room temperature. Serumseparated by centrifugationat 2500 rpm for 15 min and stored at 20c°until analyzed. Biochemical parameters, i.e. alanine amino transeferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL) were analyzed according to the reported methods.

Table 1: Effect of extracts of Ethanolic extract glycyrrhiza glabra roots on SGOT

GROUP	SGOT level mean ± SEM
Control	1760±1.02
Negative Control	2097.90± 2.468**a
Standard	1759.53±2.33**b
EEGG 200mg/kg	1735.64±1.73 *b
EEGG 400mg/kg	1750.26±1.99 ***b

Effect of EEGG on SGOT: There was significant ($p<0.001$) increase in serum SGOT in Ethanol induced group when compared to control group. There was significant ($p<0.001$) decrease in serum SGOT in Silymarin treated group when compared to control group. There was significant ($p<0.001$) decrease in serum SGOT in EEGG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.001$) decrease in serum SGOT in EEGG treated group at a dose of 400mg/kg/p.o when compared to control group.There was a significant ($p<0.01$) decrease in serum SGOT level in Silymarin treated rats when compared to ethanol induced. The EEGG at a dose of 200mg/kg/p.o showed a significant ($p<0.010$) decrease in serum SGOT level when compared to ethanol induced group. The EEGG at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in serum SGOT level when compared to Ethanol induced group.

Table 2: Effect of extracts of Ethanolic extract glycyrrhiza glabra roots on SGPT

GROUP	SGPT level mean ± SEM
Control	1875±2.11
Negative Control	2263.36± 1.46**a
Standard	1870.98± 2.65*b
EEGG 200mg/kg	1883.34±2.22**b
EEGG 400mg/kg	2090.66± 2.32**b

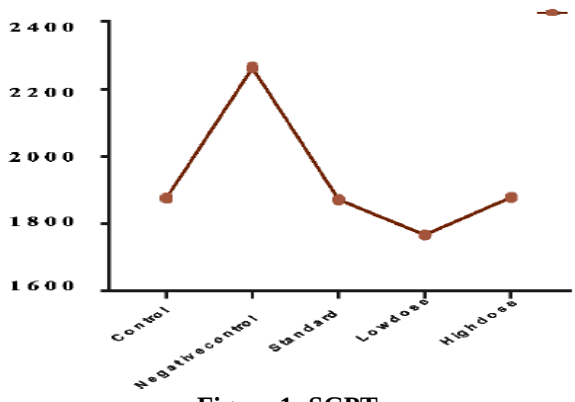

Figure 1: SGPT

EFFECT of EEGG on SGPT

There was significant ($p<0.01$) increase in serum glutamic pyruvate transaminase level in Ethanol induced rats when compared to control group. There was significant ($p<0.05$) decrease in SGPT in ethanol treated group when compared to control group.There was significant ($p<0.01$) decrease in serum SGPT in EEGG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.01$) decrease in serum SGPT in EEGG treated group at a dose of 400mg/kg/p.o when compared to control group. There was a significant ($p<0.01$) decrease in serum SGPT level in Silymarin treated rats when compared ethanol induced group. The EEGG at a dose of 200mg/kg/p.o showed a significant ($p<0.01$) decrease in serum SGOT level when compared to Ethanol induced group. The EEGG at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in serum SGOT level when compared to Ethanol induced group.

Table 3: Effect of extracts of Ethanolic extract glycyrrhiza glabra roots on Bilirubin

GROUP	Total Bilirubin mean ± SEM
Control	1.48±0.01
Negative Control	2.61 ± 0.16***a
Standard	1.47 ± 0.01*b
EEGG 200mg/kg	1.82 ± 0.03 **b
EEGG 400mg/kg	1.53 ± 0.01 **b

Effect of EEGG on Total Bilirubin: There was significant ($p<0.01$) increase in Bilirubin level in Ethanol induced group when compared to control group. There was significant ($p<0.01$) decrease in Bilirubin in Silymarin treated group when compared to control group. There was significant ($p<0.05$) decrease in Bilirubin in EEGG treated group at a dose of 200mg/kg/po when compared to control group. There was significant ($p<0.001$) decrease in Bilirubin in EEGG treated group at a dose of 400mg/kg/p.o when compared to control group. There was a significant ($p<0.01$) decrease in Bilirubin in Silymarin treated rats when compared ethanol treated. The EEGG at a dose of 200mg/kg/p.o showed a significant ($p<0.05$) decrease in serum bilirubin when compared to ethanol induced group. The EEGG at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in Bilirubin when compared to Ethanol induced group.

Table 4: Effect of extracts of Ethanolic extract glycyrrhiza glabra roots on ALP

GROUP	ALP level mean ± SEM
Control	35.06±0.12
Negative Control	53.47±0.01*a
Standard	37.8±0.03**b
EEGG 200mg/kg	35±0.72 ***b
EEGG 400mg/kg	39.98±0.11*b

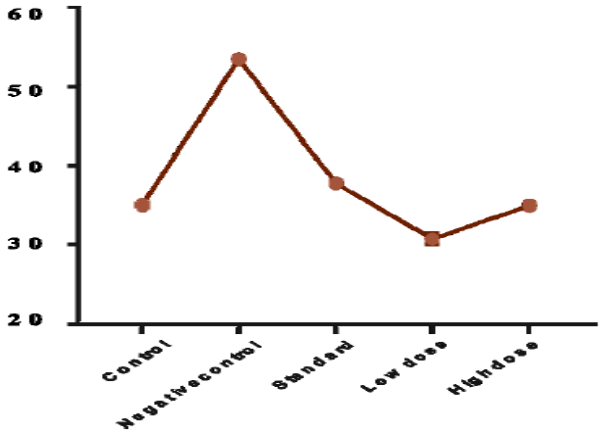

Figure 2: ALP

Effect of EEGG on ALP: There was significant ($p<0.01$) increase in ALP in ethanol induced group when compared to control group. There was significant ($p<0.01$) decrease in ALP in Silymarin treated group when compared to control group. There was significant ($p<0.05$) decrease in ALP in EEGG treated group at a dose of 200mg/kg/p.0 when compared to control group.

There was significant ($p<0.001$) decrease in Alp in EEGG treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.05$) decrease in ALP in Silymarin treated rats when compared ethanol treated. The EEGG at a dose of 200mg/kg/p.o showed a significant ($p<0.001$) decrease in ALP when compared to Ethanol induced group. The EEGG at a dose of 400 mg/kg/p.o showed a significant ($p<0.05$) decrease in ALP when compared to Ethanol induced group.

VII.CONCLUSION

The findings of this study demonstrate that *Glycyrrhiza glabra* Linn. exhibits significant hepatoprotective potential, attributed to its rich composition of bioactive compounds such as glycyrrhizin, flavonoids, and saponins. The extract effectively mitigated hepatic damage in

experimental models by restoring liver enzyme levels, reducing oxidative stress markers, and improving histopathological architecture. Additionally, the plant's antioxidant and anti-inflammatory properties play a crucial role in protecting liver cells from toxin-induced damage.

These results suggest that *Glycyrrhiza glabra* could serve as a natural alternative or complementary therapy for managing liver disorders. Its efficacy, comparable to standard hepatoprotective agents like silymarin, underscores its potential for inclusion in herbal medicine formulations. Further research is warranted to elucidate its precise molecular mechanisms and evaluate its safety and efficacy in clinical settings.

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