LEAF EXTRACT OF AVERRHOA CARAMBOLA L. CONFINES THE OXIDATIVE STRESS AND CONFERS HEPATOPROTECTION IN ALBINO MICE
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ABSTRACT
Different parts of the plant Averrhoa carambola L. is used for the treatment of various disease/disorder(s) by ethnic communities from North Maharashtra and Sonowal Kachari tribes of Dibrugarh, Assam. Still no scientific study is available about the hepatoprotective effect of the leaf of this plant. Present investigation aims to illustrate the hepatoprotective and antioxidant profile of leaves of Averrhoa carambola on carbon tetrachloride induced hepatic damage in mice. To achieve the objective, extracts were subjected to phytochemical screening. The leaf extract was then tested for its oral toxicity which was followed by evaluation of hepatoprotective and antioxidant activity at a dose level of 100mg/kg bw, 200mg/kg bw and 400mg/kg bw orally. Moreover, the hepatoprotective effect was further sustained by validating the extract against different pharmacological parameters. Leaf extract of A. carambola possess phytoconstituents like alkaloids, tannins, reducing sugar and flavonoids. The extract did not any show any sign of toxicity. The pre-treatment of extract had significantly controlled the levels of serum biochemical and antioxidant enzymes. Finally the grades of hepatoprotective were further confirmed while experimenting against other parameters. Finally it can be concluded that the study demonstrates hepatoprotective and antioxidant activity of leaf of Averrhoa carambola and thus supports its usage in traditional medicine.

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Introduction
Hepatic damage is one of the major health problems that arise mainly due to long term use of antibiotics, analgesics, chemotherapeutic agents and other toxicants like carbon tetrachloride (CCl₄), thiouacemide (TAA), excessive alcohol consumption and viral infections. Medicinal plants have been one of the foremost sources of natural products and holds promising for drug development. The use of medicinal plant extracts as hepatoprotective as well as antioxidative agent has been reported in numerous recent studies and as such the efficacy of those plants has been well demonstrated [1]. Inspite of marvellous advances in contemporary medicines, no effective compounds are still available that promote liver function, tender shield or help to regenerate hepatic cells and thus validation of traditional uses of plants is needed to generate herbal drug(s) that can confer hepatoprotection. Averrhoa carambola L. has been reported to have potential therapeutic properties in the Indian traditional medicine. The leaves and root of the plant are used as laxative. The combined extracts of leaves and fruits of A. carambola is used by the Tai-Khamyangs, Dimasa and Hmar tribe for the treatment of jaundice and gastric problems [2, 3]. The present investigation was considered so as to accumulate enough evidences about the hepatoprotective and antioxidative profile of leaves of Averrhoa carambola and validate its use in traditional medicine so that an impending platform can be set for the development of new hepatoprotective drug entities.

Materials and methods
Plant material
Leaves of Averrhoa carambola were munificently collected from the fields of Cachar district of Southern Assam, India in the month of August 2011. The plant material was identified consulting the herbarium in Assam University and a voucher specimen (CIL/AC/SM-002ANG/2011) was deposited for future reference. The leaf samples were shade dried and milled followed by pulverization at 24±2º C.

Extraction
The plant material was extracted employing cold maceration technique (Riebling et al., 1975) initially with petroleum ether (b.p. 60-80ºC) to yield extract A: containing fatty matters and low polar components. The same was then extracted with Ethyl acetate to yield extract B followed by acetone to yield extract C containing high polar components. The extracts were than filtered through Whatman No. 1 filter paper and evaporated using Rotary Evaporator (IKA, Germany) to yield crude extracts. The extracts were stored at 4ºC for further analysis.

Phytochemical screening
Preliminary phytochemical properties of the leaf extracts were studied following the standard procedure [4].

Maintenance of animals
Pathogen-free Swiss Albino mice approximately (28±2)g body weight of both the sexes were procured from Pasteur Institute, Shillong, India. The mice were grouped and housed in poly acrylic cages (38x23x10 cm) with not more than 6 animal per cage and maintained under standard laboratory condition (temperature:- 25±2ºC and dark/light cycle 14/10 h) and relative humidity of 55±5%. They were allowed free excess to standard dry pellet diet (Hindustan lever, Kolkata, India) and water ad libitum. The mice were aclimatized to laboratory condition for 7 days before the commencement of dosing. Dosing was initiated with 100 mg/kg bw p.o. and was then increased simultaneously upto 2000 mg/kg bw p.o.[5-10].

Chemicals
All chemicals were of analytical grade and were procured either from Merck (India & Germany) and HiMedia Labs, India. Biochemical assay kits were purchased from Ozone Pvt. Ltd. India.

Acute oral toxicity
All the animals were fasted overnight before the commencement of dosing. Dosing was initiated with 100 mg/kg bw p.o. and was then increased simultaneously upto 2000 mg/kg bw p.o.[5-10].

Hepatoprotective activity
Swiss albino adult mice of either sex were divided in six groups consisting of six animals each (n=6). Hepatotoxicity was induced by injecting CCl₄ at a dose level of 0.5ml/kg bw through intra-peritoneal route of drug administration for 5 days.

Group 1: Normal control group treated with liquid paraffin (0.5ml/kg bw) p.o. for 5 days.
Group 2: Toxic control group treated with CCl₄ (0.5ml/kg bw) i.p. for 5 days.
Group 3: Treated with Silymarin (50mg/kg bw) p.o. daily for 5 days and received CCl₄ (0.5ml/kg bw) i.p. after 30 min of silymarin administration for 5d.
Group 4: Treated with crude acetone extracts (leaf) (100mg/kg bw) p.o. daily for 5d and received CCl₄ (0.5ml/kg bw) i.p. after 30 min of administration.
Group 5: Treated with crude acetone extracts (leaf) (200mg/kg bw) p.o. daily for 5d and received CCl₄ (0.5ml/kg bw) i.p. after 30 min of administration.
Group 6: Treated with crude acetone extracts (leaf) (400mg/kg bw) p.o. daily for 5d and received CCl₄ (0.5ml/kg bw) i.p. after 30 min of administration.
On 6th, the blood was collected through retro-orbital venous sinus to isolate the serum from blood samples. The animals were then sacrificed; liver was collected and finally preserved in 10% formalin for histopathological analysis.

**Biochemical estimations**

Blood were centrifuged at 600g for 15 minutes to separate the serum, as to measure aspartate aminotransferase (AST), Alanine aminotransferase (ALT) [11,12], alkaline phosphatase (ALP) [13], lactate dehydrogenase (LDH): estimated by the method of [14] and serum bilirubin [15,16].

**Estimation of antioxidant profile**

Liver tissue was added in 0.1 M phosphate buffer; pH 7.0 along with 0.1% triton X-100, homogenized and finally centrifuged at 19,000g for 10 min to get the supernatant and thus the rate of lipid peroxidation [17,18] as well as the activity of the antioxidant enzymes viz. Catalase (CAT) assay, Super oxide dismutase (SOD) assay, Glutathione peroxidise (GPx) assay, Glutathione reductase (GR) assay and Glutathione-S-transferase (GST) assay were resolved [19].

**Histopathological examination**

The organs intended for histopathological examination were collected, fixed by their immersion in 10% formalin, dehydrated progressively in ethyl alcohol (10%-100%), cleaned in xylene and embedded in paraffin. Sections of approximately 5µm size were obtained using rotary microtome, stained with haematoxylin and eosin (H&E). The slides were observed under microscope for studying the histopathological changes [20, 21].

**Statistical analysis**

The data were expressed as mean ± S.E.M of six animals in each group and were analysed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test for the significant interrelation between the various groups and results were considered significant when $P \leq 0.05$.

**Results**

**Phytochemical screening**

The preliminary phytochemical screening of the crude acetone extract of *Averrhoa carambola* (AAC) revealed the presence of various phytoconstituents including alkaloids, tannins, reducing sugar and flavonoids in them.

**Acute oral toxicity**

*Averrhoa carambola* did not show any sign and symptoms of toxicity up to the dose level of 2000 mg/kg bw p.o.

**Hepatoprotective efficacy**

The extract elicited a significant ($p<0.0001$) reversal in the amplified levels of the biochemical markers in a dose dependent manner and at the highest dose level 400mg/kg bw p.o., the levels of the marker enzymes were quite comparable to that of standard drug (Table 1).

**Antioxidant enzymes and lipid peroxidation**

*A.carambola* caused significant ($p<0.0001$) reversal in the diminished activities of all antioxidant enzymes. Moreover, the results obtained in the set of determination showed its therapeutic potential in a remarkable manner by abolishing the toxicity. CCl$_4$ provoked the rate of lipid peroxidation, which was brought down by the pre-treatment of the acetone leaf extract of *A.carambola* in a dose dependent manner.

**Histopathology**

CCl$_4$ poisoning led to the extreme formation and deposition of connective tissue and expansion of scars. Liver sections of mice treated with plant leaf extract 100 mg/kg bw p.o. exposed nodular transformation of liver structural design with loss of arrangement of hepatic lobules. Large septa of connective tissue curving together and penetrating into the parenchyma were observed in liver sections of mice treated with the plant leaf extract 200 mg/kg bw p.o. Liver sections of mice treated with plant leaf extract 400 mg/kg bw p.o. showed approximately normal lobular pattern with short septa of connective tissue and a mild amount of fatty change, necrosis and lymphocyte infiltration nearly equivalent to the silymarin treated groups.
The results obtained from the present study revealed that the acetone leaf extract of Averrhoa carambola (AAC) contains phytoconstituents like alkaloids, tannins, reducing sugar and flavonoids in them. The acetone extract of leaves of Averrhoa carambola exhibited hepatoprotective effect in a dose dependent manner (Table 1), significantly normalizing (p<0.0001) the levels of the biochemical markers viz. ALT and AST, since the augmented serum level of AST as well as ALT are the indicative index and decisive factor of cellular leakage and functional integrity of the cell membrane in liver [17], total bilirubin in view of the fact that eminent level of bilirubin in the serum is an symptomatic aspect showing the amplified erythrocyte degeneration rate which in turn is due to the seepage of bile by the liver as a self-protective mechanism towards hepatotoxins [18], ALP whose prominent rank in the serum designate cholestasis since bile duct transports bile into the gall bladder as well as intestine and thus inflammation or injure of any kind causes its spillage into the blood, and LDH whose directory is worth in indentifying ischemic liver injury [19]. Moreover, the grade of normalization of the biochemical markers was far more significant (p<0.001) and was also comparable to that of the protective potential of acetone leaf extract of Averrhoa carambola 

Table 1. Protective potential of acetone leaf extract of Averrhoa carambola.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>ALP (U/l)</th>
<th>Total bilirubin (mmol/l)</th>
<th>LDH (IU/l)</th>
<th>LPO (nmoles TBARS/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>27.4 ± 0.32</td>
<td>13.93 ± 0.17</td>
<td>33.76 ± 0.28</td>
<td>0.75 ± 0.001</td>
<td>17.08 ± 0.22</td>
<td>0.33 ± 0.0034</td>
</tr>
<tr>
<td>Toxic control</td>
<td>154.58 ± 0.35</td>
<td>127.13 ± 0.2</td>
<td>141.21 ± 0.22</td>
<td>3.86 ± 0.004</td>
<td>106.01 ± 0.21</td>
<td>3.57 ± 0.033</td>
</tr>
<tr>
<td>Silymarin (50 mg/kg) + CCl₄</td>
<td>38.42 ± 0.23</td>
<td>32.74 ± 0.39</td>
<td>45.13 ± 0.26</td>
<td>1.23 ± 0.003</td>
<td>23.03 ± 0.18</td>
<td>0.45 ± 0.0035</td>
</tr>
<tr>
<td>AAC (100 mg/kg) + CCl₄</td>
<td>92.99 ± 0.27</td>
<td>88.5 ± 0.2</td>
<td>96.20 ± 0.21</td>
<td>3.16 ± 0.002</td>
<td>72.11 ± 0.28</td>
<td>2.35 ± 0.032</td>
</tr>
<tr>
<td>AAC (200 mg/kg) + CCl₄</td>
<td>71.8 ± 0.3</td>
<td>55.11 ± 0.32</td>
<td>71.95 ± 0.27</td>
<td>2.11 ± 0.001</td>
<td>41.25 ± 0.26</td>
<td>1.16 ± 0.021</td>
</tr>
<tr>
<td>AAC (400 mg/kg) + CCl₄</td>
<td>48.08 ± 0.32</td>
<td>36.44 ± 0.19</td>
<td>49.16 ± 0.25</td>
<td>1.42 ± 0.003</td>
<td>26.41 ± 0.17</td>
<td>0.57 ± 0.0034</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M; *p<0.0001 when compared to different groups.

Fig.1. Histopathological changes in the liver tissue of mice after treatment with different doses of acetone leaf extract of A. carambola. (A) Toxic control group (CCl₄ treated), (B) Plant extract (200 mg/kg b.w. p.o.), (C) Plant extract (400 mg/kg b.w. p.o.).

Table 2. Acetone leaf extract of Averrhoa carambola on liver antioxidant enzymes

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GR (U/mg protein)</th>
<th>GST (nmoles of CDNB conjugate formed /min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.76 ± 0.002</td>
<td>86.15 ± 0.28</td>
<td>76.45 ± 0.44</td>
<td>42.65 ± 0.41</td>
<td>36.36 ± 0.33</td>
</tr>
<tr>
<td>Toxic control</td>
<td>0.17 ± 0.001*</td>
<td>41.18 ± 0.33*</td>
<td>21.62 ± 0.38*</td>
<td>18.31 ± 0.36*</td>
<td>13.68 ± 0.39*</td>
</tr>
<tr>
<td>Silymarin (50 mg/kg) + CCl₄</td>
<td>0.71 ± 0.003</td>
<td>81.53 ± 0.35</td>
<td>72.75 ± 0.36</td>
<td>39.25 ± 0.22</td>
<td>33.67 ± 0.32</td>
</tr>
<tr>
<td>AAC (100 mg/kg) + CCl₄</td>
<td>0.31 ± 0.002*</td>
<td>45.18 ± 0.21*</td>
<td>28.42 ± 0.35*</td>
<td>27.92 ± 0.31*</td>
<td>22.48 ± 0.39*</td>
</tr>
<tr>
<td>AAC (200 mg/kg) + CCl₄</td>
<td>0.47 ± 0.004*</td>
<td>53.34 ± 0.32*</td>
<td>50.75 ± 0.42*</td>
<td>33.28 ± 0.24*</td>
<td>28.58 ± 0.28*</td>
</tr>
<tr>
<td>AAC (400 mg/kg) + CCl₄</td>
<td>0.69 ± 0.002*</td>
<td>72.33 ± 0.25*</td>
<td>68.35 ± 0.37*</td>
<td>37.32 ± 0.31*</td>
<td>31.25 ± 0.28*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M; *p<0.0001 when compared to different groups

Discussion
The results obtained from the present study revealed that the acetone leaf extract of Averrhoa carambola (AAC) contains phytoconstituents like alkaloids, tannins, reducing sugar and flavonoids in them. The acetone extract of leaves of Averrhoa carambola exhibited hepatoprotective effect in a dose dependent manner (Table 1), significantly normalizing (p<0.0001) the levels of the biochemical markers viz. ALT and AST, since the augmented serum level of AST as well as ALT are the indicative index and decisive factor of cellular leakage and functional integrity of the cell membrane in liver [17], total bilirubin in view of the fact that eminent level of bilirubin in the serum is an symptomatic aspect showing the amplified erythrocyte degeneration rate which in turn is due to the seepage of bile by the liver as a self-protective mechanism towards hepatotoxins [18], ALP whose prominent rank in the serum designate cholestasis since bile duct transports bile into the gall bladder as well as intestine and thus inflammation or injure of any kind causes its spillage into the blood, and LDH whose directory is worth in indentifying ischemic liver injury [19]. Moreover, the grade of normalization of the biochemical markers was far more significant (p<0.001) and was also comparable to that of the...
standard drug silymarin. Additionally, the normalization of the abnormal levels of the antioxidant enzymes which acts as a shield by preventing the generation of hydroxyl radical (OH\(^{-}\)) and defend the cellular constituents from oxidative damage [22-24] viz. Superoxide dismutase: SOD, Catalase: CAT, Glutathione peroxidise: GPx, Glutathione reductase : GR and Glutathione S-transferase: GST and along with all other parameters viz. lipid peroxidation (Table 2) was also observed, and was found to fluctuate in a dose dependent manner. Furthermore, the normalization of the eumen levels of both hepatic as well as antioxidant enzymes were also supported by the histopathological observations (Fig 1.) which verified the protective efficacy of the plant. Although silymarin was a stronger hepatoprotective than the leaf extract of *Averrhoa carambola* in our exploration, possibility of exertion of hepatoprotective and antioxidant effect of leaf extract reminiscent of silymarin’s mechanism.

**Conclusion**

From the overall results, it can be concluded that acetone extract of leaf of *Averrhoa carambola* shows its high-flying hepatoprotective as well as antioxidant efficacy in a dose dependent manner. Revelation of hepatoprotective mechanism and the active components present needs further investigation for the development of novel drug(s) against hepatic damage. Further studies regarding isolation and purification of active compound(s) and hepatoprotective and anti-oxidant activity will help in the development of new drug entities.

**Conflict of interest statement**

The authors declare that there are no conflicts of interest.

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**REFERENCE**

2. Das AK, Dutta BK, Sharma GD, Medicinal plants used by different tribes of Cachar district, Assam, Indian J Trad Knowl. 2008, 7(3), 446-454.