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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF PHYLLANTHUS ACIDUS IN WISTAR RATS

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ABSTRACT

Objectives: The research set out to examine whether or not an ethanolic root extract of Phyllanthus Acidus (PA-E) might protect rats' livers against alcohol-induced damage.

Approach: Albino Wistar rats were given a 40 percent ethanol solution orally (2 milliliters per 100 grams of body weight) in order to cause hepatotoxicity. Oral doses of 100 mg/kg and 200 mg/kg of PA-E root extract were given for a duration of 21 days. Blood samples were taken from the retro orbital plexus on day 22 to assess the following biochemical parameters: total protein, albumin, triglycerides, total cholesterol, total bilirubin, creatinine, AST, ALP, urea, and total bilirubin. The animals were killed via cervical dislocation, and then their livers and kidneys were examined histopathologically.

The results showed that ethanol significantly raised liver weight, plasma ALT, AST, ALP, bilirubin creatinine, urea, triglycerides, and total cholesterol in comparison to the control group, but PA-E extract effectively prevented a significant decline in total protein and albumin concentration. Hepato protection was confirmed by histological findings, which corroborated the biochemical results.

Results suggest that PA-E root extract protects hepatocytes from ethanol-induced liver injury through its anti-oxidative effect. Phyllanthus acidus roots have strong hepatoprotective effects, according to the current study.

Keywords: Hepatoprotective, Ethanol, *Phyllanthus Acidus*, Silymarin, *In vivo.*

INTRODUCTION

When it comes to serious health problems, liver disease is at the top. In the treatment of liver diseases, herbal medications are crucial. Modern medicine lacks effective liver hepatoprotective medications, thus traditional medical systems rely on a variety of medicinal plants and their preparations to treat hepatic problems [1]. People are increasingly turning to herbal medicines as a means of illness prevention and treatment due to their perceived efficacy and the widespread perception that these remedies are risk-free due to their "natural" composition. Scientists have used a variety of approaches to study herbs' potential to treat liver problems, and they have often established the therapeutic efficacy, action mechanisms, and processes of action of these herbs [2]. There are about 300 different remedies for jaundice and chronic liver problems in Indian traditional medicine, but only a small

number of medicinal plants have been thoroughly studied using methods recognized worldwide [3]. If we want effective herbal medicines to treat serious liver diseases, we need to carefully test medicinal plants for characteristics like antiviral activity (hepatitis B, hepatitis C, etc.), antihepatotoxicity (antioxidants, etc.), choleretic activity, and stimulation of liver regeneration [4].

In traditional medicine, the plant Phyllanthus Acidus is used. It goes by many names: gooseberry tree, wild dracaena, Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, country gooseberry, star gooseberry, starberry, arbari, West India gooseberry, and so on. The spreading, open crown of this deciduous tree can reach a height of 6–9 meters, and it has few branches. Previous research by our group has shown that Phyllanthus Acidus root ethanolic extract (PA-E) has antioxidant activity in vitro and contains alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, and no anthocyanins or glycosides [5-7].

This research looked at the possibility that PA-E may protect the livers of Wistar albino rats against ethanol-induced dysfunction.

METHODS

Materials

A herbarium specimen was used to identify the whole Phyllanthus Acidus plant, which was taken from the semi-forest regions of India. Before being shade dried to a consistent weight, fresh plant materials were carefully rinsed under running water from the faucet to eliminate any sticking contaminants. The aerial portions and the roots were cut apart. After the roots were ground and sieved (using a 40-mesh size), they were placed in an airtight container. A container with a tight lid was used for storage. Span Diagnostics Ltd., an Indian company, supplied all of the pharmaceuticals, chemicals, and reagents utilized in the biochemical estimate. Wistar albino rats, both male and female, weighing between 150 and 200 g, were obtained and kept in a typical laboratory environment.

Preparation of extract

The Soxhlet device was used to extract the dried root powder with ethanol. The full extraction operations were safeguarded by using exhaustive extraction for 10 hours. After removing the solvent by evaporation in a rotary evaporator set at 60°C, the extract was dried by placing it in a desiccator for an hour until it formed a semisolid, sticky mass with a reddish-brown hue. The ethanolic extract had a yield of 19.22%.

Acute oral toxicity study

In accordance with recommendations 423 of the Organization for Economic Cooperation and Development [8,9], healthy Wistar albino female rats weighing 150-200 g were utilized for the acute oral toxicity test. The rats were kept under conventional laboratory conditions. The albino rats were housed in typical settings in polypropylene cages. Before the trial, the animals were given unrestricted access to water, but they were also starved for 16 hours. In a typical environment, the animals will have access to a dry pellet meal and water whenever they need it, with temperatures ranging from 23±2°C to 55±10% relative humidity and a 12 hour light/dark cycle. By maintaining a constant temperature and humidity level, one may shield themselves from the effects of weather fluctuations. The animals will be given a week to adjust to the research setting before the experiments begin, and all protocols will be adhered to meticulously in line with CPCSEA standards. Institutional Animal Ethics Committee (IAEC) approval was obtained for the experimental methods.

Experimemental design:

Induction of experimental hepatotoxicity

To conduct a toxicity investigation, rats were separated into five groups of six. Each group was given a daily oral dose of 40% ethanol (2 mL/100 g body weight) for 21 days. The impact of the ethanolic root extract was assessed using silymarin, a standard medication [8,10–14].

Collection of blood samples

Under halothane anesthesia, blood samples were taken on the 22nd day by puncturing the retro orbital plexus into dry, clean vials. After 30 minutes at room temperature, the samples were allowed to clot. Hematological parameters were tested using serum that had been separated by centrifugation (2500 rpm for 15 min) and kept at -20°C with the use of an anticoagulant, EDTA.

Biochemical estimation

Using analytical kits, the biochemical parameters were measured, including serum enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as total bilirubin, creatinine, urea, triglycerides, total cholesterol, albumin, and total protein content. The liver enzyme profiles were examined using enzyme-linked immunosorbent test kits.

Span Diagnostics Ltd.'s AST test kit was used to quantify blood AST according to the technique of Reitman and Frankel (1957) [15]. Span Diagnostics Ltd.'s ALT test kit was used to estimate serum ALT according to the technique of Reitman and Frankel (1957) [15]. Span Diagnostics Ltd.'s ALP test kit was used to assess ALP activity according to the approach of Kind and King (1954) [16]. Following the methodology proposed by Fawcett and Scott (1960) [17], the diagnostic kit was used to determine serum urea. A diagnostic kit was utilized to assess serum creatinine levels. This kit was based on the approach proposed by Tietz (1987) and relied on Jaffe's (1886) color reaction [18]. The modified DMSO technique, first described by Malloy and Evelyn in 1936, was then used to estimate total bilirubin [19]. The approach proposed by Foster and Dunn (1973) [20] was used to calculate triglycerides. Allain et al., 1974 [21] is the technique used to measure serum cholesterol in this investigation. Protein concentration estimation (Lowry et al., 1951) [22]. Using an albumin test kit (Span Diagnostics Ltd.) [23], the blood albumin was calculated according to the technique described by Corcoran and Durban, 1977.

Histopathological examination

The experiment came to a close on day 22 using the cervical dislocation technique of killing the rats. The organs, including the liver and kidneys, were removed and preserved in 10% formalin. Sections were generated using a microtome that was five microns thick. After being carefully removed, the tissues were preserved in 10% formalin. They were then gradually dehydrated using ethanol ranging from 50% to 100%, rinsed in xylene, and then embedded in paraffin. Cell necrosis, fatty change, hyaline regeneration, and ballooning degeneration were observed under a microscope using sections that had been produced and stained with hematoxylin and eosin dye (Luna, 1968) [24].

Statistical analysis

The results were presented as the averages plus or minus the standard error. Analysis of variance and Duncan's Multiple Range Test were used to statistically examine any significant difference between the means of the six groups. For every test, the significance thresholds were established at $p<0.05$.

RESULTS AND DISCUSSION

Acute toxicity

According to the findings of the acute toxicity investigation, the plant extract was safe because its LD 50 values were high. The results are shown in Table 1. No toxicity or death signs were observed in the rats that were given PA -E root extract orally, even at doses as high as 2000 mg/kg body weight. The behavioral and autonomic responses of rats were unaffected by the administration of PA-E root extract. Even at 2000 mg/kg, there was no mortality, which indicated that this dosage could be the LD50. Therefore, for the aim of investigating the hepatoprotective effects of the plant extract, the therapeutic dose was determined as 1/20th, 100 mg/kg as the low dose and 1/10th, 200 mg/kg as the high dose.

Table 1: *In vivo* hepatoprotective experimental design

GROUPS	CATEGORY	TREATMENT
GROUP I	Normal control	Received water (5 ml/kg. p.o.)
GROUP II	Negative Control(Toxic Control)	40% ethanol v/v (2.0 ml/l00 g body wt., p.o.)once daily for 21 days.
GROUP III	Standard (Silymarin)	Received 40% ethanol v/v (2.0 ml/l00 g body wt., p.o.) and standard drug Silymarin (25 mg/kg. p.o.) once daily for 21 days.
GROUP IV	Low Dose (100mg/kg)	Received 40% ethanol v/v (2.0 ml/l00 g body wt., p.o.) and PA-E extract once daily for 21days.
GROUP V	High Dose(200mg/kg)	Received 40% ethanol v/v (2.0 ml/l00 g body wt., p.o.) and PA- E extract once daily for 21days.

The effect of PA-E extract on average liver weight changes in *Wistar* albino rats

The effects of PA-E and the standard medicine Silymarin on average liver weight in rats were shown in Table 2 and Fig. 1. The animals were either normal or ethanol-induced hepatotoxic. The rats in the ethanol-induced hepatotoxic group had an increase in average liver weight, according to the results. The high liver weight was reduced by administering PA -E extract, and the group treated with silymarin had their liver weight returned to a nearly normal level.

Table 2: Effect of *Phyllanthus Acidus* extract on average liver weight changes in *Wistar* albino rats

Treatment	Final Liver Weight (g/100g)
Group I Normal control	B 4.52±0.29*
Group II Negative /Toxic Control	A 6.14±0.33 *
Group III Standard (Silymarin)	a, b4.82±0.44* *
Group IV Low Dose (100mg/kg)	a, b5.18±0.39 ** *
Group V High Dose (200mg/kg)	a, b4.99±0.53 * *

Values are expressed as mean □□SE (n=six rats)
p values: * < *0.001;* ** < *0.05*
a →group I matchup with groups II, III, IV, V. b →group II matchup with groups III, IV, V.

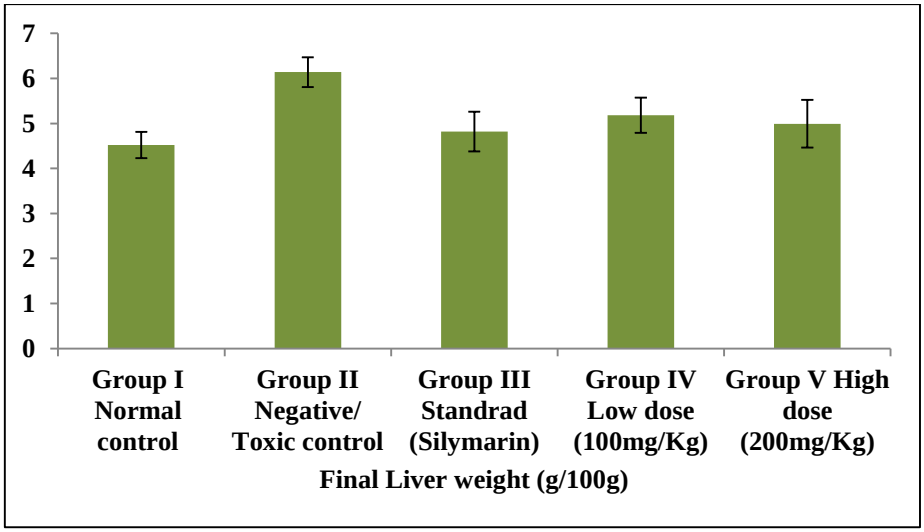


Fig. 1: The effect of PA-E root extract on average liver weight changes in rats

Effect of PA-E on liver enzymes and other parameters in ethanol-induced hepatotoxicity in *Wistar* rats

Both normal and ethanol-induced hepatotoxic rats had their blood hepatic marker enzymes examined by PA-E root extract, as shown in Table 3 and Figure 2. In Group II rats, the hepatotoxic control group had elevated levels of ALT (162.38 ± 4.62), AST (202.48 ± 4.72), and ALP (484.62 ± 8.36). Rats in Groups IV and V had significantly lower levels of AST, ALP, and ALP after receiving PA-E extract. Group IV rats that were given the lesser dosage (100 mg/kg) had ALT levels of 120.52 ± 4.22, AST levels of 126.42 ± 4.62, and ALP levels of 3006.88 ± 1.32. The ALT, AST, and ALP levels in Group V rats were 72.49 ± 3.18, 96.36 ± 4.74, and 234.26 ± 8.40, respectively, after the administration of a higher dosage of 200 mg/kg. The increased levels of the hepatic marker enzymes were reduced by

administering PA-E root extract, and silymarin brought the enzyme activity down to normal ranges. Therefore, animals treated with PA-E extract had a much reduced exposure to ethanol's harmful effects.

Table 3: Effect of the ethanolic extract of roots of *Phyllanthus Acidus* on liver enzymes (ALT, AST and ALP) by ethanol-induced hepatotoxicity in *Wistar* albino rats

TREATMENT	-1 ALT (IU. L)	-1 AST (IU. L)	-1 ALP (IU. L)
Group I Normal control	b* 60.11 ± 4.52	b* 96.98 ± 2.66	b* 220.50 ± 9.40
Group II Toxic Control	a*162.38 ± 4.62	a*202.48 ± 4.72	a*484.62 ± 8.36
Group IIIStandard (Silymarin)	a*, b* 61.12 ± 3.44	a*, b* 81.68 ± 4.12	a*, b* 201.82 ±9.34
Group IV Low Dose (100mg/kg)	a**, b** 120.52 ± 4.22	a*, b** 126.42 ±4.62	a**, b* 306.88 ±1.32
Group V High Dose(200mg/kg)	a*, b* 72.49 ± 3.18	a**, b* 96.36 ± 4.74	a*, b* 234.26 ± 8.40

Values are shown as the average plus or minus the standard error (n=6 rats). The p-values are as follows: * < 0.001; ** < 0.05.

a. Groups II, III, IV, and V will meet up with groups I. b. Groups III, IV, and V will meet up with groups II.

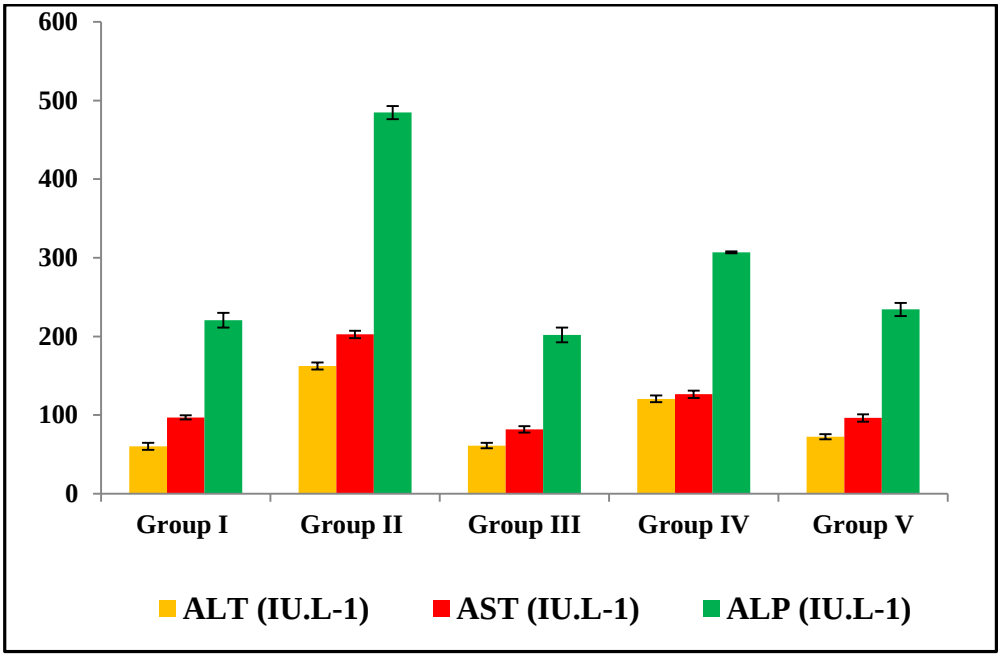


Fig. 2: Effect of plant extract (PA-E) on liver enzymes (ALT, AST, and ALP) by ethanol-induced hepatotoxicity in *Wistar* albino rats

Serum creatinine, urea, and total bilirubin levels were assessed in rats that had been caused hepatotoxicity by ethanol. The results were described in Table 4 and Fig. 3. Compared to the control group of rats (Group I), the hepatotoxic control group (Group II) exhibited elevated levels of creatinine, urea, and total bilirubin. The increased levels of creatinine, urea, and total bilirubin in rats that had been provoked to toxicity were significantly reduced after treatment with PA-E roots extract. Silimarin was able to return these levels to normal.

Table 4: Effects of plant extract (*Phyllanthus Acidus*) on serum urea, creatinine and TB in alcohol treated rats

TREATMENT	UREA -1 (milimol)	CREATININE -1 (milimol)	TB (mg/dl)
Group I Normal control	b* 7.82 ± 0.76	b* 56.44 ± 4.10	* 1.60±0.08
Group II Toxic Control	a*	a*	a*

	20.28 ± 1.42	82.20 ± 6.56	5.12 ± 0.10
Group III Standard (Silymarin)	a*, b* 7.56 ± 1.12	a*, b* 59.16 ±6.24	a*, b* 1.50 ± 0.08
Group IV Low Dose (100mg/kg)	a*, b* 10.22 ± 1.18	a*, b* 68.78± 5.36	a**, b* 2.38 ± 0.10
Group V High Dose (200mg/kg)	a**, b* 8.11± 1.04	a**, b* 60.63 ± 5.50	a*, b* 1.94 ± 0.08

Values are shown as the average plus or minus the standard error (n=6 rats). The p-values are as follows: * < 0.001; ** < 0.05. Group I will be paired with groups II, III, IV, and V. Group II will be paired with groups III, IV, and V.

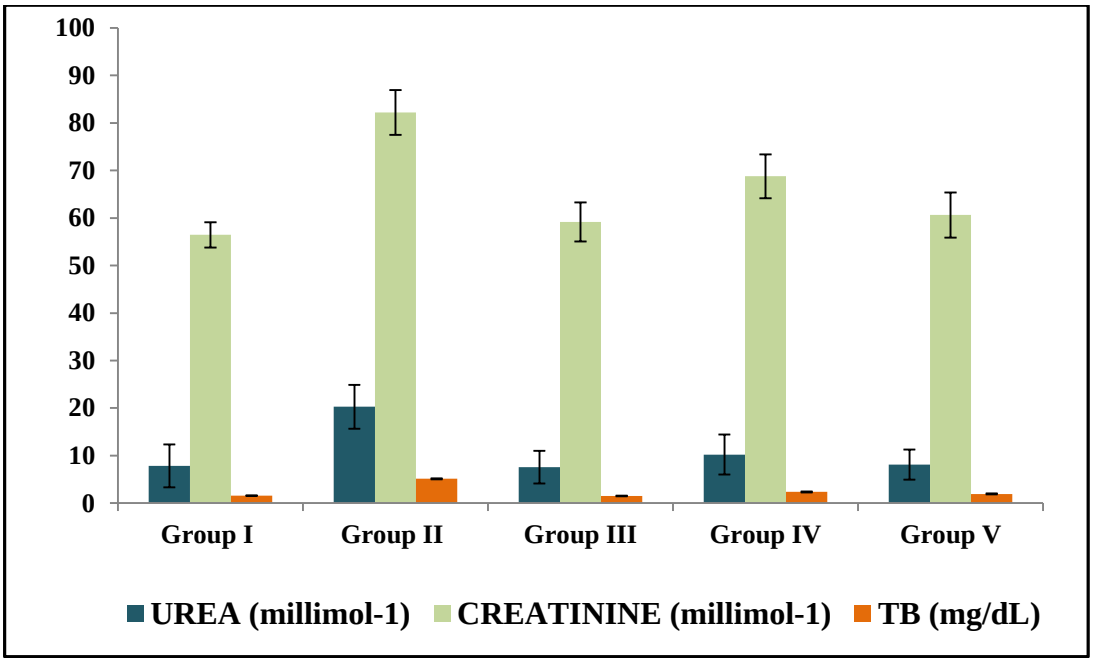


Fig. 3: Effects of the plant extract (PA-E) on serum urea, creatinine, and total bilirubin in alcohol treated rats

We can see how PA-E root extract affected blood TG and TC levels in rats that were caused hepatotoxicity by ethanol in Table 5 and Figure 4. When compared to the rats in Group I, the hepatotoxic (negative control) group (Group II) exhibited elevated levels of TG at 92.45 ± 4.88 and TC at 212.44 ± 3.46. Rats in Groups IV and V had significantly lower levels of TG and TC after being orally administered PA-E extracts. In Group IV rats, the levels of TG and TC were significantly reduced (74.26 ± 4.22 and 124.66 ± 3.28, respectively) when the lower dose of PA-E root extract was administered orally, in comparison to the Group II hazardous group. The level of TG and TC in Group V rats was significantly reduced (60.62 ± 3.28 and 99.42 ± 2.56) after an oral administration of a higher dosage of PA-E root extract. The levels of TG and TC were significantly reduced to nearly normal in Group III, which was treated with silymarin.

Table 5: Effects of plant extract on serum TG and TC in ethanol treated rats

TREATMENT	TG (mg/dl)	TC (mg/dl)
Group I (Normal control)	b* 58.33 ± 2.04	b* 95.89 ± 1.94
Group II (Toxic Control)	a* 92.45 ± 4.88	a* 212.44 ± 3.46
Group III Standard (Silymarin)	a*, b* 58.22 ± 3.56	a*, b* 93.75 ± 2.05
Group IV Low Dose	a*, b**	a*, b**

(100mg/kg)	74.26 ± 4.22	124.66 ± 3.28
Group V High Dose (200mg/kg)	a**, b* 60.62 ± 3.28	a*, b* 99.42 ± 2.56

The data represent the average plus or minus the standard error of six rats. The p-values indicate that the groups I and II were matched with groups II, III, IV, and V, and group II was matched with groups III, IV, and V. The significance levels were *<0.001 and **<0.05, respectively.

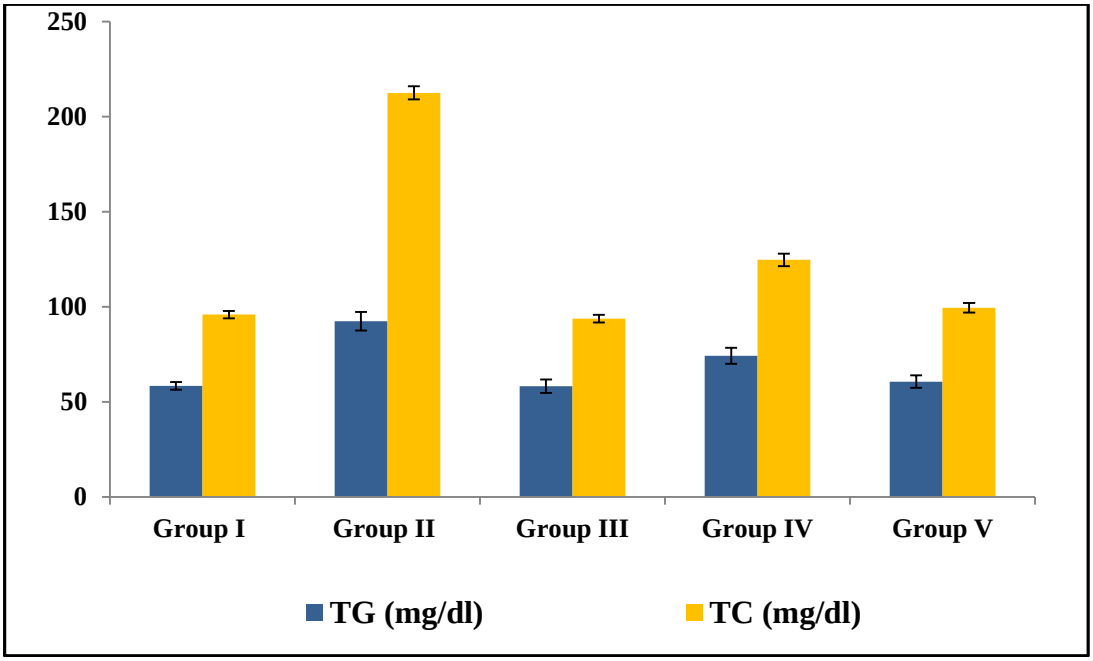


Fig. 4: Effects of the plant extract on serum TG and TC in the ethanol treated rats

Both normal and ethanol-induced hepatotoxic rats were used to illustrate the effects of PA-E root extract on total protein and albumin levels in blood (Table 6 and Fig. 5). A decrease of 5.68 ± 1.32 TP and 5.30 ± 1.15 albumin was seen in the hepatotoxic control group. The levels of TP and ALB were significantly elevated in Groups IV and V rats after oral administration of PA-E extract. Group IV rats exhibited a decent rise in TP and albumin levels of 7.29 ±1.33 and 6.85±1.20, respectively, after being orally administered the lower dosage of PA-E extract. When given orally, a large dosage of PA-E root extract nearly brought the TP level back to normal in rats from Group V. The levels of TP and albumin in rats treated with silymarin were much higher than in the control group.

Table 6 Effects of plant extract on serum TP and ALB in alcohol treated rats

TREATMENT	**TP mg/dl)**	**ALBUMIN (mg/dl)**
Group – I (Normal control)	7.81 1.21^{b*}	7.18 ± 1.35^{b*}
Group – II (Toxic Control)	5.68± .32^{a*}	5.30 ± 1.15^{a*}
Group – III Standard (Silymarin)	8.01± 1.44$^{a*, b*}$	7.35 ± 1.26$^{a*, b*}$
Group – IV Low Dose (100mg/kg)	7.29 ±1.33$^{a*, b*}$	6.85± 1.20$^{a**, b*}$
Group – V High Dose (200mg/kg)	7.85 ± 1.45$^{a*, b*}$	7.34 ± 1.28$^{a*, b*}$

The numbers represent the average plus or minus the standard error of six rats. The p-values indicate that the groups I and II matched with each other, whereas the groups III and IV matched with each other.

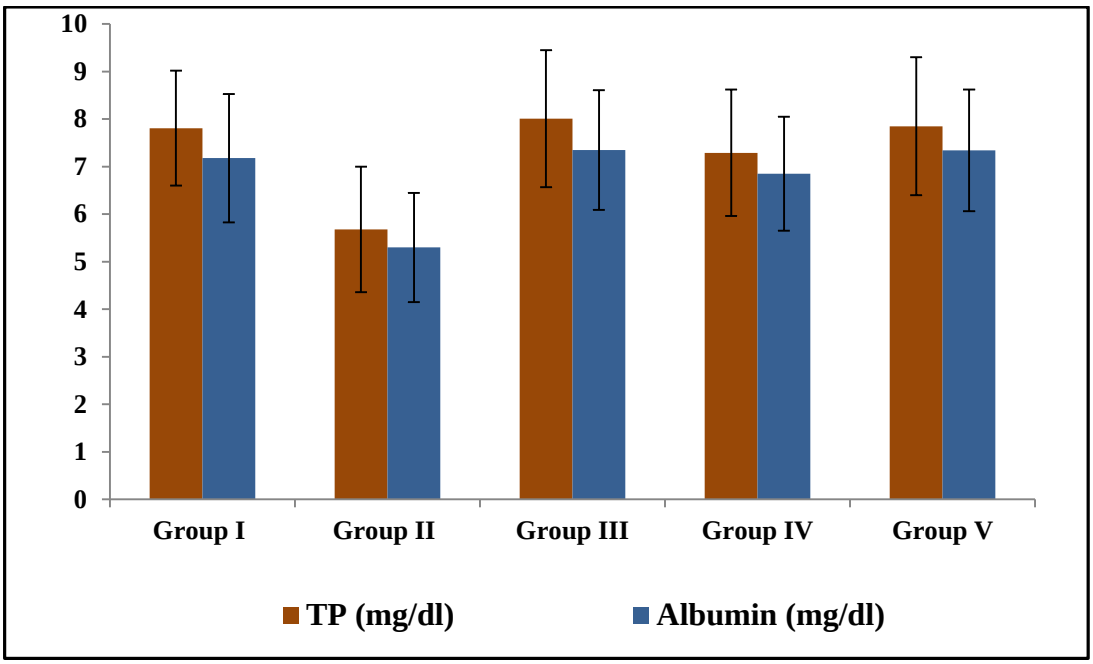
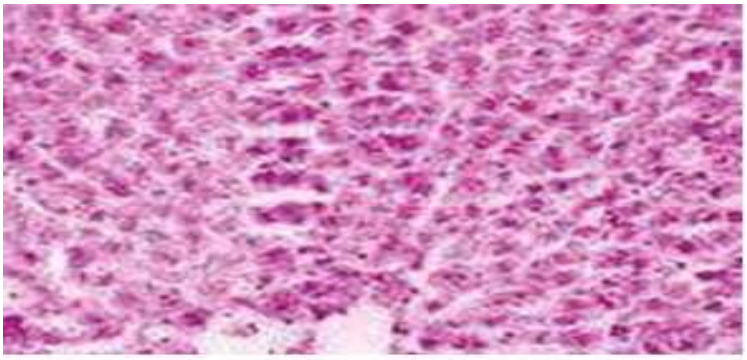


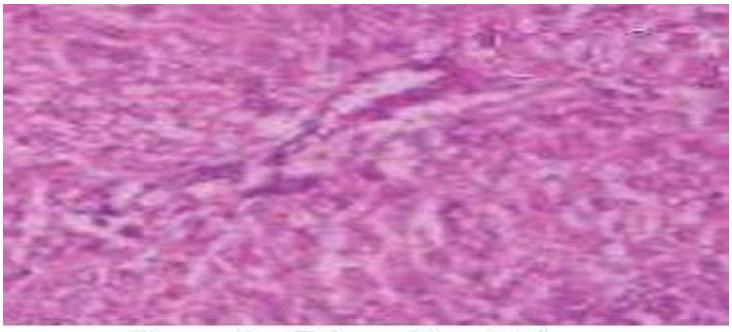
Fig. 5: Effects of the plant extract on serum TP and ALB in the alcohol treated rats

Histopathological results of liver and kidney

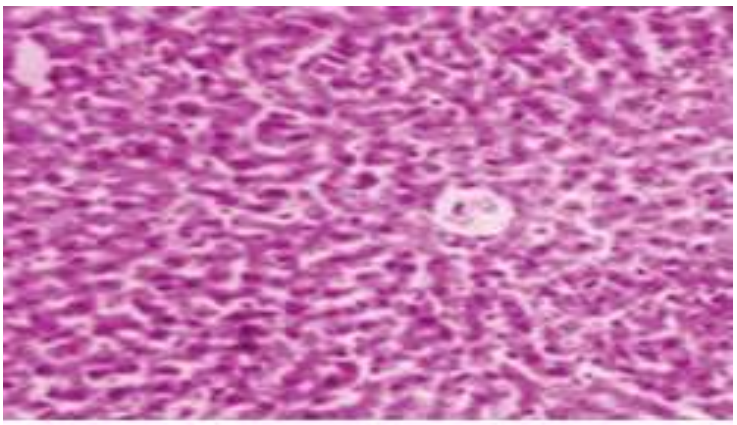
Histopathological analyses of ethanol-intoxicated rats' livers and kidneys showed the precise cellular alterations that took place. The livers of the rats in the control group that were otherwise healthy showed classic features of the organ, including a large nucleus, a well defined central vein, and a nucleolus. Severe toxicity, inflammatory cell collection, endothelial cell swelling, and clogged blood vessels were seen in the toxic control alcohol treated rat liver (Group II). Inflammation was significant in the rats treated with low doses (Group IV). Group V, which consisted of rats given high doses, had little inflammatory cells around the portal tract. Group III rats given silymarin had significant regeneration after ethanol toxication, with no signs of necrosis and intact cellular boundaries.



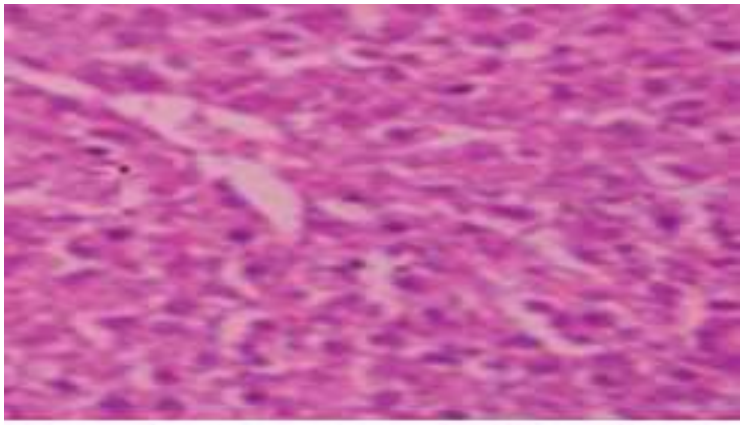
Group 1- Normal Control



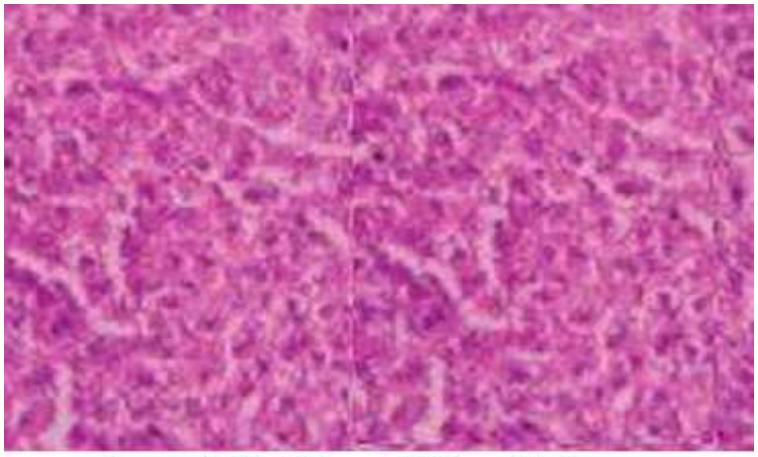
Group 2 : Ethanol treated



Group 3- Silymarin



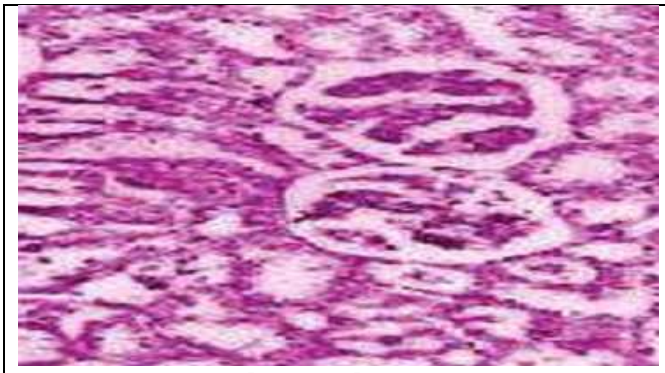
Group 4: Low Dose (100 mg/Kg)



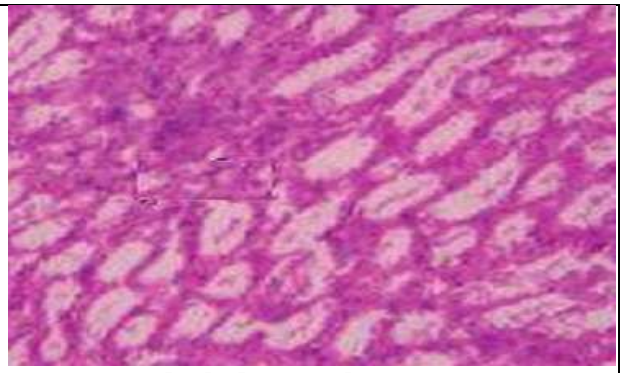
Group 5: High Dose (200 mg/Kg)

Figure: Histological studies in liver tissue

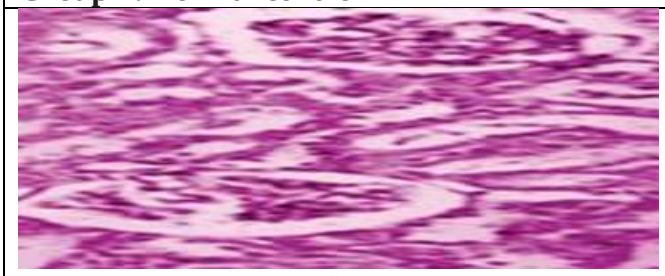
Group II rats given alcohol had enlarged tubules and hazy enlargement of tubules as revealed by histological examinations of kidney tissue. Group V rats given a high dosage of alcohol (200 mg/kg) showed a dramatic reduction in the alterations and an almost normal look of the kidneys. The kidneys of the control rats treated with the experimental drug exhibited no abnormalities histologically.



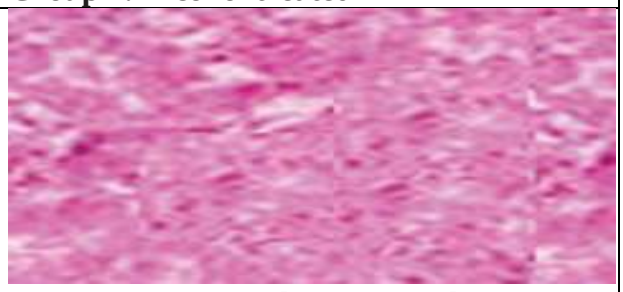
Group 1: Normal control



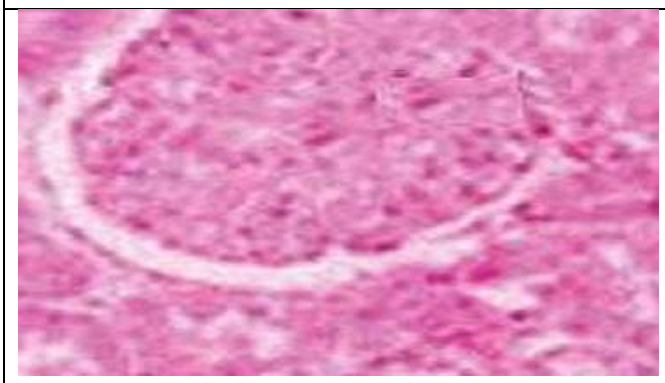
Group 2: Alcohol treated



Group 3: Silymarin



Group 4: Lower dose (100mg/kg)



Group 5: High dose (200mg/kg)

Figure: Histological studies in kidney tissue

CONCLUSION

This folk medicinal plant, Phyllanthus Acidus, has notable hepatoprotective activity, according to the results of the current study. This could be because it contains phytochemicals like polyphenols, flavonoids, and alkaloids, which have free radical scavenging and antioxidant properties. It backs up the long-held beliefs that this herb may cure a variety of liver problems. These results demonstrate that Phyllanthus Acidus is effective in preventing and treating liver diseases by preserving the hepatocytes' functional state. In light of the promising findings, more studies may be conducted to determine the phytoconstituents and assess their hepatoprotective properties after isolation, identification, characterization, and standardization. Research on the specific phytoconstituents and how they protect the liver should also be undertaken.

AUTHORS' CONTRIBUTION

This work is co-authored by a research scholar and a research guide.

COMPETING INTERESTS

There is no perceived bias or conflict of interest, according to the writers.

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None.

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