HEPATOPROTECTIVE ACTIVITY OF SAUROPUS ANDROGYNOUS LEAVES AGAINST PARACETAMOL AND ETHANOL INDUCED HEPATOTOXICITY IN RATS.

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ABSTRACT

The hepatoprotective activity of ethanolic extract of the leaves of *Sauropus androgynous* was investigated for paracetamol and ethanol induced hepatotoxicity in rats. Wistar albino rats were divided into five groups of 6 animals each and are given orally with the following treatment for 10 and 21 days respectively. The Normal control group was given 0.3% starch solution 1 ml/kg b.w, Paracetamol at a dose of 2 g/kg b.w, *p.o*, and 30% Ethanol at a dose of 1 ml/100 g b.w, was given for inducing hepatotoxicity. Silymarin (50 mg/kg, *p.o*.) was given as reference standard for both the methods. Two doses of *S. androgynous* leaves extract i.e., 200 and 400 mg/kg *p.o.* was tested for hepatoprotective activity. The treatment was given for 10 days in Paracetamol induced hepatotoxicity and 21 days for Ethanol induced hepatotoxicity model. After 24 h of last treatment blood was collected by cardiac puncture and analysed for various serum parameters like serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) in different groups. In both the methods, ethanolic extract of *Sauropus androgynous* showed dose dependent significant decrease in SGPT, SGOT, ALP, total bilirubin and increase in total protein levels. Hepatoprotective effect was also confirmed by histopathological studies of liver which showed less damage in extract treated groups when compared with Paracetamol (2 g/kg) and 30% Ethanol (1 ml/100g). The extract found to have significant hepatoprotective activity in both Paracetamol and Ethanol induced hepatic injury models.

Keywords: Hepatoprotective activity, *Sauropus androgynous*, Paracetamol, Ethanol, Hepatic injury.

INTRODUCTION

Liver is a vital organ in human body that plays major role in the metabolism and elimination of xenobiotics from the body. The major function of the liver are carbohydrates, proteins and fat metabolism, detoxification, secretion of bile and storage of vitamin (Ward and Daly et al., 1999). Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But the risk of liver intoxication is due to the higher exposure to environmental toxins, pesticides, pharmaceuticals, abused by poor drug habits, alcohol and frequent use of chemotherapeutic leads into the various liver ailments like cirrhosis (liver failure), hepatic encephalopathy, enlarged liver, viral hepatitis A, B, C, D, E and alcoholic liver disease (Waugh et al., 2002). Liver damage is always associated with cellular necrosis, depletion in the tissue lipid peroxidation and depletion in the tissue glutathione (GSH) levels. The disorders associated with fat (fatty liver) and bilirubin (jaundice) metabolism are commonly seen. In the absence of reliable modern hepatoprotective drugs there are a number of traditional drugs are recommended for treatment of liver disease. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme test, drug induced liver injury is responsible for 5% of all acute failure. We have witnessed limitation of allopathic system of medicine. Lately herbal medicine has gained momentum and its evident from the certain herbal remedies peaked at par with synthetic drugs.

Sauropus androgynous (Phyllanthaceae), an edible plant, has efficacy as an antibacterial, aphrodisiac, antioxidant, cytotoxic, lactation enhancer, antidiabetic, anti-cholesterol and hypoglycaemic (Kumar et al. 2004). *Sauropus androgynous* contains sauroposide and some secondary metabolites like alkaloids, flavonoids, phenols, terpenoids, glycosides and some vitamins *i.e*., carotenoids, thiamine, ascorbic acid and alpha-tocopherol. In view of this, the present work is aimed to investigate the hepatoprotective activity of ethanolic extract of *Sauropus androgynous* against Paracetamol and Ethanol induced hepatotoxicity in rats.

MATERIALS AND METHODS:

Plant material: The fresh leaves of the plant *Sauropus androgynous* were collected from garden of Bharathi College of Pharmacy, garden of Botany department of Bharathi College and local areas of Mandya district, Karnataka. The plant was identified, Confirmed and authenticated by Mr. H.M. Mahesh, Botanist, Department of Botany, Bharathi College of post-Graduation and Research Centre, Bharathinagara, Karnataka.

Preparation of extract: The leaves of *Sauropus androgynous* were collected and dried in shade. The dried material was then reduced to coarse powder using an electrical grinder, the powdered drug was subjected to hot continuous extraction with 70% ethanol in a Soxhlet extractor for 72 hours at 50^0C. The extract was air dried and weighed, 60 g of powdered leaves of *Sauropus androgynous* yields 6.15 g of dried extract.

Phytochemical screening: The qualitative chemical investigation of ethanolic extracts of leaves of *Sauropus androgynous* was carried out to check the presence of various phytoconstituents as per standard tests (Harborne1998), (Chaturvedi et al. 2012) indicates the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides, steroids, carbohydrates and vitamins.

Experimental animals: Healthy male wistar albino rats of approximately same age (10 to 12 weeks), weighing between 150-200 g were taken for experiment, procured from Venkateshwara breeders, Bangalore. The animals were acclimatized by keeping them in animal house facility of Bharathi College of Pharmacy, Bharathinagara. They were housed in polypropylene cages containing bedding material as husk and maintained under standard husbandry conditions and 12hrs light and 12hrs dark cycle. They were fed with commercial pelleted rat chow with water ad. libitum. The animals were maintained in accordance with the CPCSEA guidelines. The hepatoprotective activity studies were conducted after obtaining the approval from Institutional Animal Ethical committee (IAEC) with a reference no**.** BCP/PCOL/04/2021 dated 15/08/2021.

Selection of dose: The dose of 200 mg/kg and 400 mg/kg was used (Kritishanti and Islamie 2019).

ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY

Paracetamol induced hepatotoxicity (Punnagai et al. 2020).

Male wistar albino rats of weighing between 150-200 g were divided into five group of six animals each. Group I was maintained as normal control vehicle 0.3% starch solution (1 ml/kg) was given. Group II receives Paracetamol 2 g/kg body weight by *p.o,* at every 72hr for 10 days. Group III animals were treated with silymarin (50 mg/kg *p.o*,) which serves as standard for 10 days. Group IV animals were treated with ethanolic extract of *Sauropus androgynous* (EESA) (200 mg/kg) and Group V animal were treated with ethanol extract of *Sauropus androgynous* (400 mg/kg) for 10days. Group III, IV and V simultaneously administered Paracetamol (2 g/kg) 1hr after the assigned treatment for every 72hr. After 10 days, the animals were then anaesthetized diethyl ether, and blood was collected by cardiac puncture, serum is separated by centrifuging at 3000 rpm for 15 min and analysed for various biochemical parameters and histopathological studies.

Ethanol induced hepatotoxicity (Vuyyala et al. 2019), (Lodhi et al. 2014).

Male wistar albino rats of weighing between 150-200 g were divided into five group of six animals each. Group I was maintained as normal control vehicle 0.3% Starch solution (1 ml/kg) was given. Group II receives 30% Ethanol 1 ml/100gm body weight by *p.o,* for up to 21 days. Group III animals were treated with silymarin (50 mg/kg *p.o*,) which serves as standard for 21 days. Group IV animals were treated with ethanolic extract of *Sauropus androgynous* (200 mg/kg) and Group V animal were treated with ethanol extract of *Sauropus androgynous* (400 mg/kg) for 21 days. Group III, IV and V simultaneously administered 30% ethanol 1 ml/100 gm 1hr after the assigned treatment for 21days. After 21 days, the animals were then anaesthetized diethyl ether, and blood was collected by cardiac puncture, serum is separated by centrifuging at 3000 rpm for 15min and analysed for various biochemical parameters and histopathological studies.

EVALUATION: The SGPT, SGOT, ALP, total bilirubin and total protein was estimated in the present study from the blood serum using biochemical enzymatic kits as marker of liver injury (Dufour et al. 2012), (Thomas 1998), (Burtis 1999), (Carobene et al. 2013), (Heerspink et al. 1980).

HISTOPTHOLOGICAL EVALUATION

For histopathological studies, liver tissues were fixed at 10% neutral formalin. Liver tissue was trimmed with the aid of a rotary microtome and embedded in paraffin wax. Afterward, tissue sections were stained with hematoxylin and eosin dye. The histological section was evaluated by light microscopy.

STATISTICAL ANALYSIS

All the values were expressed as mean ± S.E.M and statistical significance was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test $p<0.05$ have been considered as significant.

RESULTS

Hepatoprotective activity of *Sauropus androgynous* on Paracetamol induced hepatic damage in rats:

Paracetamol in dose of 2 gm/kg b.w produced acute hepatic damage. Paracetamol significantly increased the serum level of SGPT, SGOT, ALP, total bilirubin and decreased the level of total protein. It was observed that, EESA significantly lowered Paracetamol induced levels of SGPT, SGOT, ALP, Total bilirubin and increased the level of total protein. The levels of various serum marker enzymes were depicted in Table 1 & figure 1.

Histopathological observations:

Histopathological observations of Normal control group (Figure 2A) showed normal appearing hepatocytes. Liver section of Paracetamol control group (Figure 2B) showed ballooning degeneration of hepatocytes, lymphocytic infiltration. Liver section of rats treated with Paracetamol + Silymarin 50 mg/kg b.w (Figure 2C) showed normal appearing hepatocytes, mild lymphatic infiltration. Liver section of rats treated with Paracetamol + EESA 200 mg/kg b.w (Figure 2D) showed normal appearing hepatocytes, moderate lymphatic infiltration. Liver section of rats treated with Paracetamol + EESA 400 mg/kg b.w (Figure 2E) showed normal appearing hepatocytes, mild lymphatic infiltration, indicating the hepatoprotective activity of ethanolic extract of *Sauropus androgynous* (EESA).

Table 1: Effect of EESA on serum SGPT, SGOT, ALP, Total protein, Total bilirubin in Paracetamol induced hepatotoxicity.

Groups	Serum analysis				
	SGPT (U/L)	**SGOT (U/L)**	**ALP (IU/L)**	**Total protein (g/dl)**	**Total bilirubin (mg/dl)**
Normal control	47.1 ± 2.59	102.4 ± 1.26	179.7 ± 9.59	9.73 ± 0.32	0.57 ± 0.05
Paracetamol 2 gm/kg	69.1 ± 5.21***	164.8 ± 18.8***	27.2 ± 12.23***	7.11 ± 0.63***	1.09 ± 0.14***
Paracetamol + Silymarin 50 mg/kg	51.1 ± 2.71**	116.0 ±1.95***	184.6 ± 3.55**	9.18 ± 0.28**	0.72 ± 0.05**
Paracetamol + EESA 200 mg/kg	55.0 ± 3.02*	127.1 ± 5.23*	193.7 ± 2.91*	8.73 ± 0.26*	0.79 ± 0.03*
Paracetamol + EESA 400 mg/kg	53.3 ± 2.57**	122.5 ± 6.78**	187.7 ± 2.93**	8.97 ± 0.44*	0.76 ± 0.01*

All values are expressed as mean ± SEM. The statistical significance is analyzed using one- way ANOVA followed by Dunnett's multiple comparison test. Paracetamol control group was compared to normal control group and treated groups (Paracetamol + Silymarin 50 mg/kg, Paracetamol + EESA 200 mg/kg & Paracetamol + EESA 400 mg/kg) were compared to paracetamol control group and the P- values, ***$p< 0.001$, **$p<0.01$ & *$p<0.05$ are significant.

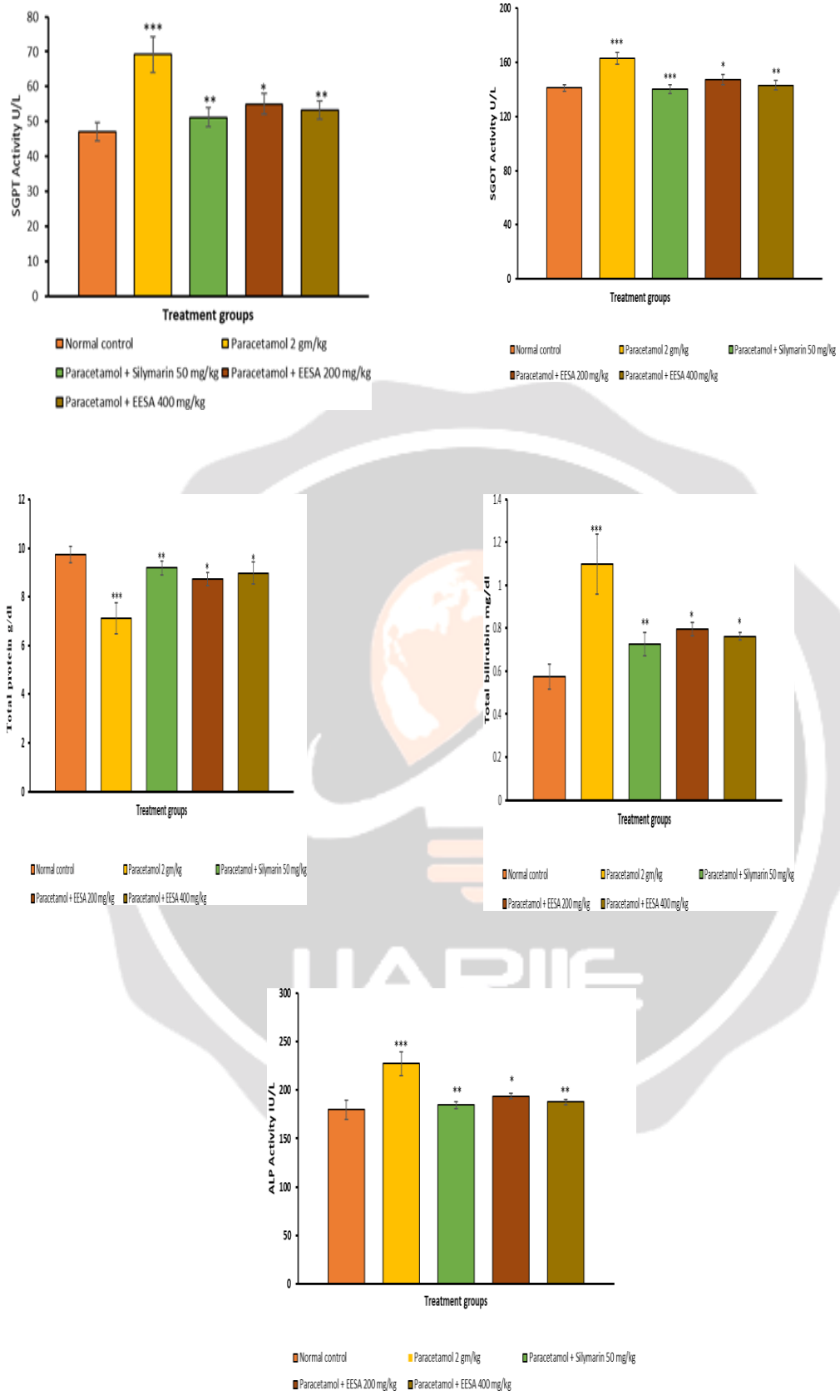


Figure 1: Effect of EESA on serum SGPT, SGOT, ALP, Total protein, Total bilirubin in paracetamol induced hepatotoxicity

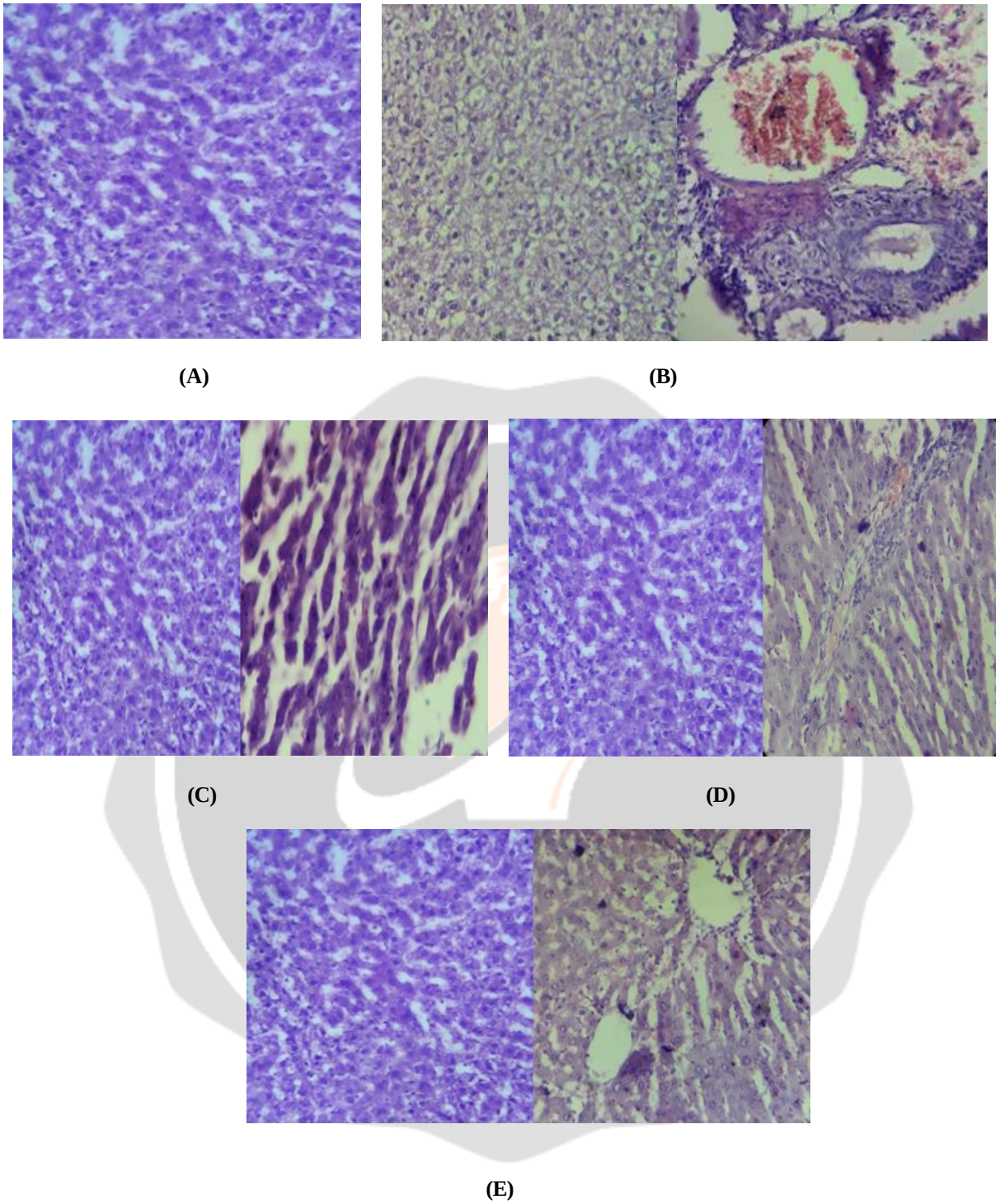


Figure 2: Effect of EESA on serum SGPT, SGOT, ALP, Total protein, Total bilirubin in Paracetamol induced hepatotoxicity. (A) Normal control, (B) Paracetamol Control (2 g/kg), (C) Paracetamol + Silymarin 50 mg/kg, (D) Paracetamol + EESA 200 mg/kg, (E) Paracetamol + EESA 400 mg/kg.

Hepatoprotective activity of *Sauropus androgynous* on Ethanol induced hepatic damage in rats:
30% Ethanol in dose of 1 ml/100 g b.w produced acute hepatic damage. Ethanol significantly increased the serum level of SGPT, SGOT, ALP, Total bilirubin and decreased the level of total protein. Ethanol was given as a toxicant. It was observed that, EESA significantly lowered ethanol induced levels of SGPT, SGOT, ALP, total bilirubin and increased the level of total protein. The levels of various serum marker enzymes were depicted in Table 2 & Figure 3.

Histopathological observations:

Histopathological observations of Normal control group (Figure 4A) showed normal appearing hepatocytes. Liver section of Ethanol control group (Figure 4B) showed ballooning degeneration of hepatocytes with few hepatocytes showing nuclear pyknosis and moderate infiltration of lymphocytes in the portal triad**.** Liver section of rats treated with Ethanol + Silymarin 50 mg/kg b.w (Figure 4C) showed normal appearing hepatocytes without any nuclear pyknosis and very less lymphocytic infiltration. Liver section of rats treated with Ethanol + EESA 200 mg/kg b.w (Figure 4D) showed normal appearing hepatocytes with mild lymphatic infiltration without ballooning degeneration of hepatocytes. Liver section of rats treated with Ethanol + EESA 400 mg/kg b.w (Figure 4E) showed normal appearing hepatocytes with less lymphatic infiltration, indicating the hepatoprotective activity of ethanolic extract of *Sauropus androgynous* (EESA).

Table 2: Effect of EESA on serum SGPT, SGOT, ALP, Total protein, Total bilirubin in Ethanol induced hepatotoxicity (n=6).

Groups	Serum analysis				
	SGPT (U/L)	**SGOT (U/L)**	**ALP (IU/L)**	**Total protein (g/dl)**	**Total bilirubin (mg/dl)**
Normal control	41.17 ± 1.42	128.31 ± 2.28	167.22 ± 12.02	9.11 ± 0.48	0.53 ± 0.032
30% Ethanol 1 ml/100g	54.60 ± 1.69***	144.61 ± 2.10***	208.83 ± 2.28***	6.64 ± 0.50**	1.13 ± 0.08***
Ethanol + Silymarin 50 mg/kg	42.08 ± 2.05***	130.31 ± 2.18***	176.99 ± 2.82**	9.24 ± 0.64**	0.60 ± 0.02***
Ethanol + EESA 200 mg/kg	47.27 ± 1.32*	135.79 ± 1.64*	185.32 ± 1.63*	8.57 ± 0.26*	0.79 ± 0.04*
Ethanol + EESA 400 mg/kg	45.53 ± 2.67**	133.20 ± 3.01**	181.86 ± 2.49**	9.01 ± 0.35**	0.71 ± 0.02**

All values are expressed as mean ± SEM. The statistical significance is analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Ethanol control group was compared to normal control group and treated groups (Ethanol + Silymarin 50 mg/kg, Ethanol + EESA 200 mg/kg & Ethanol + EESA 400 mg/kg) were compared to Ethanol control group and the P- values, ***p< 0.001, **p<0.01 & *p<0.05 are significant.

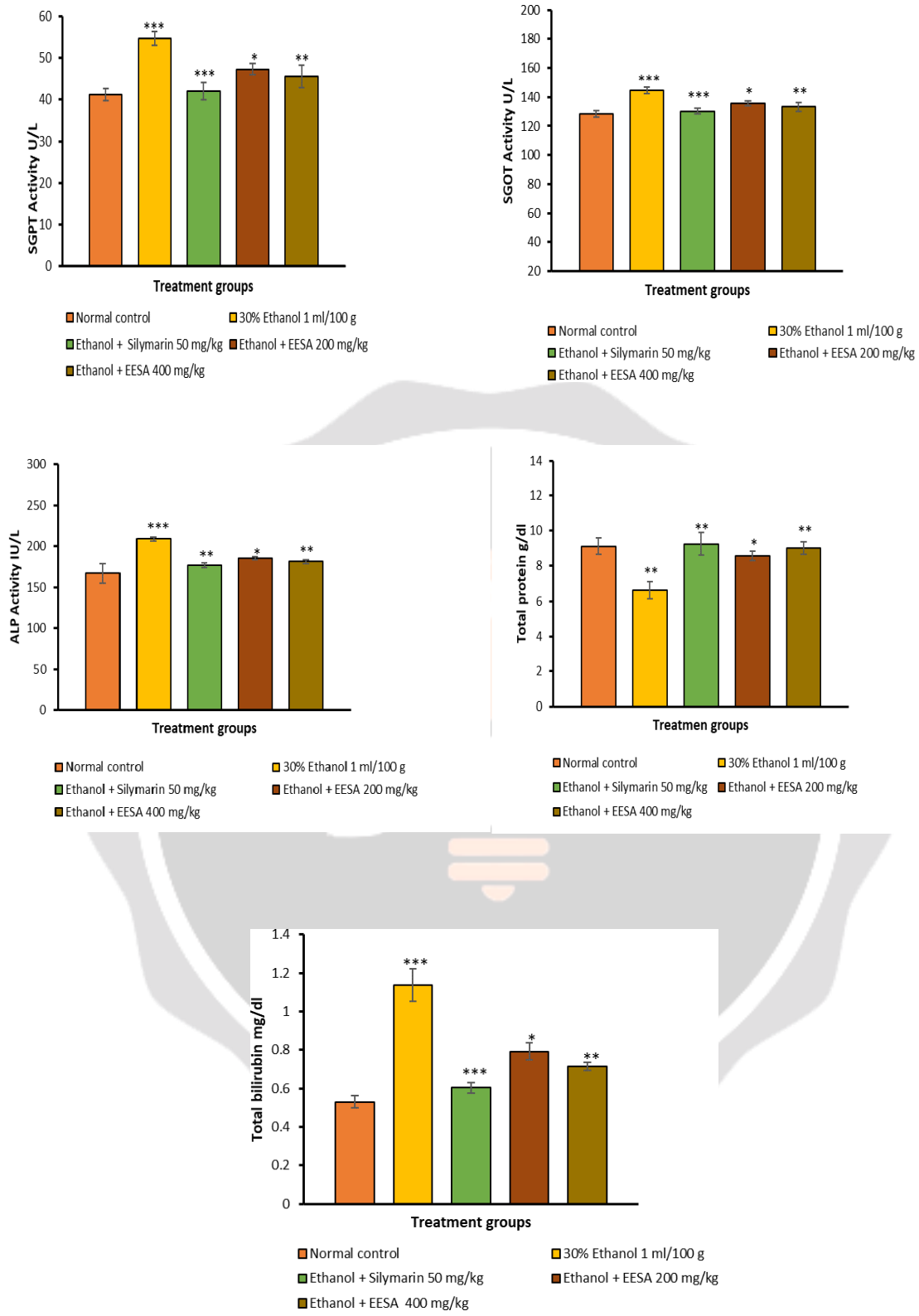
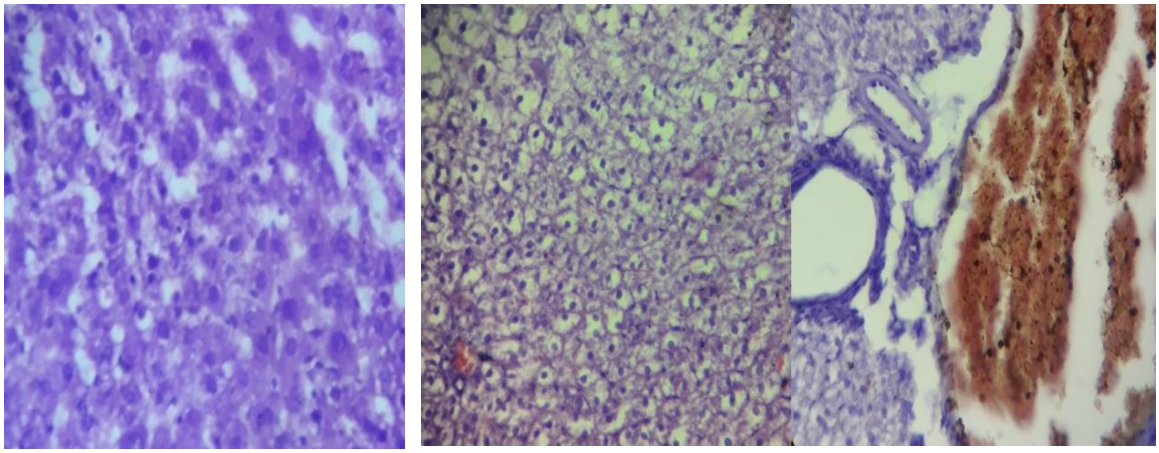
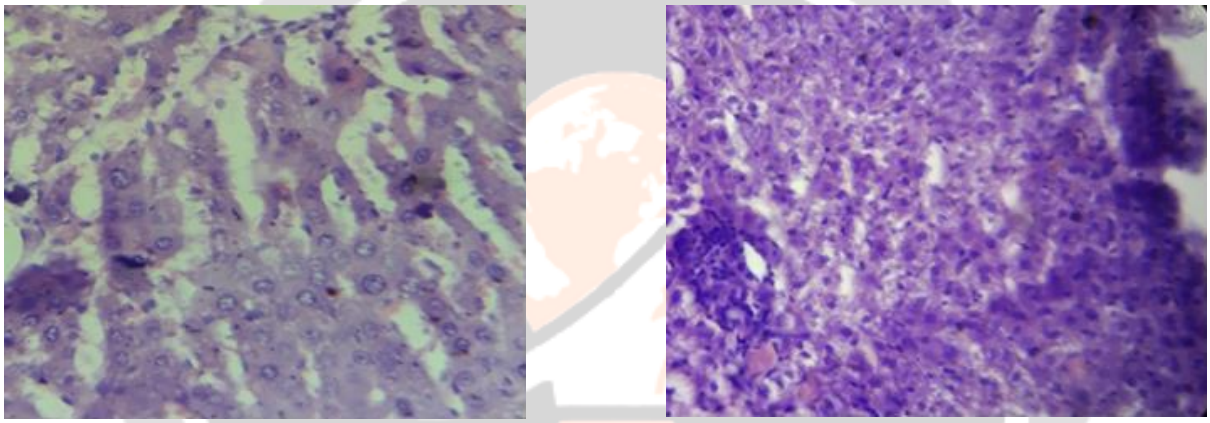


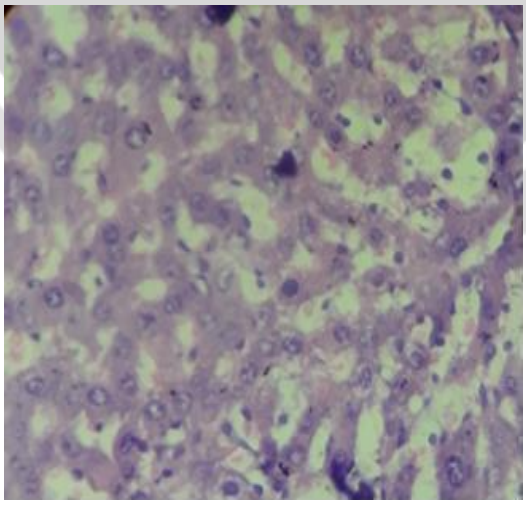
Figure 3: Effect of EESA on serum SGPT, SGOT, ALP, Total protein, Total bilirubin in Ethanol induced hepatotoxicity.



(A) **(B)**



(C) **(D)**



(E)

Figure 4: (A) Normal control, (B) Ethanol Control (1 ml/100g), (C) Ethanol + Silymarin 50 mg/kg, (D) Ethanol + EESA 200 mg/kg, (E) Ethanol + EESA 400 mg/kg.

DISCUSSION

Hepatotoxicity is the injury or liver damage caused by exposure to the drugs also called as toxic liver or toxic hepatitis. It is an adverse drug reaction that may be uncommon but serious. Drug induced liver injury is responsible for 5% of all hospital admission and 50% of all acute liver failures. The liver susceptible to injury by chemicals because of liver is the major organ in charge of breaking down fats, proteins, carbohydrates, drugs, and practically anything that enters our body. Since the metabolism of drugs occurs in the liver, the toxic effects of the drug effect liver. Chemicals (e.g., drugs, smoking, alcohol) and toxins (plant, viral, or bacterial) induce changes to the biochemical pathways in the liver cells, or induce an immune response, or make the liver cells extremely sensitive to the defence missiles or cytokines in the body. The cells of bile duct are also affected by exposure to hepatotoxic drugs.

Over the centuries, a number of medicinal plants have been exploited for the treatment of disorders associated with the liver or for the control of hepatotoxic aspects of diseases. These medicinal plants owe their activities due to the presence of phytoconstituents and may exert hepatoprotective effect by interfering generally with the pathways inducing toxicities or specifically with certain components of the pathway.

The present study has planned to evaluate the hepatoprotective activity of *Sauropus androgynous* leaves in scientific biological methods using ethanolic extract.

The initial phase of the study was the collection, authentication of the plant material and it was dried in shade to prevent the evaporation of volatile constituents and it was powdered. The powder was used for preparing ethanolic extract by Soxhlet method. The prepared ethanolic extract was dried and the percentage yield was found to be 10.25%. The phytoconstituents observed are carbohydrates, proteins, amino acids, alkaloids, flavonoids, glycosides, tannins and vitamins.

Flavonoids have beneficial effects in the hepatotoxic conditions and hence it is observed that leaves of *Sauropus androgynous* having hepatoprotective property but there is no pharmacological work was done for hepatoprotective activity. Based on this evidence the leaves of *Sauropus androgynous* are selected in the present study against two animal models. In the assessment of hepatoprotective activity Paracetamol and Ethanol induced hepatotoxicity models were used. SGPT, SGOT, ALP, total protein, total bilirubin was estimated. Histopathological observations have been carried out.

In this study, rats were treated with Paracetamol and Ethanol to develop a significant hepatic damage, which was observed a substantial increase in the activities of serum SGPT, SGOT, ALP, total bilirubin and decrease in total protein level. This is an indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Liver damage was assessed by histopathological examination, reports revealed that administration of Paracetamol and Ethanol has shown various ballooning degeneration of fatty cyst, nuclear pyknosis, infiltration of lymphocytes.

The animals treated with leaves of *Sauropus androgynous* has decreased the elevated levels of biochemical markers like SGPT, SGOT, ALP, total bilirubin & increase in total protein levels. Also, histopathological observation showed that the hepatic globular architecture was normalized, fewer lymphocytic infiltration was seen and hepatocytes appeared to be normal. These observations suggest that the leaves of *S. androgynous* possessed significant hepatoprotective activity against Paracetamol and Ethanol induced hepatotoxicity.

CONCLUSION

The present study was designed to evaluate the hepatoprotective activity of *Sauropus androgynous* in Paracetamol and Ethanol induced hepatotoxicity in experimental rats. On the basis of results, it has been concluded that the ethanolic extract of *Sauropus androgynous* of dose 200 mg/kg and 400 mg/kg has possesses dose dependent hepatoprotective property on paracetamol induced hepatotoxicity than compared to Ethanol induced hepatotoxicity. These could be due to the presence of different types of active principles, each with single or diverse range of biological activities. The present study provides the benefits of using leaves of *Sauropus androgynous* in prevention and treatment of hepatotoxicity.

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