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Evaluate The Blood Detoxifying And Hepatoprotective Activity Of Semecarpus Anacardium Linn in Wistar Rats

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A B S T R A C T:

This study explores the potential of Semecarpus anacardium Linn leaf ethanolic extract as a natural remedy for liver damage and blood toxicity caused by acetaminophen overdose. Using a well-established animal model, male Wistar rats were divided into five groups: a healthy control group, an acetaminophen-induced toxicity group, a standard treatment group receiving N-acetylcysteine (NAC), and two treatment groups administered ethanolic extract of S. anacardium leaves (EESA) at doses of 200 mg/kg and 400 mg/kg. Liver function was assessed by measuring serum levels of key enzymes (ALT, AST, ALP) and total bilirubin, while blood profiles (CBC) and liver tissue histology were also evaluated. The findings showed that EESA, particularly at the higher dose, significantly reduced liver enzyme levels and improved overall liver architecture. Haematological improvements were also observed, including normalization of red and white blood cell counts and platelet levels. These results suggest that S. anacardium possesses promising hepatoprotective and blood-purifying properties, likely due to its antioxidant and anti-inflammatory constituents. This study supports the traditional use of this plant in liver-related disorders and encourages further investigation into its active compounds and mechanisms of action.

Keywords: Semecarpus anacardium Linn; hepatoprotective activity; Wistar rats; acetaminophen-induced hepatotoxicity; blood detoxification; ethanolic extract; antioxidant activity; liver enzymes; histopathology; N-acetylcysteine.

Introduction

Liver is the largest gland and second largest organ in the human body. which plays a key role in metabolism, detoxification, and excretion of different endogenous and exogenous chemicals and it is the most significant organ in the human body.[1] Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites through detoxifying and eliminating them. The liver, a major organ of metabolization and excretion, is susceptible to a number of pathologies, classified as cirrhosis, acute chronic hepatitis and hepatitis. The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. [2] It is involved in almost all biochemical path ways to growth, to fight against infections, diseases, nutrient supply, energy provision etc. [3] The major functions of liver are carbohydrate, protein and fat metabolism, detoxification, storage of vitamin and formation of bile. [4] Liver detoxifies drugs, alcohol, chemical compounds, heavy metals, infectious organisms, as well as toxin by way of products from the blood. Furthermore, the liver eliminates blood contaminants and waste products such as chemical substances, drugs, viruses, bacteria, parasites, fungi, pesticides and herbicides, fat, meals components, alcohol, and dead cells. It metabolizes substances in the bloodstream earlier than they are distributed to the exclusive components of the body in which they're needed.[5]

Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In spite other tremendous advances made in allopathic, no effective hepatoprotective medicine is available.[6]
Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. [7,8] In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations (Hikino and Kiso, 1988; Handa et al., 1989; Sharma et al., 1991; Evans, 1996). [12,14]

Semecarpus anacardium Linn. (Anacardiaceae) also known as Bhallatak and marking nut tree. It typically grows in tropical and subtropical deciduous forest of the Indian subcontinent, including parts of India, Sri Lanka, and Nepal. *Semecarpus anacardium Linn*. is known for its diverse bioactive properties.[15] Scientific studies have demonstrated that extracts from its fruit , leaves and nut possess anti-inflammatory, antioxidant, antimicrobial, anti-cancer, activities. Due to these properties, the plant is being investigated for potential therapeutic applications in conditions such as hepatoprotection , inflammatory diseases .[16] Its unique chemical composition makes it a promising candidate for pharmacological research and drug development.[18] Therefore, the present study was aimed at determining the hepatoprotective and blood detoxifying activity of ethanol extract of *Semecarpus anacardium* leaves (EESA) using the Acetaminophen induced liver damage rats as the animal model .

Material and Method

2.1 Experimental animals

Wistar albino male rats (150-250gms) obtained from the animal house, Department of Pharmacology, Vidyabharati College of Pharmacy Amravati, Maharashtra 444602 . The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk bedding . Animals were housed at a temperature of 24±2 C and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chew. All animal experiments were carried out according to NIH guide lines after getting approval of the Institute's Animal Ethics Committee (IAEC) accordance with the guidelines.

2.2 Plant material

The fresh leaves of the plant material would be collected from the area like medicinal garden of Shivaji College of Science Amravati. The plant was identified by a botanist, and voucher specimen was deposited in the Department of Botany in Vidya Bharati Mahavidyalaya Amravati. After authentication, the plants were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

2.3 Preparation of plant extract:

Plant leaves were washed in running tap water for removing of dust particle and foreign particles and shade dried for 8 days. Shade dried leaves were used for preparation of powder with electric blender. These powders were stored in plastic container for further use. Around 25 gm powder used for extraction in 250 ml ethanolic solvents. The extraction was done by simple extraction techniques till dark coloration of the solvent and discolorations of powder extract. The solvents were evaporated to complete dryness by rotavator and stored in Eppendorf's tube at 4 ˚C for further use.

2.4 *Chemicals*

Acetaminophen , N-acetylcysteine (NAC;) were used in the study. All other chemicals and reagents used were of analytical grade.

2.5 Experimental design

A total 30 Albino Wistar male rats were randomly divided into five groups each group contain six animals and were kept in the experimental period of 14 days, as follows,

- Group I (vehicle)
  Animals received 0.9% NaCl solution at 0.5 mL/rat with normal diet.
- Group II (Negative control)
  Animals received Acetaminophen alone at 2gm/kg BW (p.o.) dissolved in the vehicle.
- Group III (Standard control)
  It serving as positive control received 100 mg/kg NAC.
- Group IV (Treatment 1)
  Animals received (Acetaminophen (2gm/kg) + Semecarpus anacardium extract (200mg/kg))
  BW, p.o. dissolved in the vehicle.
  Group V (Treatment 2)
  Animals received Acetaminophen (2mg/kg) + Semacarpus anacardium extract (400mg /kg) dissolved in the vehicle.

At the end of the experimental period, On 15th days the animals were subjected to diethyl ether and anaesthetised . Blood samples were collected from tail vein for estimation of biochemical parameters such as ALT, AST, ALP, and total bilirubin concentration and CBC . Serum was separated by centrifuging blood at 2500 rpm for 10 min and the levels of AST, ALT, ALP, total bilirubin were analyze .

2.6 Histopathological studies:

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with haematoxylin and eosin (H–E) dye for photo microscopic observation, including cell necrosis, fatty change .[14]

2.7 Statistical analysis

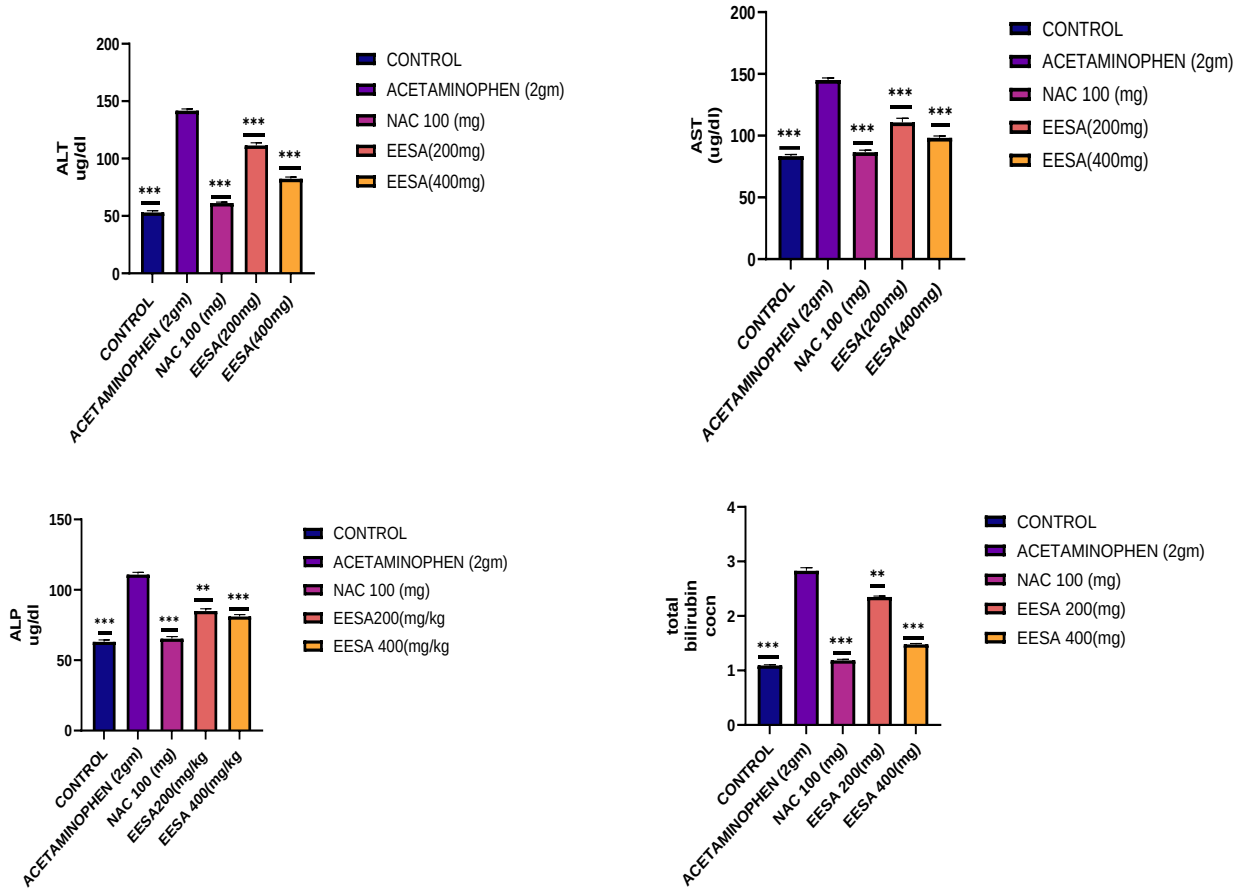
The results were expressed as means ± standard errors of mean (SEM). The data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's test to determine the level of significance. A value of *$p<0.05$, **$p<0.01$, ***$p<0.001$ were consider statistically significant compare to the negative control group.

Result

Biochemical characterization

The present studies were performed to assess the hepatoprotective and blood detoxifying activity of various solvent derived leaf ethanolic extracts of semecarpus anacardium linn in rats against acetaminophen as hepatotoxin which cause liver damage. Altering liver microsomal membranes of hepatocytes and cell damage results release of enzymes AST, ALP and ALT. Estimation of levels of AST, ALT, ALP, bilirubin concentration in serum is used to assess hepatic function in rats. The hepatic enzymes AST, ALT, ALP, bilirubin concentration and in serum was significantly (P<0.05). The toxic effect of acetaminophen was controlled in the animals treated with ethanolic extracts of Semecarpus anacardium linn (200mg/kg & 400mg /kg) was taken.Among the six groups ethanolic extracts was effectively controlled the liver damage induced by acetaminophen way of restoration of the levels of liver function .

(Table no 1: Effect of Acetaminophen induced by (2gm/kg) treated with Ethanolic extract of Semecarpus anacardium linn leaves (EESA200 mg/kg, 400 mg/kg), and N acetyl cysteine (100 mg/kg) on A) ALT B) AST C) ALP D) Total bilirubin concentration. Values are mean ± SEM (n = 6) and analysed with one-way ANOVA followed by Dunnet's test using Graph pad prism version 10.2, p<0.5*, **p <0.01, ***p<0.001 were consider statistically significant compare to the acetaminophen (negative control) group.)



(**Fig no 1**.This figure illustrate the serum ALT, AST, ALP and total bilirubin concentration levels across experimental groups Acetaminophen induced hepatotoxicity. Both NAC and EESA significantly reduced serum levels, with EESA showing a dose-dependent protective effect. The results suggest EESA has potential hepatoprotective activity comparable to NAC.**)**

3.2 Haematological analysis

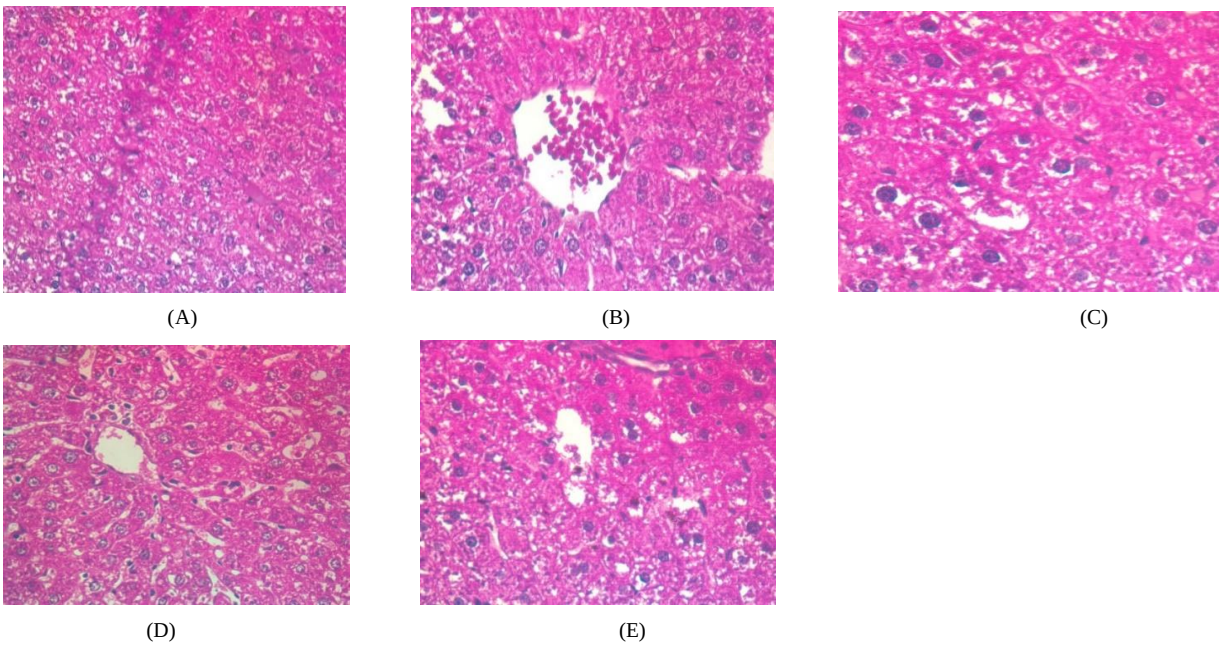
The results of the haematological indices showed that RBC, HGB, MCH, PLT and WBC were reduced compared to the (negative control) Acetaminophen induced. While this reduction was insignificant ($p > 0.01$) for RBC, GB and MCH, it was marginally significant ($p < 0.05$) for PLT and WBC. Only MCHC increased in the effluent treated rats but was insignificantly ($p > 0.05$) different from the negative control (APAP induced).

Parameters/ Groups	RBC	WBC	Haemoglobin	Platelet Count	MCH	MCHC
Control	**6.5±0.3***	**8.0±0.6****	**13.5 ± 1.13***	**2.5± 0.2****	**27.0±0.7***	**33.5±1.2***
Acetaminophen	**3.2 ± 0.4**	**14.7 ± 0.9**	**7.4 ± 0.6**	**1.5 ± 0.3**	**15.7± 0.9**	**26.6 ± 1.4**
N-acetyl cysteine	**6.3±3.5***	**7.5± 0.6****	**11.2 ± 05***	**2.0 ± 0.2***	**23.0±0.6***	**29.2±1.5***
EESA 200	**4.9 ±0.2***	**12.2± 0.5***	**9.2 ± 0.4***	**3.1 ±0.2****	**19.5 ±0.7***	**28.5 ±1.0***
EESA400	**5.9±0.3***	**10.0±0.4****	**10.6 ± 0.5***	**2.8±0.2****	**21.7±0.6***	**29.1±1.0***

(**Table no.2.** Values are mean ± SEM (n = 6) and analysed with one-way ANOVA followed by Dunnet's test *p<0.05, **p <0.01, were consider statistically significant compare to the acetaminophen (negative control) group).

3.3 Estimation of Histopathological studies:

Anatomy of liver was studied Immediately after sacrificing the animals. A small portion was fixed in 10% neutral buffered formalin as described by luna. This section of 4-5 µm were taken, stained with haematoxylin and Eosin and histology was studied. The result was expressed in mean ± SEM, Student t test, was used for statistical significance between groups. Rats in all group were killed and livers were dissected out and fixed and processed to paraffin blocks and alcohol dehydration and Xylene clearance. Liver tissue was sectioned at 5 µm thick and stained with haematoxylin and eosin for routine observation. The section was stained and then sent for grossing.



(A) (B) (C)

(D) (E)

(Figure 2: A) Section of liver tissue of control rat showing normal hepatic cells with central vein. **B)** Section of infected liver tissue of rat when given acetaminophen to induce damage showing severe necrosis, massive vascular degeneration and dilated blood sinusoids are filled with red blood cells. **C)** Section of treated liver tissue of rat with NAC. **D)** Section of treated liver tissue of rat with Ethanoic extract of leaves *Semecarpus anacardium* 200 mg/kg a moderate reduction and partial restoration of hepatocytes was observed **E)** Section of treated liver tissue of rat with Ethanoic extract of leaves *Semecarpus anacardium* 400 mg/kg showing normal hepatocytes with regenerating hepatocytes, diminution of fibrosis and reduction in fibrotic area was observed .

Discussion

The present study investigated the hepatoprotective and blood detoxifying effects of Semicarpus anacardium extract in acetaminophen-intoxicated rats. The extract was administered in two doses—200 mg and 400 mg—to evaluate its dose-response relationship. Biochemical results demonstrated that both doses mitigated liver damage to varying extents, with the 400 mg of EESA dose showing a significantly greater hepatoprotective effect. In the 200 mg group, a moderate reduction in liver enzyme levels and partial restoration of hepatic function were observed, suggesting the onset of protective activity potentially due to antioxidant and anti-inflammatory constituents. In contrast, the 400 mg dose resulted in a marked decrease in ALT, AST, ALP, and total bilirubin levels, approaching values seen in the untreated control group. This improvement may be attributed to the higher concentration of bioactive phytochemicals exerting enhanced free radical scavenging, membrane-stabilizing, and anti-inflammatory effects. The mechanism by which Semicarpus anacardium exerts its protective effect may involve several bioactive compounds, such as flavonoids, tannins, and phenols , which are known for their antioxidant and hepatoprotective properties. These phytochemicals likely function by neutralizing reactive oxygen species, enhancing cellular antioxidant defences, and preserving membrane integrity. While the findings of this study provide compelling evidence of the extract's therapeutic potential, further investigations are warranted to isolate the active constituents and elucidate the precise molecular mechanisms involved.

Haematology is the study of blood's structure and function, important for understanding how drugs, chemicals, or herbal extracts affect the body. Blood transports oxygen, nutrients, and removes waste through organs like the kidneys and liver. Red blood cells (RBCs), or erythrocytes, carry oxygen to tissues. Acetaminophen can reduce RBC levels, potentially causing anaemia and toxicity. However, another study showed increased RBCs after giving ethanol extract of Semicarpus anacardium to rats. Acetaminophen also raises white blood cell (WBC) counts, indicating inflammation and immune response due to liver damage, which is consistent with earlier studies linking high WBC levels (leucocytosis) to liver injury. Acetaminophen slightly lowered haemoglobin (Hb) levels, likely due to liver issues affecting red blood cell production or damage to the cells themselves. However, mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) mostly stayed the same. Treatment with N-acetylcysteine (NAC), a known antidote for acetaminophen toxicity, helped improve blood parameters. White blood cell (WBC) counts returned to normal, showing reduced inflammation. Platelet counts also improved, suggesting better liver function. Hb levels stabilized, and RBC values like MCV and MCHC moved back to normal, highlighting NAC's ability to reduce oxidative and inflammatory stress.

Semecarpus anacardium Linn treatment showed positive effects on key blood parameters. It reduced WBC counts, improved RBC and platelet levels, and helped maintain or increase haemoglobin and MCV values. These changes indicate its anti-inflammatory, antioxidant, and hematopoietic properties, along with a protective role in liver function and immune regulation.
The histological observation of the liver showed distortion in cell architectures of the acetaminophen induce damage showing severe necrosis, massive vascular degeneration and dilated blood sinusoids are filled with red blood cells. Administration of EESA extract almost comparable to NAC showed the capability to increasing number of viable cells and declining of hepatic enzyme levels in serum. A possible mechanism of ethanolic extract of Semecarpus anacardium protective effects against acetaminophen induced hepatotoxicity has been observed.

Conclusion

This study highlights the significant hepatoprotective and blood detoxifying potential of ethanolic extracts from *Semecarpus anacardium Linn* against acetaminophen-induced toxicity. Administered at doses of 200 mg/kg and 400 mg/kg, these extracts were effective in reducing liver damage and enhancing blood detoxification in rat models. Their therapeutic effects are likely due to antioxidant and anti-inflammatory properties, which help combat oxidative stress and cellular damage caused by acetaminophen. The results showed a dose-dependent protective response, with the higher dose offering greater protection. Additionally, the research emphasizes the potential of *Semecarpus anacardium Linn* and *Anacardium occidentale* as alternative treatments for drug-induced liver injury. It not only contributes to understanding the mechanisms of acetaminophen toxicity but also underscores the value of plant-based therapies in developing liver protection strategies. Further studies should aim to identify the active compounds responsible and clarify their mechanisms, supporting the development of new approaches to manage hepatotoxicity and detoxification.

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