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Research Article

# HEPATOPROTECTIVE ACTIVITY OF *AVERRHOA CARAMBOLA* STEM ETHANOLIC EXTRACT ON $CCL_4$ INDUCED LIVER DAMAGE IN RATS

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**ABSTRACT**

Objective: Various parts of *Averrhoa carambola* of family Oxalidaceae is used in traditional folkloric medicine. The present study aimed to evaluate the protective effect of *Averrhoa carambola* stem ethanolic extract against $CCl_4$ induced hepatic damage in rat. *Averrhoa carambola* stem ethanolic extract; ACSEE (250mg); ACSEE (500mg) were administered orally for 10 days in rats and compared with standard silymarin (100mg/kg) orally.

Results: The results showed significant decrease in ALT, AST and ALP levels in Extract (ACSEE) treated groups which were increased due to $CCl_4$ induced liver damage and are comparable with standard drug. Histopathological study of liver tissue reveals the hepatoprotective activity of *Averrhoa carambola* stem ethanolic extract.

Conclusion: The *Averrhoa carambola* stem ethanolic extract posses hepatoprotective activity and further research work is under process for separation of components from *Averrrhoa carambola* stem ethanolic extract to understand the active phytoconstituents.

**Keywords:** *Averrhoa carambola*, $CCl_4$, Histopathology, Serum enzyme levels, Sylimarin.

## INTRODUCTION

*Averrhoa carambola* of family Oxalidaceae is commonly known as star fruit, carambola and in local name Kamrakh (hindi), Ambanamkaya (telugu). Its nutritive values are also useful to the human health. Various parts of tree has been used in traditional folkloric medicine [1]. It also reported to have anti inflammatory activity of leaves, antimicrobial activity of fruit extract, anti hypoglycaemic activity of fruits, antioxidant activity of fruit and residue extracts, hypocholesterolaemic and hypolipidaemic activity of fruits, metabolic effects of enzymes of fruits, antiulcer activity [2].

The liver performs many functions and target organ for toxic drug-induced lesions. It transforms and excretes many drugs and toxins. These substances are frequently converted into inactive form by reactions that occur in the hepatocytes. Trasformations that occur in the liver that render many drugs water soluble and they readily excreted by the kidneys [3]. The physiological response to injury results such as necrosis, cholestasis, steatosis, inflammation and fibrosis.

Hepatitis is an autoimmune disorder, produce inflammation in the liver, leads to injury or distruction of hepatocytes. In most common hepatitis cause of hepatitis is viral infection. Specific viruses incite the immune system to fight off infections. Specific immune factors become over-produced that cause injury. Hepatitis is caused by drugs, alcohols, chemicals and environmental toxins.

Hepatoprotective activity of drugs is evaluated by various methods like Carbon tetrachloride induced hepatotoxicity [4], paracetamol induced hepatotoxicity [5], and ethanol induced hepatotoxicity [6]. Carbon tetrachloride ($CCl_4$) is the chemical which induce hepatotoxicity through lipid peroxidation by its free radical derivative ($CCl_3$, $CCl_3O_2$). Excessive productions of the reactive species manifests in tissue thiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury [7].

$CCl_4$ induced liver toxicity begins with the change in the endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite $CCl_3$ radical is produced by microsomal oxidase system binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue [8]. Results of hepatotoxicity increase the serum enzyme levels such as aspartate amino transferase, alanine aminotransferase and alkaline phosphate.

Present study was conducted to evaluate the protective effect of *Averrhoa carambola* stem ethanolic extract against $CCl_4$ induced hepatic damage in rat.

## MATERIALS AND METHODS

### Collection of Plant Material and Authentification

The stem of *Averrhoa carambola* was procured from Guwhati, Assam. The authentification of the plant was done by Prof. Dr. K. Madhva Chetty, Dept. of Botany, Sri Venkateswara University,Tirupathi. The Voucher specimen was deposited in the herbarium of our department.

### Preparation of Extract

Freshly collected plant material was shade dried at room temperature and coarsely powdered in Wiely mill.The powdered stem (450 g) was extracted with ethanol using Soxhlet apparatus. The crude extract was evaporated to dryness in a rotary film evaporator (Roteava, Equitron Medica instrument, India) and was found to be 6 g.

### Physiochemical studies of Extract

The ethanolic stem extract of *Averrhoa carambola* (ACSEE) was subjected for evaluation of oraganoleptic characters, solubility and phytochemical tests.

### Drugs and Chemicals

Silymarin (Allied Fabri Chem Private limited, Hyderabad) used as the standard hepatoprotective drug, Ethanol and $CCl_4$ (Aman Scientific Products, Vijayawada) were obtained from the institute store house and were of analytical grade. SGOT, SGPT and ALP enzyme kit (Span diagnostics limited, Surat ) were purchased.

### Animals

Rats of either sex weighing about 150-200 g [9] were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25$^0$C 12hr light/dark cycle and given standard pellet diet and water. Before performing the experiment the ethical clearance was obtained from Institutional Animal Ethics Committee.

### Acute oral toxicity studies

Acute oral toxicity study was carried out for ethanolic stem of extract *Averrhoa carambola* stem extract using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423 in Female Wister rats[10].

### Evaluation of Hepatoprotective activity

For evaluation of hepatoprotective activity of the first day, all animals were randomly divided into five groups of six animals each. Each grop of animals were treated with respective vehicles or drugs for10 days, along with suspension of $CCl_4$ in liquid paraffin (1:2v/v, 1ml of $CCl_4$/kg, s.c.) to induce liver damage 24hr before start of treatment [11-14].

Group-1: Normal (3ml/kg, s.c. liquid paraffin) for10 days.
Group-2: Control (suspension of $CCl_4$ in liquid paraffin 1:2v/v, 1ml of $CCl_4$/kg, s.c.) for 10 days.
Group-3: ACSEE (250mg/kg p.o for 10 days) along with $CCl_4$.
Group-4: ACSEE (500mg/kg p.o for 10 days) along with $CCl_4$.
Group-5: Silymarin (100mg/kg p.o for 10 days) along with $CCl_4$.

On 11th day, the blood samples were collected by retro-orbital puncture from each animal for estimation of hepatic enzyme levels. Blood samples were centrifuged for 15 mins at 3000 rpm to separate the serum. Alkaline phosphates (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) were estimated using standard kits.

## RESULTS AND DISCUSSION

### Preliminary Physicochemical Screening

The ACSEE was screened for various physicochemical test as per the reported methods and found that the ethanolic extract was deep brown in colour, having pungent smell and soluble in ethanol and water. The phytochemical tests confirmed the presence of saponins, alkaloids, flavanoids and tannins.

### Acute oral toxicity studies

The ACSEE was screened for toxicity studies according to OECD guidelines 423 taking three female wister rats with staring dose of 2000 mg/kg body weight. The animals were observed for 14 days and were found to be safe upto a dose of 2000 mg/kg body weight. So, the ACSEE comes under the category of Class 5 or unclassified. The $LD_{50}$ was found to be 5000mg/kg body weight. The testing doses were selected as 1/10th and 1/20th of 2000 mg/kg body weight i.e. 250 mg/kg body weight and 500 mg/kg body weight respectively.

### Evaluation of Hepatoprotective activity

Hepatoprotective activity of *Averrhoa carambola* stem ethanolic extract was evaluated by $CCl_4$ induced hepatotoxicity in rats and estimating serum hepatic enzyme levels. The results are given in Table 1.

Hepatic cells appear to participate in a variety of enzymatic metabolic activities and administration of $CCl_4$ damage hepatic cells and elevate serum level of AST, ALT, ALP and bilirubin significantly. Significant ($p<0.001$) increase in heptic enzyme levels were observed in control group. Treated animals with ACSEE (250mg/kg) and ACSEE (500mg/kg) showed reduction in serum enzyme levels and were comparable with standard silymarin.

The results of histopathological studies of normal rat liver showed normal hepatocytes, sinusoids. Liver section of rat treated with $CCl_4$ exhibited severe necrosis, disappearance of hepatocytes and areas of inflammation with increased sinusoidal spaces. Liver section of rat treated with ACSEE (250mg) and $CCl_4$ exhibited mild degree of necrosis, reduced sinusoidal dilation and less inflammation. Liver section of rat treated with ACSEE (500mg) and $CCl_4$ showed normal hepatocyte appearance with normal sinusoids with no inflammation. Liver section of rat treated with silymarin (100mg) and $CCl_4$ exhibited normal hepatocytes. The results are given in Fig. 2A, Fig. 2B, Fig. 2C,Fig. 2D and Fig. 2E respectively.

**Table 1: Effect of *Averrhoa carambola* stem ethanolic extract on $CCl_4$ induced hepatotoxicity in rats**

| Treated groups | Heptic enzyme levels | | |
|---|---|---|---|
| | AST(IU/L) | ALT(IU/L) | ALP(IU/L) |
| Normal | 94.83±1.701a*** | 84.83±1.701a*** | 70.17±2.960a*** |
| Control(CCl4) | 149.0±1.983b*** | 159.0±1.983b*** | 111.5±3.253b*** |
| ACSEE(250mg) | 128.0±2.191b*** | 138.0±2.191b*** | 96.83±2.182b*** |
| ACSEE(500mg) | 85.00±1.065b*** | 95.00±1.065b*** | 85.00±1.461b*** |
| Silymarin(100mg) | 70.17±2.455b*** | 86.00±1.633b*** | 77.00±2.206b*** |

Values are in mean±S.E.M(n=6); ns-Non significant,*p<0.05, **p<0.01,***p<0.001. a= control compared with Normal, b=All test groups compared with Control using One way ANOVA followed by Dunnet's "t" test.

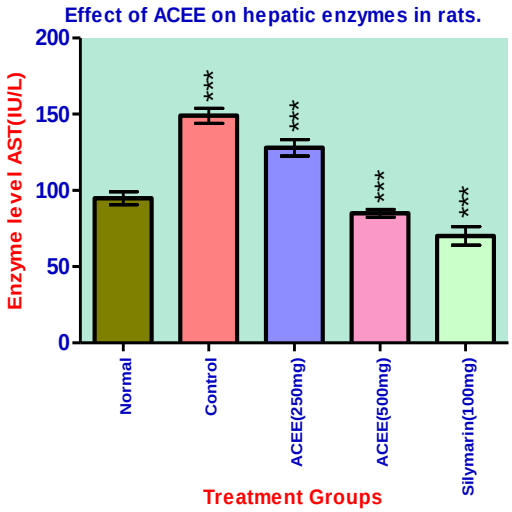


**Fig. 1A: Effect of ACSEE on serum AST levels**

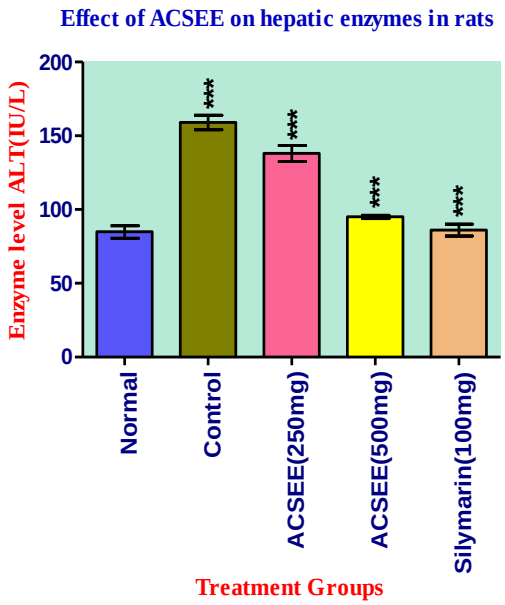


**Fig. 1B: Effect of ACSEE on Serum ALT levels**

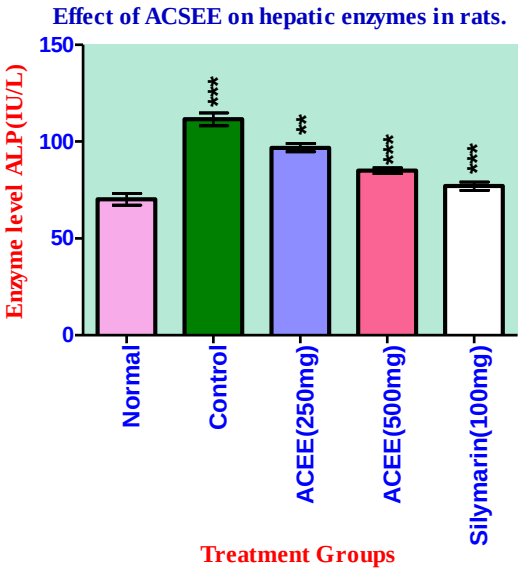


**Fig. 1C: Effect of ACSEE on ALP enzyme levels**

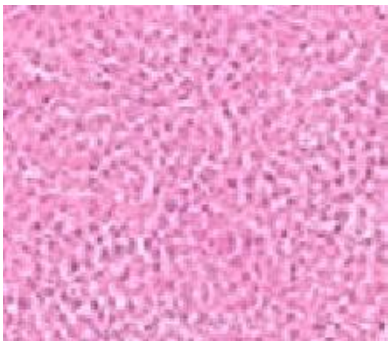

**Fig. 2A: Vehicle treated group**

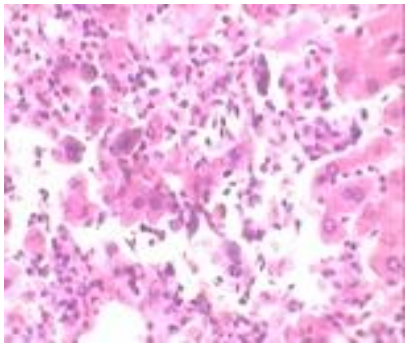

**Fig. 2B: Control group treated with $CCl_4$ normal hepatocytes and sinusoids exhibited severe necrosis with disappearance of hepatocytes and areas of inflammation, increased sinusoidal spaces.**

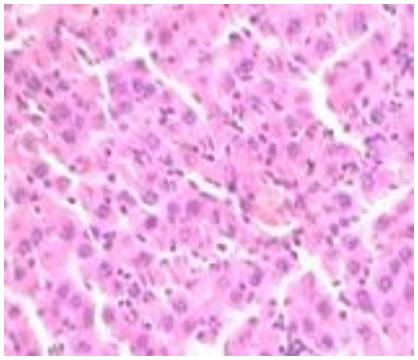

**Fig. 2C: ACSEE 250mg/kg exhibited mild degree of necrosis and reduced sinusoidal dilation**

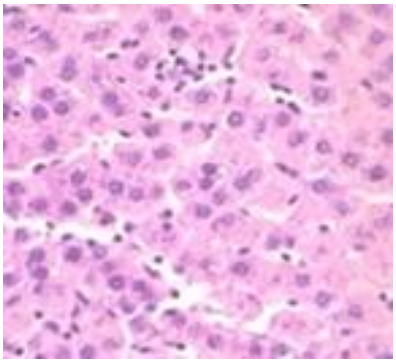

**Fig. 2D: ACSEE 500mg/kg exhibited normalization of cells and reduced sinusoidal along with mild inflamogens**

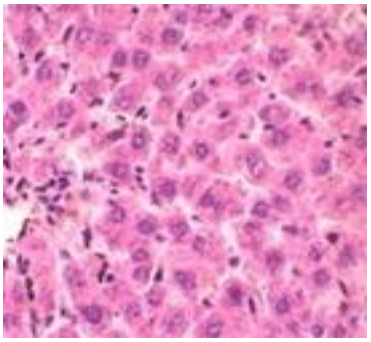

**Fig. 2E: Silymarin(100mg/kg)exhibited almost normal hepatocytes**

## CONCLUSION

Presented study concludes that *Averrhoa carambola* stem ethanolic extract posseshepatoprotective activity and presence of phytoconstituents like saponins, alkaloids, flavanoids and tannins in extract as reported previously. The active constituents alone or in combination may be responsible for the hepatoprotective activity. Further research work is under process for separation of components from *Averrrhoa carambola* stem ethanolic extract to understand the active phytoconstituents.

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