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# Hepatoprotective effect of *Averrhoa carambola* fruit extract on carbon tetrachloride induced hepatotoxicity in mice

A.K. Azeem<sup>1\*</sup>, Molly Mathew<sup>2</sup>, Chandramohan Nair, Dilip.C<sup>1</sup>

<sup>1</sup>Al Shifa College of Pharmacy, Poonthavanam P.O, Kizhattur, Perinthalmanna, Malappuram Dist, Kerala, India 679329

<sup>2</sup>Malik Deenar College of Pharmacy, Kasaragod, Kerala

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## ABSTRACT

**Objective:** To investigate the hepatoprotective activity of *Averrhoa carambola* fruit extract against carbon tetrachloride induced hepatic injury. **Methods:** Hepatotoxicity was induced on albino mice by intraperitoneal administration of CCl<sub>4</sub>, half an hour after the administration of the last dose of the extract of *Averrhoa carambola* fruit. Aqueous extract of the fruit of *Averrhoa carambola* was administered at a dose of 0.9 g/kg body weight once daily for seven days. The hepatic injury and its prevention was assessed by the estimation of serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphates (ALP), glutathione level and histopathological studies of liver. **Results:** Pre-treatment of mice with the fruit extract of *Averrhoa carambola* significantly reduced serum levels of ALT, AST and ALP enzyme and significantly increased the liver reduced glutathione levels 24 h after the administration of carbon tetrachloride. A marked improvement in the enzyme activities and the liver reduced glutathione level was observed in the pre-treated mice 4 days after the administration of carbon tetrachloride. Histopathological studies provided supportive evidence for the biochemical analysis. **Conclusions:** The aqueous extract of the fruit of *Averrhoa carambola* has hepatoprotective effect against carbon tetrachloride induced liver damage in mice.

## 1. Introduction

*Averrhoa carambola* (Family: Oxalidaceae) is a small evergreen tree about 9 m height with close drooping branches. It is found in tropical countries especially in South India, Sri Lanka, Myanmar, Java and China. The fruit is sweet and sour; it is thermogenic, febrifuge, antipyretic, antiscorbutic and tonic. It is useful in traditional Ayurvedic medicine as a diaphoretic, diuretic, expectorant, antidiarrhoeal, antiemetic and in acute dyspepsia. It alleviates hemorrhoids, intermittent fever, liver dysfunctions and various kinds of poisoning<sup>[1]</sup>. The activity of the drug is thought to be due to the presence of 5-hydroxy methyl furfural<sup>[2]</sup>, certain volatile principles<sup>[3]</sup> polyphenolic antioxidants<sup>[4]</sup> and so on. Carbon tetrachloride is a widely used chemical to induce liver damage in experimental studies, and its toxicity has been studied extensively. The resulting hepatic injury is characterized by leakage of cellular enzymes into the blood stream and by centrilobular

necrosis<sup>[5,6]</sup>. The present study is undertaken to establish the claimed hepatoprotective activity of the ripe fruit of *Averrhoa carambola* which is very extensively used by the common people of South India.

## 2. Materials and methods

### 2.1. Fruit extract

Whole fruit of *Averrhoa carambola* was collected from Kottayam District, Kerala, and its identity was confirmed by comparing it with the authentic samples kept at Govt. Ayurveda College Thiruvananthapuram, Kerala. The whole fruit was cut into pieces and refluxed in distilled water for one hour to prepare the aqueous extract.

### 2.2. Animals

Healthy 6–8 weeks old male Albino mice (30–35g) were maintained on a standard laboratory diet. Animals were fasted for 18 h prior to administration of carbon tetrachloride (CCl<sub>4</sub>). All protocols used in this study were approved by the Institutional ethics committee.

\*Corresponding author: A.K. Azeem, Asstant Professor, Al Shifa College of Pharmacy, Poonthavanam P.O, Kizhattur, Perinthalmanna, Malappuram Dist, Kerala, India 679329.

Tel: +919447005783

E-mail: akazeem80@gmail.com

### 2.3. Chemicals

Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphates (ALP) were purchased from Randox, UK. 5,5'-di-thio bis-2-nitrobenzoic acid (DTNB) was purchased from Sigma Chemical Co., USA. All other chemicals were of analytical grade.

### 2.4. Hepatoprotective study

Mice were randomly divided into eight groups with 6 mice in each. Group I served as normal control and was given distilled water orally. Group II, the drug control group, was given the plant extract orally for 7 days and sacrificed on the following day. Mice were administered 0.9 g/kg of the plant extract once daily, which is equivalent to 0.2 mL/30 g mouse<sup>[7]</sup>. Groups III and IV were given carbon tetrachloride (0.5 mL/kg in olive oil) intraperitoneally and the mice in group III were sacrificed 24 h later. Group IV mice were sacrificed four days after the administration of carbon tetrachloride. Mice in group V and VI were given the plant extract orally for 7 days. On the seventh day, carbon tetrachloride was administered intraperitoneally half an hour after administration of the last dose of the plant extract. Mice in group V were sacrificed 24 h later and those of group VI were sacrificed 4 days after the administration of carbon tetrachloride. Groups VII and VIII were administered the same dose of carbon tetrachloride as in the same way and half an hour later the plant extract was administered orally. Mice in group VII were sacrificed 24 h later. Mice in group VIII were administered the plant extract for two more days at 24 h intervals and sacrificed four days later. Blood samples were drawn from ether anaesthetized mice by cardiac puncture and liver tissue was excised for the determination of reduced glutathione and a part was fixed in buffered formalin for histopathological assessment of liver damage.

### 2.5. Biochemical parameters

Liver damage was assessed by the estimation of serum activities of ALT, AST and ALP using commercially available test kits (Randox, UK). Liver reduced glutathione level (GSH) was assessed by the method of Sedlak and Lindsay.

### 2.6. Histopathological assessment<sup>[8]</sup>

Histopathological assessment of liver damage was done after staining the slides of liver tissue with haematoxylin and eosin.

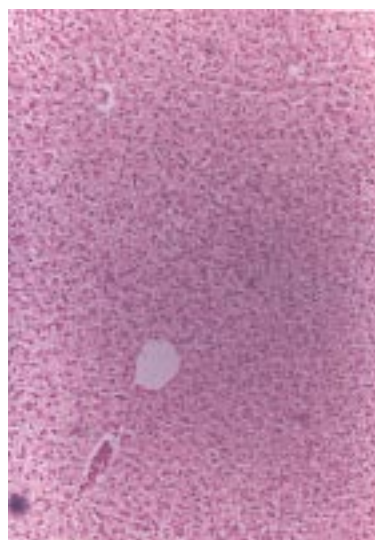
### 2.7. Statistical analysis

Statistical comparisons of data were made by means of Student's *t*-test.  $P < 0.05$  was regarded as significant.

## 3. Results

There was no significant change in the activities of serum ALT, AST, ALP and liver reduced glutathione levels in group II mice compared to group I (Table 1). A significant increase ( $P < 0.001$ ) in the activities of serum enzymes and a significant

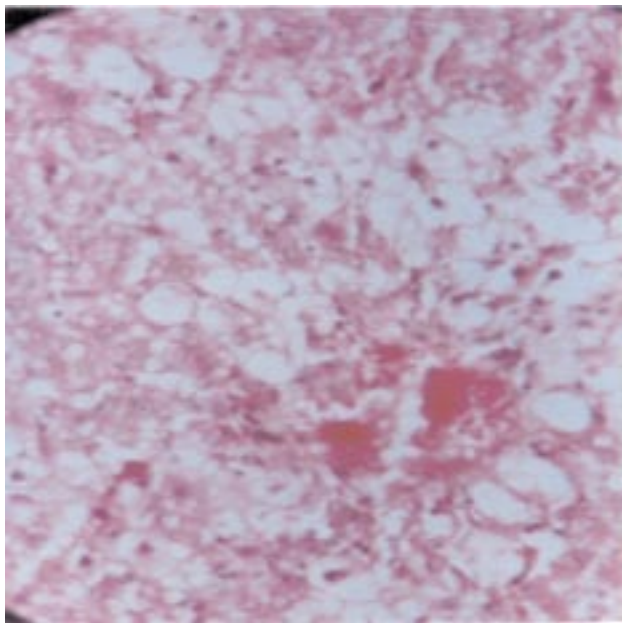
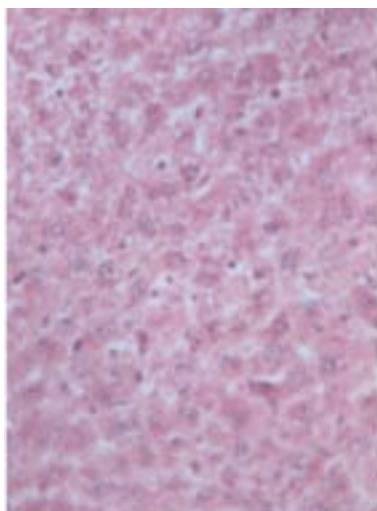
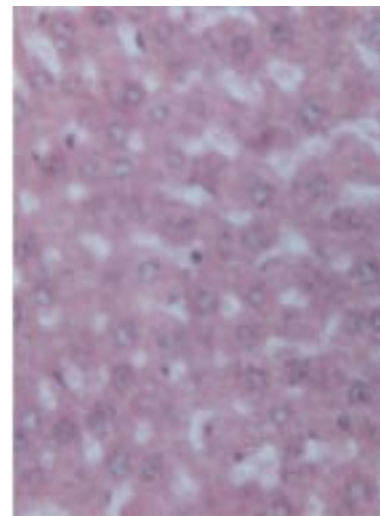
decrease ( $P < 0.001$ ) in the liver reduced glutathione level occurred within 24 h of exposure to carbon tetrachloride (group III). Post-treatment with fruit extract of *Averrhoa carambola* (group VII) decreased the CCl<sub>4</sub> induced alterations in ALT, AST and ALP by 8.70%, 20.96% and 68.82%, respectively while pre-treatment (group V) decreased the respective enzyme levels by 21.40%, 47.36% and 71.12%, 24 h after the administration of CCl<sub>4</sub> compared to group III. The percentage increase in liver reduced glutathione level in the post-treated group was 27.53% whereas it was increased by 42.32% in the pre-treated group. The same pattern was observed in the serum enzyme activities and liver reduced glutathione levels 4 days after the administration of CCl<sub>4</sub>. The percentage decrease in ALT, AST, ALP and increase in GSH levels were 8.28%, 10.39%, 26.65% and 77.27%, respectively in the post-treated group (group VIII) and 33.59%, 63.94%, 57.28% and 93.76%, respectively in the pre-treated group (group VI) compared to group IV (Table 1). Histopathological studies also provided supportive evidence for the biochemical analysis. The drug control group (group II) showed the normal parenchymal architecture with cords of hepatocytes, portal tracts, and central veins without noticeable alterations compared to the normal control group (Figure 1). Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes in the livers of mice treated with carbon tetrachloride (group III, Figure 2). The toxin mediated changes in livers of mice pre-treated with the drug extract (group V, Figure 3), 24 h after the administration of CCl<sub>4</sub> were of much less intensity than those observed in the livers of carbon tetrachloride treated mice (group III). Though the extent of cellular necrosis was less compared to the carbon tetrachloride group, post-treatment was not as good as the pre-treatment. A similar pattern was observed in the liver histopathology of mice 4 days after the administration of CCl<sub>4</sub> (Group VIII, Figure 4). Compared to the post-treated groups, pre-treated groups showed a faster recovery. The areas of necrosis were much less in the pre-treated group, 4 days after the administration of CCl<sub>4</sub> (group VI). In the post-treated group, the extent of damage was less compared to CCl<sub>4</sub> treated mice, but the areas of necrosis were visible<sup>[9]</sup>.



**Figure 1.** Liver section of normal control mice showing normal hepatocyte and normal architecture (Haematoxylin and eosin  $\times 10$ ).

**Table 1**Effect of *Averrhoa carambola* extract on the activity of serum enzymes and hepatic levels of reduced glutathione in mice( $\bar{x}\pm s$ ) (n=6).

| Group | ALT (U/L)                 | AST (U/L)                   | ALP (U/L)               | GSH (mg/g)             |
|-------|---------------------------|-----------------------------|-------------------------|------------------------|
| I     | 5.06±0.65                 | 12.35±0.83                  | 14.23±2.06              | 2.92±0.22              |
| II    | 3.92±0.71 <sup>a</sup>    | 9.60±1.22 <sup>a</sup>      | 10.25±0.86 <sup>a</sup> | 2.62±0.11 <sup>a</sup> |
| III   | 974.82±28.80 <sup>b</sup> | 1 347.40±90.34 <sup>b</sup> | 68.54±3.58 <sup>b</sup> | 1.02±0.14 <sup>b</sup> |
| IV    | 31.62±2.38 <sup>b</sup>   | 45.20±5.24 <sup>b</sup>     | 27.32±5.27 <sup>b</sup> | 1.45±0.07 <sup>b</sup> |
| V     | 766.15±44.40 <sup>d</sup> | 709.23±43.12 <sup>d</sup>   | 19.72±3.18 <sup>e</sup> | 1.45±0.13 <sup>c</sup> |
| VI    | 21.00±1.76 <sup>f</sup>   | 16.30±1.62 <sup>g</sup>     | 11.67±1.85 <sup>f</sup> | 2.80±0.08 <sup>g</sup> |
| VII   | 890.00±48.88              | 1 064.90±94.48 <sup>c</sup> | 21.37±3.27 <sup>e</sup> | 1.29±0.00              |
| VIII  | 29.00±2.13                | 40.50±7.34                  | 20.08±1.96              | 2.57±0.14 <sup>g</sup> |

<sup>a</sup>: no significant difference compared to group I;<sup>b</sup> $P<0.001$  compared with group II;<sup>c</sup> $P<0.05$ , <sup>d</sup> $P<0.01$ , <sup>e</sup> $P<0.001$  compared with group III;<sup>f</sup> $P<0.01$ , <sup>g</sup> $P<0.001$  compared with group IV.**Figure 2.** Liver section of CCl<sub>4</sub>-treated mice demonstrating perivenular necrosis of hepatocyte, congested sinusoids and inflammation (Haematoxylin and eosin×40).**Figure 3.** Liver of *Averrhoa carambola* pre treated group 24 h after administration of CCl<sub>4</sub> showing normal architecture, congested central vein and sinusoids; mild inflammation (Haematoxylin and eosin×40).**Figure 4.** Liver of CCl<sub>4</sub> and *Averrhoa carambola* post treated rat showing normal architecture, congested central vein and sinusoids (Haematoxylin and eosin×40).

#### 4. Discussion

GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals including CCl<sub>4</sub>. The significant impairment of hepatic GSH status associated with a substantial hepatocellular damage induced by CCl<sub>4</sub> suggested the determinant role of hepatic GSH in the development of carbon tetrachloride toxicity<sup>[10]</sup>. Cell injury induced by xenobiotics occurs only if mitochondrial GSH is depleted<sup>[11]</sup>. In an earlier study, mice were fasted for 16 h to minimize the individual variation among mice of the same group where reduced glutathione level was concerned. Increase in hepatic GSH level in *Averrhoa carambola* fruit extract treated mice could either be due to an effect on the de novo synthesis of GSH, its regeneration or both. As a consequence, hepatic GSH level could be sufficient to counteract the increased formation of free radicals as in the case of carbon tetrachloride toxicity. The dosage of the plant extract was calculated according to an internationally accepted calculation where the normal therapeutic dosage of humans was extrapolated to mice. The observed protective

effect of the plant extract against carbon tetrachloride may be attributed to the presence of flavonoids<sup>[12]</sup>, ascorbic acid, carotenoids, tannins or lignans among the plant constituents. Flavonoids are known to be antioxidants, free radical scavengers and antilipoperoxidants leading to hepatoprotection<sup>[14]</sup>. The mechanism by which *Averrhoa carambola* fruit exerts its protective action against carbon tetrachloride induced alterations in the liver may be due to the antioxidant effect exerted by any of the constituent. The plant extract was also capable of bringing about an accelerated recovery in the post-treatment regimen indicated that the protective effect might not be due to an antioxidant property alone. The histopathological observations suggest the possibility of the plant extract being able to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of ALT, AST and ALP into the circulation.

In conclusion, the results of the present study indicated that under the present experimental conditions, aqueous extract of *Averrhoa carambola* showed hepatoprotective effect against carbon tetrachloride induced liver damage in mice.

### Conflict of interest statement

We declare that we have no conflict of interest.

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