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THE ETHANOL EXTRACT OF *EUPHORBIA HETEROPHYLLA* PROTECTS LIVER CELLS AGAINST ETHANOL-INDUCED TOXICITY DUE TO ITS ANTIOXIDANT POTENTIALS IN WISTAR RATS

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Keywords:

Euphorbia heterophylla, Hepatoprotective activity, Ethanol, Serum Enzymes, Bilirubin, Total protein, Liver enzymes

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ABSTRACT: Background: The herbal drugs used in traditional medicine are proven more effective and safe compared to synthetic drugs for managing lifestyle diseases, including liver toxicity. **Objective:** The present investigation aimed to evaluate the hepatoprotective potentials of ethanol extract of Euphorbia heterophylla leaves against ethanol-induced liver damage in rats. **Methodology:** The acute oral toxicity study was conducted according to guidelines No 425 prescribed by OECD. The extract was proved safe up to 2000mg/kg. Ethanol-induced hepatotoxicity in rats model was used to assess the protective properties of ethanol extract of *Euphorbia heterophylla*. The serum, such as (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, albumin, total protein, ions was determined. And other parameters like clotting time, liver weight, liver histopathology, and liver antioxidant enzymes were also examined. **Results:** In toxic control animals treated with ethanol alone, there were variations in the parameters mentioned above. But in the animals treated with ethanol extract of *Euphorbia heterophylla* (EEEH) and standard drug silymarin, all the parameters were normal, possibly due to their beneficial property in protecting the liver against ethanol-induced hepatotoxicity. **Conclusion:** The results of the present research study suggest that the ethanol extract of *Euphorbia heterophylla* possesses significant hepatoprotective activity.

INTRODUCTION: Liver damage is always associated with cellular necrosis, increased tissue lipid peroxidation, and depletion in the tissue glutathione (GSH) levels. In addition, serum levels of many biochemical markers like Aspartate Amino Transaminase (AST), Alanine Amino Transaminase (ALT), triglycerides, cholesterol, bilirubin, and alkaline phosphatase are elevated.

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With time, damage to the liver results in scarring (cirrhosis), which can lead to liver failure, a life-threatening condition. Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury. Drug-induced hepatic injury is the most common reason cited for withdrawal of an approved drug.

It is necessary to identify drug-related liver injury because early detection can decrease the severity of hepatotoxicity [1, 2]. As per survey made by World Health Organization (WHO) survey, about 18,000 people die yearly due to liver diseases. The common ailments of liver diseases are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, fulminant hepatic failure and

certain tumors like hepatoma. It is estimated that two billion people worldwide are infected with hepatitis B, and about 350 million of these have the chronic form of the disease. In modern medicine, some of the most promising or studied drugs utilized for the liver that are chosen and analyzed critically are colchicine, corticosteroids, interferons and thalidomide. Curcumin and thalidomide is very attractive newly discovered protective and curative compound on experimental hepatic diseases. Unfortunately, clinical studies are lacking for the same [3]. In spite of phenomenal growth of modern medicine, few synthetic drugs are available to treat hepatic disorders. However, several herbs/herbal formulations are claimed to have beneficial activity in treating hepatic disorders.

But, they need to be validated in the light of science to ensure their ability to conserve therapeutic effectiveness in the formulation form. Herbal products today symbolize safety in contrast to synthetic, which are considered unsafe to humans and the environment. About 600 commercial preparations with claimed liver-protecting activity are available worldwide. About 100 Indian medicinal plants belonging to 40 families are used for herbal formulation. A few reports on the Hepatoprotectiveactivityare cited here, e.g. *Apium graveolens linn*, *Boerrhavia difffusa linn*, *Euphorbia antisyphilitica*, *Rubia cardifolia* and *Solanum lyratum* [4].

The herbal plant *Euphorbia heterophylla* is effective in treating hepatitis due to its antioxidant property. The whole Euphorbia heterophylla family Euphorbiaceae plant contains tannins, alkaloids, flavonoid, sterol, quinine, lignin and coumarin [5, 6]. The plant is used as a superoxide scavenging agent and in wound healing, anticoagulant, anticancer, nociceptive and antimicrobial. The literature review indicated that the Hepatoprotective activity of this species has not been evaluated. Hence, the present study is designed to investigate leaves of *Euphorbia heterophylla* for its Hepatoprotective activity [7, 8].

MATERIALS AND METHODS:

Chemicals: The study's chemicals and reagents were all analytical. The hepatotoxin Ethanol was purchased from Bangalore's Sigma-aldrich Chemical Pvt. Ltd.) The conventional medication Silymarin was purchased from the Bangalore-based Himalya Dug Company. (Nice Chemicals Bangalore) and Estimation Kits from SPAN Diagnostics for AST, ALT, ALP, serum bilirubins, sodium, potassium, and glutathione peroxidase.

Plant Material: The whole plant of *Euphorbia heterophylla* had been collected in the surroundings of Bangalore. The leaves were identified, collected and authenticated by Dr. V Ramarao, Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. The authenticated plant material was separated from other plant parts, cleaned, washed and dried for further use.

Preparation of Ethanol Extract of *Euphorbia heterophylla*: The shade-dried leaves were powdered and sieved through No. 22 mesh after being ground into powder. Using petroleum ether, 350 g (approx.) of coarse powder was defatted, and the remaining marc was extracted with chloroform water [9].

Preliminary Phytochemical Investigation of Ethanol Extract of *Euphorbia heterophylla*: According to Khandelwal's guidelines, the preliminary phytochemical analysis of the ethanol extract of *Euphorbia heterophylla* had been carried out [10].

Animals: The healthy albino Wistar male rats were purchased from *in-vivo* Biosciences, Bangalore and housed under typical conditions, including a standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India), water available at all times, relative humidity of 55 10%, and 12 hour light/dark cycles. The rats were acclimated for a week under the aforementioned ambient circumstances after being randomly assigned to various groups and before the experiment began. The Institutional Animals Ethics Committee, East West College of Pharmacy, Bangalore, accepted the study protocol (Ref No.EWCP/CPCSEA/IAEC/2018/04).

Evaluation Hepatoprotective Property of EEEH: The ethanol-induced liver damage in wistar rats model [11, 12] was used to evaluate the hepatoprotective activity of ethanol extract of *Euphorbia heterophylla*. The experimental design was as follows:

After 21 days of treatment, experimental period blood samples were collected individually for all the animals by retro-orbital puncture method and estimated for aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, direct bilirubin, total bilirubin, Sodium, chlorides and Potassium. The clotting time was determined for blood samples by the capillary tube method. Later all the animals were sacrificed by cervical dislocation, liver samples were collected, and the individual weights of the livers were estimated. A part of the liver sample was evaluated for histopathological examination, and another part was homogenized and estimated for lipid peroxidation and antioxidant enzymes glutathione peroxidase, glutathione reductase, and catalase-peroxidase [13, 14].

Sl. no.	Name of Group	Treatment
I	Normal	Administered with normal saline 2ml/kg i.p
II	Toxic control	Administered with 40 % ethanol (3.76 g/kg, p.o) twice a for 25 days and then 2 % vehicle (Tween 20) for next 21 days
III	Standard Control	Administered with 40 % ethanol (3.76 g/kg, p.o) twice a for 25 days and then silymarin (25mg/kg per day, p. o.) for next 21 days
IV	EEEH-100mg	Administered with 40 % ethanol (3.76 g/kg, p.o) twice a for 25 days and low dose of ethanol extract of *Euphorbia heterophylla* (100 mg/kg,p.o) for next 21 days
V	EEEH-200mg	Administered with 40 % ethanol (3.76 g/kg, p.o) twice a for 25 days and medium dose of ethanol extract of *Euphorbia heterophylla* (200 mg/kg,p.o) for next 21 days
VI	EEEH-400mg	Administered with 40 % ethanol (3.76 g/kg, p.o) twice a for 25 days and high dose of ethanol extract of *Euphorbia heterophylla* (400 mg/kg,p.o) for next 21 days

Determination of Lipid Peroxidation and Liver Antioxidant Enzymes: The liver samples were dissected out and washed using ice-cold saline solution. The pieces of liver samples were subjected to homogenization using tissue homogenizer within 0.1M Tris- Hcl buffer (at pH 7.4).

The homogenate was centrifuged and collected supernatant solution was used for the determination of lipid peroxidation and liver antioxidant enzymes such as Glutathione Peroxidase (GPX), Catalase Peroxidase (CAP), Glutathione reductase (GRD). The homogenate was also determined for Lipid Peroxidation (LOP) activity in the liver [15, 16].

Histopathological Evaluation: Two animals from each group were sacrificed by being put down at the conclusion of the research. The liver was quickly removed and ice-cold saline rinsed after exsanguinations. Before sectioning, the liver samples were dehydrated in a graduated series of alcohol, fixed with 10% formaldehyde, and embedded in paraffin wax.

The tissue was dewaxed, sliced into about 5 m thick slices, and rehydrated. The sections were then stained with hematoxylin-eosin dye and examined under a light microscope for histological alterations. Each sample was examined under a 100X magnification.

Statistical Analysis: The data obtained from the study were subjected to statistical analysis by one way ANOVA followed by Turkey multiple comparisons test, and results were expressed in terms of Mean±SEM values. Statistical analysis was performed using GraphPad prism.

RESULTS:

Phytochemical Investigation: The ethanol extract of *Euphorbia heterophylla* was tested for preliminary phytochemical investigation. According to the study's findings, it revealed that ethanol extract contains alkaloids, flavonoids, tannins, and phenolic chemicals.

Assessment of Hepatoprotective Potentials of EEEH:

Effect of EEEH on Liver Weight: In the present study, a significant ($P<0.05$) increase in weights of rat liver was observed, which may be due to damage induced by ethanol administration in toxic control animals compared to normal animals. In animals treated with reference standard Silymarin and EEEH (200mg/kg and 400mg/kg), there was a significant ($P<0.05$) reduction liver weight compared to toxic animals (see **Table 1**).

TABLE 1: EFFECT OF *EUPHORBIA HETEROPHYLLA* ETHANOL EXTRACT ON CLOTTING TIME AND LIVER WEIGHT AGAINST ETHANOL-INDUCED LIVER DAMAGE IN RATS

Treatment	Weight of liver (gm)	Prothrombin Time (secs)
Normal Control	6.11±0.061	194.35±8.22
Toxic Control	8.45[a]±0.08	511.38[a] ±18.32
Standard (Sylimarin)	6.08[b] ±0.21	189.71[b] ±9.20
METC 100 mg/kg	7.94±0.16	475.57±8.55
METC 200 mg/kg	7.21[c] ±0.12	364.28[c] ±7.24
METC 400 mg/kg	5.82[c] ±0.02	205.16[c] ±5.91

Values are mean ± S.E.M, n=6 symbols represent statistical significance. [a]p<0.05, Toxic control vs Normal control [b]p<0.05, Standard vs Toxic control and [c]p<0.05, extract treated vs Toxic control.

Effect of EEEH on Clotting Time: In the current study, the prothrombin time was prolonged due to a deficiency of clotting factors toxic animals compared to the normal group due to ethanol-induced liver injury. The dose-dependent significant (P<0.05) reduction in clotting time was observed animals treated with standard silymarin while EEEH (200mg/kg & 400mg/kg) Animals treated with silymarin and ethanol extract have shown a significant decrease in clotting time compared to positive toxic animals indicating that ethanol extract can reverse complications of hepatotoxicity **Table 1**.

Effect of EEEH on Serum Enzymes: In our study, the serum enzymes ALT, AST and ALP were significantly (P<0.05) elevated toxic control group due to the administration of ethanol compared to animals of normal group as a result liver damage while EEEH (200mg/kg and 400mg/kg) & standard drug silymarin significantly (P<0.05) reduced concentration serum enzymes in therapeutic animals.

The effect of ethanol extract was comparable standard drug and it was dose. Treatment with silymarin and ethanol extract significantly reduced serum concentrations of enzymes ALT, AST and ALP & reduced bilirubin levels in blood compared to toxic animals **Table 2.**

TABLE 2: EFFECT OF *EUPHORBIA HETEROPHYLLA* ETHANOL EXTRACT ON SERUM ENZYMES, TOTAL BILIRUBIN AND TOTAL PROTEIN AGAINST ETHANOL-INDUCED LIVER DAMAGE IN RATS

Treatment	Serum parameters				
	SGPT (IU/ml)	SGOT (IU/ml)	SALP (IU/ml)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Normal Control	61.25± 2.532	129.8± 2.467	77.71± 2.459	0.3482± 0.01609	5.348± 0.1421
Toxic Control	152.3[a] ± 1.512	237.6[a] ± 5.781	203.0[a] ± 6.664	0.8494[a] ± 0.02191	2.706[a] ± 0.1026
Standard (Silymarine)	59.86[b] ± 2.002	129.0[b] ± 3.233	83.04[b] ± 2.910	0.3844[b] ± 0.009558	5.220[b] ± 0.05639
EEEH 100 mg/kg	149.2± 1.253	218.7± 5.248	198.5± 5.443	0.8312± 0.02553	3.270± 0.1776
EEEH 200 mg/kg	133.2[c] ± 1.555	187.0[c] ± 5.387	133.6[c] ± 2.612	0.6236[c] ± 0.02192	3.972[c] ± 0.1333
EEEH 400 mg/kg	87.98[c] ± 1.471	135.9[c] ± 4.753	86.50[c] ± 3.866	0.3814[c] ± 0.02126	4.87[c] ± 0.09151

Values are mean ± S.E.M, n=6 symbols represent statistical significance. [a]p<0.05, Toxic control vs Normal control [b]p<0.05, Standard vs Toxic control and [c]p<0.05, extract treated vs Toxic control.

Effect of EEEH on Direct Bilirubin & Total Bilirubin: In the current investigation, the administration of ethanol-induced hepatic injury serum direct bilirubin and total bilirubin were significantly (P<0.05) increased in toxic control animals as compared to normal group of animals while there was significant (P<0.05) reduction of direct bilirubin and total bilirubin was observed in animals treated with standard drug silymarin and EEEH (200mg/kg and 400mg/kg) compared to toxic alone animals. The results were equivalent to normal the effect of ethanol extract was dose-dependent. EEEH reduced bilirubin levels in blood shows the increased detoxification in therapeutic animals compared to toxic, which could be due to possible protection given by ethanol extract **Table 2.**

Effect of EEEH on Total Protein and Albumin: In drug-induced liver toxicity, total protein reduction is observed due to decreased albumin synthesis due to cirrhosis.

In present study, in toxic control group animals administered with ethanol significant (P<0.05) reduction of serum total protein and albumin was observed due to liver damage compared to normal animals but administration of silymarin and EEEH (200mg/kg and 400mg/kg) caused dose-dependent significant (P<0.05) rise in total protein and albumin therapeutic group compared to toxic animals and the results **Table 2.**

Effect of EEEH on Serum Ions: In current research, ethanol induced liver damage may cause ascites and hence there was significant (P<0.05) reduction serum ionic concentration was observed in toxic control animals when compared to animals of normal group but serum ionic concentrations were significantly (P<0.05) increased in animals of therapeutic groups treated with silymarin and EEEH (200mg/kg and 400mg/kg) when compared to toxic animals. The effect of extract was dose-dependent and comparable to standard **Table 3.**

TABLE 3: EFFECT OF *EUPHORBIA HETEROPHYLLA* ETHANOL EXTRACT ON SERUM IONS AGAINST ETHANOL INDUCED LIVER DAMAGE IN RATS

Treatment	Serum ions		
	Sodium (mE/L)	Potassium (mE/L)	Chlorides (mE/L)
Normal Control	138.5±1.3	5.73±0.74	80.76±3.025
Toxic Control	239.21[a] ±4.866	3.02[a] ±0.88	140.4[a]±3.22
Standard (Silymarin)	136.54[b] ±2.2	5.840[b] ±0.89	83.68[b] ±2.7
EEEH 100 mg/kg	218.2±1.92	3.23±0.82	136.3±4.31
EEEH 200 mg/kg	182.6[c] ±3.1	4.35[c] ±0.86	113.4[c] ±3.65
EEEH 400 mg/kg	142.2[c] ±2.7	5.74[c] ±0.82	84.48[c] ±3.71

Values are mean ± S.E.M, n=6 symbols represent statistical significance. [a]p<0.05, Toxic control vs Normal control [b]p<0.05, Standard vs Toxic control and [c]p<0.05, extract treated vs Toxic control.

Effect on Liver Antioxidant Enzymes: There was a significant (P<0.05) reduction concentration of liver antioxidant enzymes GPX, CAP and GRD in toxic control animals treated with ethanol alone compared to normal animals. While animals of therapeutic groups treated with sylimarin and EEEH (200mg/kg and 400 mg/kg), have exhibited a significant (P<0.05) rise in liver antioxidant enzyme compared to toxic animals **Table 4.**

TABLE 4: EFFECT OF *EUPHORBIA HETEROPHYLLA* ETHANOL EXTRACT ON THIOLS AND LIVER ANTIOXIDANT ENZYMES AGAINST ETHANOL INDUCED LIVER DAMAGE IN RATS

Treatment	Antioxidant Enzymes (mg/g)			
	Catalase Peroxidase	Glutathione reductase	Glutathione Peroxidase	Lipid Peroxidase
Normal Control	59.68±1.640	3.756±0.3302	12.39±1.22	8.323 ±0.96
Toxic Control	31.87[a]±1.037	2.336[a]±0.2713	7.56[a] ±0.81	17.91[a] ±0.12
Standard (Silymarine)	50.16[b] ±1.370	3.806[b] ±0.2856	11.88[b] ±1.081	10.92[b] ±0.67
EEEH 100 mg/kg	32.36±3.450	2.440±0.3047	7.547 ±1.015	16.26 ±0.71
EEEH 200 mg/kg	37.11[c] ±3.403	3.662[c] ±0.6461	9.077[c] ±0.7279	13.72[c] ±0.82
EEEH 400 mg/kg	49.11[c] ±2.393	4.968[c] ±0.5225	11.65[c] ±1.010	11.12[c] ±1.3

Values are mean ± S.E.M, n=6 symbols represent statistical significance. [a]p<0.05, Toxic control vs Normal control [b]p<0.05, Standard vs Toxic control and cp<0.05, extract treated vs Toxic control.

Effect on Lipid Peroxidation in Liver: The activity of enzyme lipid peroxidase significantly (P<0.05) increased in ethanol alone treated toxic animals compare to normal animals while concentration of lipid peroxidase was significantly reduced in therapeutic groups treated with standard drug sylimarin and TCME (200mg and 400mg/kg) indicates the ability of the extract to reduce free radical-mediated damages **Table 4.**

Histopathological Evaluation: The histo-pathological examination of liver samples revealed the protection to the liver tissue by the ethanol extract against ethanol-induced damage.

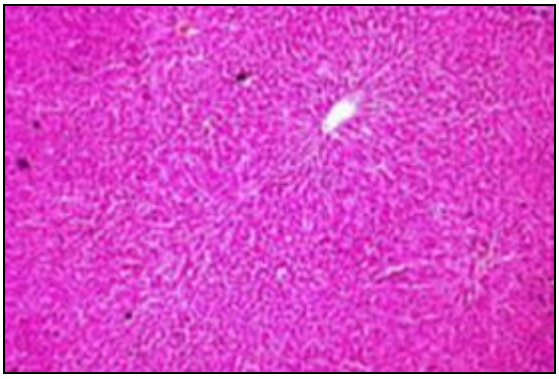

FIG. 1: HISTOPATHOLOGY OF LIVER SAMPLE FROM NORMAL GROUP

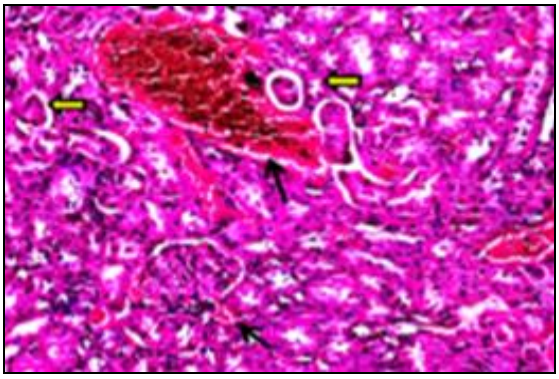

FIG. 2: HISTOPATHOLOGY OF LIVER SAMPLE FROM TOXIC GROUP

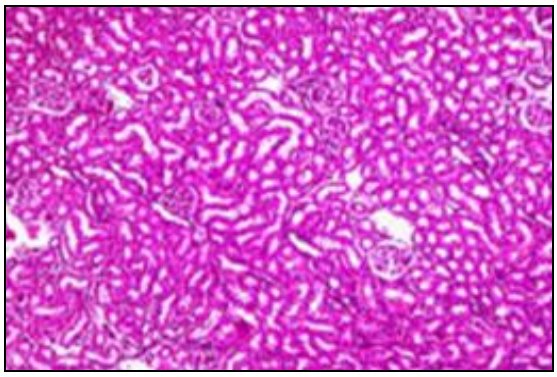

FIG. 3: HISTOPATHOLOGY OF LIVER SAMPLE FROM STANDARD

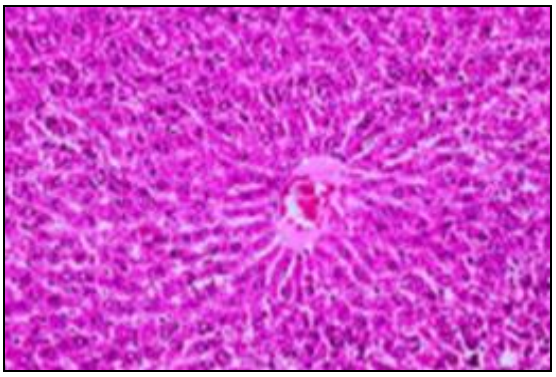

FIG. 4: HISTOPATHOLOGY OF LIVER SAMPLE GROUP FROM EEEH (200MG/KG)

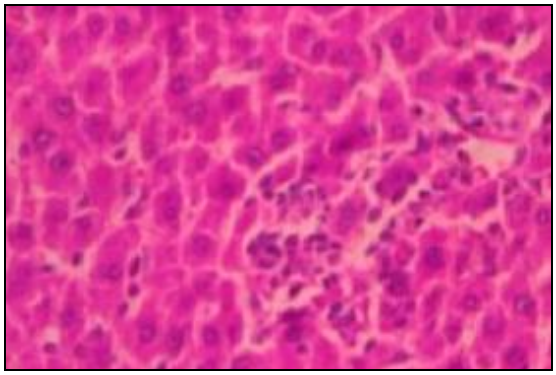

FIG. 5: HISTOPATHOLOGY OF LIVER SAMPLE FROM EEEH (400MG/KG)

The administration of ethanol was caused the complete loss of the normal architecture of livers in positive control animals with the appearance of vacuolated hepatocytes and degenerated nuclei. The pathological changes like vacuolization, fatty degenerations and coagulative necrosis of liver cells were found to be severe in the centrilobular region. The hepatotoxic metabolite ethanol produced excessive formation and deposition of fibrous tissue and results in development of scars. The nodular transformation of rat liver treated with EEEH 100mg/kg has shown that large septa of fibrous tissue flowing together, which penetrated the parenchyma cells, were found. But sections of liver samples belonging to therapeutic groups treated with high doses of ethanol extract showed almost normal lobular patterns with tiny and a mild degree of fatty degenerations, necrosis and infiltration of lymphocyte which was more or less comparable to the standard drug silymarin-treated groups.

DISCUSSION: Ethanol is a hepatotoxicant to induce liver damage since it is clinically relevant. This liver damage is associated with several reactions of free radicals, such as reactive oxygen species (ROS) which causes elevation in MDA and GST content while reducing the level of SOD. The

elevation and reduction of enzymatic and non-enzymatic markers of serum were also associated with this condition. Alcoholic liver disease was normally found in liver histology. The plant *Euphorbia heterophylla* was enriched with antioxidants that could revert and lower the free radicals level. It had shown that the beneficial effects of this phytochemical in preventing the ethanol-induced hepatotoxicity are mediated by the antioxidant effects [16, 17].

The disturbance in the metabolism of carbohydrates, fats and proteins is the main consequence of liver toxicity which leads to fatty change or fatty characterized by the deposition of fat in liver. Hence, the liver's total weight increases due to the deposition of fat and triglycerides in drug-induced hepatic damage [18]. But administration of ethanol extract and silymarin could normalize the weight of livers in therapeutic groups indicates their liver protective properties. The liver produces all the clotting factors associated with blood clotting mechanism and has a main role in regulating normal prothrombin or clotting time. In liver disorders synthesis of clotting factors will be affected; hence, clotting time is prolonged [18]. Administration with silymarin and ethanol extract has normalized clotting time.

Storage of various serum enzymes like ALT, AST and ALP is one of the liver's important functions. ALT and AST transaminases are involved in transamination reactions of various amino acids, while alkaline Phosphatase (ALP) is isoenzyme synthesized mainly by liver and has an important role in the dephosphorylation of biomolecules. These enzymes are leaked into the blood in hepatotoxicity due to liver parenchyma damage; hence their concentrations in serum were found to be elevated [19, 20]. Another very important role of liver is the detoxification of bilirubin which is breakdown product of haem an iron components of hemoglobin. The bilirubin uptake by liver parenchyma cells from the blood and conjugates with glucuronic acid in presence of enzyme glucuronyl transferase. Later conjugated bilirubin gets excreted through bile. In liver toxicity total bilirubin and direct bilirubin concentration are increased in serum due to reduced ability of liver parenchyma cells [21]. Treatment with silymarin and ethanol extract significantly reduced serum concentrations of enzymes ALT, AST and ALP indicating the enhanced storage function and also reduced bilirubin levels in blood shows the increased detoxification in therapeutic animals compared to toxic group which could be due to possible protection given by ethanol extract. Serum total protein, also called as total protein or plasma total protein is synthesized by the liver and is a important biochemical test for assessing liver function. The albumin and globulin that are produced in liver are the main components of total protein in the plasma [22].

The total protein and serum albumin level was increased by ethanol extract treated animals indicated its ability to reverse the hepatic damage caused by ethanol. The two main complications of hepatotoxicity are ascites and edema which are due to the accumulation of fluids in extra-vascular sites of the body. In these complications, serum ions sodium, potassium and chlorides move blood into extra-vascular tissues, finally leading to reduction in these ionic concentrations in blood [23, 24]. In our study, animals treated with ethanol extract and sylimarin exhibited significant increase of ions sodium, potassium and potassium which shows the property of the ethanol extract to reduce ascites and edema may be by regenerating the liver cells.

The ability of the living system to counteract free radical-mediated damages is a natural antioxidant mechanism in which glutathione Peroxidase, Catalase Peroxidase, Glutathione S transferase, glutathione reductase, and Lipid peroxidase are produced in the affected organ/tissue. The liver antioxidant enzymes include Glutathione Peroxidase (GPX), Catalase Peroxidase [25]. (CAP), Glutathione S transferase (GST), Superoxide dismutase (SOD) and Glutathione reductase (GRD). In the present study, there was a significant increase in the synthesis of liver antioxidant enzymes found in animals treated with EEEH, indicating its potential to protect the liver cells against ethanol-induced free radical damage. The drug-induced hepatotoxicity is mainly due to oxidative stress and free radicals-mediated damage. Hence free radical scavenging and antioxidant mechanisms are more important in reversing or prevent drug-induced liver toxicity [26, 27]. The extract of *Euphorbia heterophylla* had been reported for its antioxidant activity. In the present

study methanol extract of *Euphorbia heterophylla* could reduce most of the complications of ethanol-induced hepatotoxicity and also significantly increase liver antioxidant enzymes such as glutathione Peroxidase, Catalase Peroxidase, Glutathione S transferase, glutathione reductase and lipid peroxidase which may be the possible mechanism of action of the extract. Further studies are required to correlate the hepatoprotective potentials of the extract with increased glutathione concentrations and also to isolate and evaluate hepatoprotective principles from the ethanol extract. Hence the possible mechanism of the beneficial liver-protecting property of our extract due to its potent antioxidant activity. The Histopathological studies supported the results of biochemical tests, showing less damage in the cytoarchitecture of the liver.

CONCLUSION: The results obtained from estimating biochemical parameters suggest that ethanol extract of *Euphorbia heterophylla* leaves possess significant hepatoprotective properties in ethanol-induced liver toxicity in rats model.

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CONFLICTS OF INTEREST: We hereby declare that there is no conflict of interest.

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