Effects of *Phyllanthus acidus* (L.) Skeels fruit on carbon tetrachloride-induced acute oxidative damage in livers of rats and mice

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**Objective:** The present study was undertaken with a view to validate the traditional use of *Phyllanthus acidus* (L.) Skeels fruit as a hepatoprotective agent.

**Methods:** The 70% ethanolic extract of *P. acidus* fruit (100, 200 and 400 mg/kg, p.o.), and reference drug silymarin (100 mg/kg, p.o.) were given to rats of different groups respectively once a day for 5 d and the carbon tetrachloride (CCl₄) (2 mL/kg, subcutaneously) was given on days 2 and 3. Serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) were assessed along with liver histopathological examination. The effects on oxidative stress markers such as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were also assessed in liver tissue homogenate to evaluate in vivo antioxidant activity. In addition, the effects on hexobarbitone-induced sleeping time were observed and the free radical-scavenging potential was determined by using 2,2-diphenyl-1-picrylhydrazil (DPPH) in mice.

**Results:** *P. acidus* extracts and silymarin exhibited a significant hepatoprotective effect as evident from the decreases of serum AST, ALT and ALP levels and LPO and increases in the levels of TP, GSH, SOD, CAT, and GPx compared with control group (P<0.01 or P<0.05). The biochemical results were supplemented with results of histopathological sections of the liver tissues. *P. acidus* extracts considerably shortened the duration of hexobarbitone-induced sleeping time in mice compared with control group (P<0.01) and showed remarkable DPPH-scavenging activity.

**Conclusion:** The present findings suggest that the hepatoprotective effect of *P. acidus* against CCl₄-induced oxidative damage may be related to its antioxidant and free radical-scavenging potentials.

**Keywords:** *Phyllanthus,* plant extracts; carbon tetrachloride; oxidative stress; liver function tests; animal experimentation; rats; mice

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Chemical-induced liver injury depends mostly on the oxidative stress in hepatic tissue and underlies the pathology of numerous diseases, including cancer. There is still a lack of effective therapeutics; hence, a treatment with antioxidants has been proposed for prevention and/or attenuation of injury\(^1\). Carbon tetrachloride (CCl\(_4\))-induced acute liver damage is the best characterized system of xenobiotic-induced hepatotoxicity and is a commonly screening model to evaluate the hepatoprotective potential of drugs with antioxidant properties\(^2\). Silymarin, used as reference standard in present study, provides significant protection against CCl\(_4\)-induced alterations of hepatic markers involved in normal function, oxidative stress and liver histopathology\(^3\).

The plants of genus *Phyllanthus* (Euphorbiaceae) have long been used in traditional systems of medicine to treat chronic liver diseases\(^4\). More than 35 species of this genus have been reported as endemic to India, which are predominantly used as a remedy for hepatic disorders\(^5\). A number of experimental studies have demonstrated the liver protective potential of *Phyllanthus* plants in different *in vitro* and *in vivo* systems\(^6\). However, several plants of this genus remain unexplored. Systematic investigation of such plants might yield fruitful results in our quest to discover new and promising hepatoprotective agents, which may be developed as pharmaceutical entities or as simple adjuncts to existing therapies. With this view, we selected *Phyllanthus acidus* (L.) Skeels (*P. acidus*) for our present study.

*P. acidus*, commonly known as “harfarauro”, “star gooseberry” or “mayom”, is a small tree and is cultivated as a fruit tree in many Asian countries. The fruit of *P. acidus* has been reported to be a rich source of ascorbic acid, fibers and carotenoids\(^10\). Several parts of this plant have been used in folk medicine. The roots and seeds are cathartic. The fruit is a liver tonic and a blood purifier and is used in several vitiolated conditions of jaundice, bronchitis, constipation, vomiting, biliousness, urinary concretions and piles in Ayurvedic system of medicine\(^11\). The leaves are useful to treat fever, piles, small pox, blood vomiting, itching and gum infection\(^12\). Several therapeutic properties including antiviral\(^13\), antibacterial\(^14\), neuroprotective\(^15\), anti-fibrosis\(^16\), and anticancer\(^17\) activities have also been reported for *P. acidus*. An aqueous extract of *P. acidus* has been reported to be effective against woodchuck hepatitis virus-DNA polymerase\(^18\). The hepatoprotective activity of *P. acidus* leaves against CCl\(_4\)-induced acute liver damage in rat model has been reported\(^19\).

In the ethnomedical claims, the fruit of *P. acidus* is used for the treatment of different diseases including hepatopathy\(^11\). To the best of our knowledge there is no scientific report available in support of its hepatoprotective activity. Therefore, to justify the traditional claims we have assessed the hepatoprotective effect of *P. acidus* fruit on CCl\(_4\)-induced hepatotoxicity rat model.

### 1 Materials and methods

#### 1.1 Experimental animals

Wistar albino rats (200 to 220 g) and Swiss albino mice (20 to 25 g) of either sex were used for the studies. The animals were grouped and housed in polyacrylic cages with not more than 6 per cage and maintained under standard laboratory conditions of temperature (25±2 °C) and relative humidity (55±5%) with dark and light cycle (12/12 h). They were acclimatized to laboratory conditions for 7 d before commencing the experiment and allowed free access to standard pellet diet (H. P. Agro Industries, India) and water ad libitum. Animal studies were approved by the Institutional Animal Ethics Committee (379/01/ab/CPSEA) and conducted as per the regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSEA).

#### 1.2 Plant materials and extraction

The fresh *P. acidus* fruits were collected in May 2008 from University campus, Sagar (India) and authenticated by the taxonomist of Botany Department of the Dr. H. S. Gour University, Sagar (India). A voucher specimen (No. Bot/H/4322) has been deposited in the same department for future reference. The fruits were cut into small pieces, dried at room temperature and then subjected to size reduction to a coarse powder by using grinder and stored for further use.

The fruit powder (500 g) was extracted with 70% ethanol by cold maceration (10 d)\(^20\). After filtration, the extracts were concentrated under reduced pressure in a rotary evaporator to obtain dark brownish green semisolid extracts (yield: 12.4% w/w). For dosing, the crude extracts were uniformly suspended in 2% gum acacia and given orally at 100, 200 and 400 mg/kg body weight to animals.

#### 1.3 Chemicals and reagents

CCl\(_4\), 2,2-diphenyl-1-picrylhydrazil (DPPH), thiobarbituric acid (TBA) and 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Silymarin was obtained as a gift sample from Serum International Ltd., Pune, India. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin estimation kits were procured from Span Diagnostics, Surat, India. All other chemical and reagents used were of analytical grade.

#### 1.4 Preliminary phytochemical screening

Preliminary phytochemical analysis of *P. acidus* extracts was performed to identify the nature of phytoconstituents\(^21\).

#### 1.5 Acute toxicity studies

Healthy adult male albino mice (18 to 22 g) were used for acute toxicity studies as per Organization for Economic Cooperation
and Development (OECD) guidelines (Guideline 423: Acute Toxic Category Method)\textsuperscript{[23]} On the basis of these studies, the doses of 100, 200 and 400 mg/kg per os (p.o.) were selected for in vivo experiments.

1.6 CCl\textsubscript{4}-induced hepatotoxicity

1.6.1 Experimental protocol The experiment was conducted according to the method procedures described previously\textsuperscript{[23]}. Rats were randomly divided into six groups, each consisting of six rats. Group I (normal control) rats received distilled water (1 mL/kg, p.o.) daily for 5 d and olive oil (1 mL/kg, subcutaneously (s.c.)) on days 2 and 3. Group II (CCl\textsubscript{4} control) rats received distilled water (1 mL/kg, p.o.) daily for 5 d and CCl\textsubscript{4}: olive oil (1 : 1, 2 mL/kg, s.c.) on days 2 and 3. Group III rats were treated with the reference drug silymarin (100 mg/kg, p.o.) daily for 5 d and received CCl\textsubscript{4}: olive oil (1 : 1, 2 mL/kg, s.c.) on days 2 and 3, 30 min after administration of silymarin. Groups IV to VI were treated with ethanolic extracts of *P. acidus* at 100, 200 and 400 mg/kg, p.o., respectively, for 5 d and received CCl\textsubscript{4}: olive oil (1 : 1, 2 mL/kg, s.c.) on days 2 and 3, 30 min after administration of the extracts.

On the sixth day, under ether anesthesia, blood and liver samples of rats were collected. The blood samples were allowed to clot for 30 min and the serum was separated by centrifugation at 3 000×g at 4 °C. The livers were immediately taken out and washed with ice-cold normal saline, stored at −80 °C and processed for determination of oxidative stress markers and histopathological studies.

1.6.2 Determination of liver function markers Activities of the serum enzymes ALT, AST\textsuperscript{[24]} and ALP\textsuperscript{[24]} were determined. Total bilirubin (TB)\textsuperscript{[24]} and serum total protein (TP) contents\textsuperscript{[27]} were also determined to assess the CCl\textsubscript{4}-induced acute liver injury.

1.6.3 Determination of oxidative stress markers Fresh liver samples were perfused with ice-cold normal saline to completely remove all the red blood cells followed by homogenization with 0.2 mol/L phosphate buffer using homogenizer. Then the homogenate was centrifuged at 2 500×g for 10 min at 4 °C and used for the analysis of oxidative stress markers. Lipid peroxidation was assayed by measuring the malondialdehyde (MDA) content\textsuperscript{[28]} in the tissue homogenate. Levels of hepatic reduced glutathione (GSH)\textsuperscript{[29]} and antioxidant enzymes such as superoxide dismutase (SOD)\textsuperscript{[30]}, catalase (CAT)\textsuperscript{[31]}, and glutathione peroxidase (GPx)\textsuperscript{[32]} in the homogenate were measured accordingly.

1.7 DPPH scavenging assay DPPH was used in this assay to assess the free radical-scavenging (antioxidant) property of *P. acidus* extracts\textsuperscript{[25]}. Briefly, methanol solution of *P. acidus* extracts (50 to 300 μg/mL) was mixed with 0.1 mmol/L DPPH methanol solution at a ratio of 3 : 1. The contents were mixed vigorously and allowed to stand at 20 °C for 30 min. Then the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher radical-scavenging activity. The inhibitory concentration (IC\textsubscript{50}) value (the concentration required to scavenge 50% DPPH free radicals) was calculated. Ascorbic acid, a known antioxidant, was used as a positive control. All the tests were carried out in triplicate.

1.8 Hexobarbipne-induced sleeping time studies Six groups of Swiss albino mice were used for this study (8 per group). Food was withdrawn on the preceding night of the experiment. Normal control and CCl\textsubscript{4} control groups received 2% gum acacia for 5 d, p.o.; test groups I to IV received *P. acidus* extracts at 100, 200 and 400 mg/kg for 5 d; test groups II to IV received *P. acidus* extracts at 100, 200 and 400 mg/kg for 5 d. CCl\textsubscript{4} (50 μL/kg, p.o.) in vehicle (olive oil) was given to CCl\textsubscript{4} control group and test groups I to IV, 1 h after the respective treatment on day 5. All the six groups of animals were given hexobarbipine (60 mg/kg, intraperitoneally (i.p.)), 2 h after CCl\textsubscript{4}/vehicle treatment. The time between loss of righting reflex and its recovery was recorded\textsuperscript{[34]}.

1.9 Statistical analysis The data of present study were expressed as \( \bar{x} \pm s \). The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using Instat software (version 2.1). The minimum level of significance was set at \( P<0.05 \).

2 Results

2.1 Preliminary phytochemical screening On preliminary phytochemical analysis, the *P. acidus* extracts showed the presence of flavonoids, glycosides, phenolic compounds, proteins, saponis and phytosterols.

2.2 Acute toxicity studies In acute oral toxicity study, the *P. acidus* extracts did not show any sign and symptoms of toxicity or mortality up to 2 000 mg/kg body weight, which could be considered relatively safe.

2.3 Effects of *P. acidus* extracts on CCl\textsubscript{4}-induced hepatotoxicity

2.3.1 Histopathological examination of the liver tissues The hepatoprotective effect of *P. acidus* extracts was confirmed by histopathological examination of the liver tissues of control and treated animals. The histological architecture of CCl\textsubscript{4}-treated liver sections showed necrosis, ballooning degeneration, and the loss of cellular boundaries. However, administration of *P. acidus* extracts and silymarin almost normalized these defects in the histological architecture of the liver tissues, showing marked hepatoprotective effect. Liver sections of normal control rats showed normal hepatic cells with well-preserved cytoplasm, well
brought out central vein and prominent nucleus and nucleolus (Figure 1A). Liver section of CCl₄ control rats showed massive fatty changes, necrosis, ballooning degeneration, and broad infiltration of the lymphocytes and the loss of cellular boundaries (Figure 1B). Liver section of rats treated with CCl₄ and silymarin showed well brought out central vein, hepatic cell with well-preserved cytoplasm, and prominent nucleus and nucleolus (Figure 1C). Liver section of rats treated with CCl₄ and P. acidus ethanolic extracts showed well brought out central vein, hepatic cell with well-preserved cytoplasm, and prominent nucleus and nucleolus (Figure 1D).

2.3.2 Effects of P. acidus extracts on liver function markers The effects of P. acidus extracts on serum enzymes, TB and TP levels in CCl₄-induced hepatotoxicity model rats were shown in Table 1. Elevated AST, ALT, ALP and TB and decreased TP level due to CCl₄ administration (2 mL/kg, s.c.) were significantly prevented with P. acidus extracts and silymarin treatment compared with CCl₄ control group ($P<0.05$ or $P<0.01$). There is no statistically significant difference between P. acidus-treated groups and silymarin-treated group.

2.3.3 Effects of P. acidus extracts on oxidative stress markers Reduced activities of enzymatic and non-enzymatic antioxidants (SOD, CAT, GPx and GSH, respectively) and enhanced lipid peroxidation were observed in CCl₄ control group (Table 2), whereas the P. acidus extracts- and silymarin-treated groups showed marked rise in antioxidant levels with significant reduction in lipid peroxidation compared with CCl₄ control group ($P<0.01$). There is no statistically significant difference between P. acidus extracts-treated groups and silymarin-treated group.

2.4 DPPH-scavenging potential of P. acidus extracts The P. acidus extracts exhibited a maximum DPPH radical-scavenging activity of 87% at a concentration of 155 μg/mL. The IC₅₀ value was found to be 68.2 μg/mL, which is approximately two times of that of ascorbic acid (IC₅₀ = 32.8 μg/mL) used as reference antioxidant.

Figure 1  Histological observations of rat livers with CCl₄-induced hepatotoxicity
A: Liver section of normal control rats; B: Liver section of CCl₄ control rats; C: Liver section of rats treated with CCl₄ and silymarin; D: Liver section of rats treated with CCl₄ and P. acidus ethanolic extracts.

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Aspartate transaminase (IU/L)</th>
<th>Alanine transaminase (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
<th>Total bilirubin (mg/L)</th>
<th>Total protein (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>6</td>
<td>148.27±8.96</td>
<td>67.38±9.67</td>
<td>177.28±2.69</td>
<td>6.3±0.1</td>
<td>50.9±0.9</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>282.29±7.87**</td>
<td>249.18±6.98**</td>
<td>293.35±6.17**</td>
<td>17.9±0.2**</td>
<td>42.2±0.1**</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>173.43±7.93△△</td>
<td>145.89±10.84△△</td>
<td>199.46±3.68△△</td>
<td>7.3±0.1△△</td>
<td>61.1±1.3△△</td>
</tr>
<tr>
<td>Group IV</td>
<td>6</td>
<td>247.67±12.24△</td>
<td>180.12±0.86△△</td>
<td>241.48±4.67△△</td>
<td>10.2±0.2△</td>
<td>45.6±0.2△</td>
</tr>
<tr>
<td>Group V</td>
<td>6</td>
<td>235.79±8.28△△</td>
<td>170.47±7.67△△</td>
<td>232.28±5.27△△</td>
<td>9.4±0.1△△</td>
<td>49.8±0.2△△</td>
</tr>
<tr>
<td>Group VI</td>
<td>6</td>
<td>199.28±6.64△△</td>
<td>152.27±11.38△△</td>
<td>202.91±6.96△△</td>
<td>7.6±0.2△△</td>
<td>51.4±0.1△△</td>
</tr>
</tbody>
</table>

** $P<0.01$, vs group I; △ $P<0.05$, △△ $P<0.01$, vs group II; Group I: normal control; Group II: CCl₄ control; Group III: silymarin (100 mg/kg); group IV: P. acidus (100 mg/kg); group V: P. acidus (200 mg/kg); group VI: P. acidus (400 mg/kg).
2.5 Effects of *P. acidus* extracts on hexobarbitone-induced sleeping time  A significant increase in hexobarbitone-induced sleeping time was observed in mice after CCl\textsubscript{4} administration. Pretreatment with *P. acidus* extracts at different dose levels (100, 200 and 400 mg/kg, p.o.) and silymarin (100 mg/kg, p.o.) considerably shortened the duration of hexobarbitone-induced sleeping time as compared with CCl\textsubscript{4} control group, indicating the hepatoprotection (Table 3). There is no statistically significant difference between *P. acidus*-treated groups and silymarin-treated group.

### Table 2  Effects of ethanolic extracts of *P. acidus* fruit on oxidative stress markers in Wistar rats with CCl\textsubscript{4}-induced acute hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Malondialdehyde (nmol/mg of tissue)</th>
<th>Glutathione (μmol/g of protein)</th>
<th>Superoxide dismutase (U/mg)</th>
<th>Catalase (U/mg)</th>
<th>Glutathione peroxidase (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>6</td>
<td>0.48±0.31</td>
<td>5.13±0.06</td>
<td>42.37±0.27</td>
<td>127.65±2.62</td>
<td>32.42±2.87</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>1.76±0.25</td>
<td>3.48±0.05</td>
<td>18.36±0.89</td>
<td>65.42±3.86</td>
<td>33.58±0.94</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>0.86±0.08</td>
<td>4.91±0.06</td>
<td>36.67±1.35</td>
<td>121.74±8.62</td>
<td>48.82±4.97</td>
</tr>
<tr>
<td>Group IV</td>
<td>6</td>
<td>1.69±0.36</td>
<td>3.59±0.07</td>
<td>22.14±1.42</td>
<td>79.13±7.33</td>
<td>35.23±0.53</td>
</tr>
<tr>
<td>Group V</td>
<td>6</td>
<td>1.36±0.12</td>
<td>3.72±0.06</td>
<td>26.65±1.29</td>
<td>88.83±8.96</td>
<td>39.02±0.51</td>
</tr>
<tr>
<td>Group VI</td>
<td>6</td>
<td>1.14±0.34</td>
<td>3.88±0.05</td>
<td>32.43±1.16</td>
<td>101.82±2.63</td>
<td>43.21±1.05</td>
</tr>
</tbody>
</table>

\* \* \* \* \*P<0.01, \* \*P<0.05, \* \* \*P<0.01, \* \* \* \*P<0.001 vs group I; \* \* \*P<0.05, \* \* \* \*P<0.01 vs group II; Group I: normal control; group II: CCl\textsubscript{4} control; group III: silymarin (100 mg/kg); group IV: *P. acidus* (100 mg/kg); group V: *P. acidus* (200 mg/kg); group VI: *P. acidus* (400 mg/kg).

### Table 3  Effects of ethanolic extracts of *P. acidus* fruit on hexobarbitone-induced sleeping time in CCl\textsubscript{4}-intoxicated albino mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Sleeping time (min)</th>
<th>Hepatoprotection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8</td>
<td>21.65±2.14</td>
<td>-</td>
</tr>
<tr>
<td>CCl\textsubscript{4} control</td>
<td>8</td>
<td>101.20±3.84</td>
<td>-</td>
</tr>
<tr>
<td>Test group I (silymarin 100 mg/kg)</td>
<td>8</td>
<td>40.52±3.12</td>
<td>76.27</td>
</tr>
<tr>
<td>Test group II (<em>P. acidus</em> extracts 100 mg/kg)</td>
<td>8</td>
<td>81.87±6.47</td>
<td>24.99</td>
</tr>
<tr>
<td>Test group III (<em>P. acidus</em> extracts 200 mg/kg)</td>
<td>8</td>
<td>64.79±3.98</td>
<td>45.76</td>
</tr>
<tr>
<td>Test group IV (<em>P. acidus</em> extracts 400 mg/kg)</td>
<td>8</td>
<td>58.48±2.87</td>
<td>53.72</td>
</tr>
</tbody>
</table>

\* \* \* \*P<0.01, \* \* \* \*P<0.05, \* \* \* \* \*P<0.001 vs normal control group; \* \* \* \*P<0.05, \* \* \* \* \*P<0.01 vs CCl\textsubscript{4} control group.

3 Discussion and conclusion

The present study demonstrates the hepatoprotective action of *P. acidus* fruit against CCl\textsubscript{4}-induced liver injury in Wistar rats. Since the biochemical and histological changes associated with CCl\textsubscript{4} are almost similar to acute viral hepatitis, CCl\textsubscript{4}-induced hepatotoxicity was chosen as an experimental model\footnote{}\footnote{35}. It is well established that CCl\textsubscript{4} is biotransformed by the cytochrome P-450 (CYP-450), especially by CYP-2E1 to a highly reactive trichloromethyl free radical. This free radical in turn reacts with oxygen to form a trichloromethylperoxy radical, which may attack on the membrane lipids of endoplasmic reticulum more readily than the trichloromethyl free radical. The trichloromethylperoxy radical leads to elicit lipid peroxidation, the disruption of Ca\textsuperscript{2+} homeostasis, elevation of hepatic enzymes, reduction of protein synthesis and finally results in cell death\footnote{38}. The elevated levels of AST, ALT, ALP and serum bilirubin are diagnostic indicators of acute liver injury\footnote{37}. In the present study, administration of CCl\textsubscript{4} caused considerable hepatocellular damage as indicated by the massive elevations of AST, ALT, ALP and bilirubin compared with normal control animals. Administration of *P. acidus* extracts at different dose levels attenuated the increased levels of the serum markers induced by CCl\textsubscript{4} and caused a subsequent recovery towards normalization. *P. acidus* extracts also increased the level of serum TP, which further indicates its hepatoprotective action. The histopathological observations of liver tissues further supported our claims.

It has been hypothesized that one of the principal causes of CCl\textsubscript{4}-induced hepatopathy is the formation of lipid peroxides by the free radical derivatives of CCl\textsubscript{4}. Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl\textsubscript{4}-induced toxicity\footnote{38}. In the present study, significant rise in the MDA level was observed, which suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals\footnote{39}. Treatment with *P. acidus* extracts considerably prevented lipid peroxidation, indicating its antioxidant action. Scavenging of DPPH is related to the inhibition of lipid peroxidation\footnote{39}. The free radical-scavenging property of *P. acidus* extracts was further confirmed by DPPH assay.

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accompanied by a set of endogenous
antioxidant enzymes such as SOD, CAT and GPx. Regarding non-enzymatic antioxidants, 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 CCl₄.⁴⁰

The reduced activities of SOD, CAT and GPx and GSH level as observed in our study point out the hepatic damage in rats administered with CCl₄. But the extracts-treated groups showed significant increase in the levels of these antioxidants, which further indicated the antioxidant activity of *P. acidus* extracts.

The damage imposed by CCl₄ on hepatocytes causes a loss of drug metabolizing enzymes of the liver which results in the prolongation of sleeping time induced by short acting barbiturates, like hexobarbitone⁴¹. Treatment with drugs that stimulates hepatic drug metabolizing enzymes considerably shortens the duration of hexobarbitone-induced sleeping time⁴². Thus the reduction in hexobarbitone-induced sleeping time caused by *P. acidus* extracts in the present study demonstrates its capacity to stimulate hepatic drug metabolizing enzymes.

*P. acidus* fruit has been reported to be a rich source of ascorbic acid, fibers and carotenoids.⁴⁰ The hepatoprotective and antioxidant action of ascorbic acid against acetaminophen-induced toxicity has already been reported⁴³. In addition, our preliminary phytochemical analysis showed the presence of phenolic compounds and flavonoids in *P. acidus* extracts. The hepatoprotective property of some phenolics and flavonoids has been reported against xenobiotic-induced hepatotoxicity in animals.⁴⁴ These compounds also exert antioxidant or *in vitro* free radical-scavenging effects.⁴⁵,⁴⁶,⁴⁷ *Phyllanthus emblica* fruit (Indian gooseberry), closely related with *P. acidus*, is also reported to contain ascorbic acid, flavonoids, tannins and phenolic compounds as chief antioxidant and hepatoprotective compounds.⁴⁵,⁴⁶,⁴⁷ Taking into account of the fact, the presence of such phytoconstituents in *P. acidus* extracts might be responsible for the offered hepatoprotective and antioxidant effects.

In conclusion, the results of biochemistry and histological studies collectively demonstrate that *P. acidus* fruit has significant hepatoprotective activity against CCl₄-induced acute liver toxicity in rats. The hepatoprotective action of *P. acidus* is certainly associated with their antioxidant properties acting as a scavenger of free radicals. The improvement in liver injury and liver functions by *P. acidus* extracts may be due to the presence of flavonoids and phenolic compounds which are reported to offer significant protection against liver toxicity. These preliminary findings on hepatoprotective and antioxidant actions reported herein would lend support to the use of *P. acidus* fruit as a hepatoprotective agent. Further studies are in progress in our lab to characterize the active principles and for better understanding the mechanism of action.

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REFERENCES


西印度醋栗对四氯化碳引起的大鼠和小鼠急性过氧化肝损伤的保护作用

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目的：证实西印度醋栗 (Phyllanthus acidus (L.) Skeels) 的果实在传统医学中作为保肝药使用的药用用途。
方法：连续 5d 分别给予各组大鼠西印度醋栗果实的 70% 乙醇提取物 (100, 200 和 400 mg/kg 口服) 及对照药水飞蓟素 (100 mg/kg 口服) 并于第 2 天及第 3 天皮下注射四氯化碳 (2 mL/kg)。测定血清天冬氨酸氨基转移酶 (aspartate transaminase, AST)、丙氨酸氨基转移酶 (alanine transaminase, ALT)、碱性磷酸酶 (alkaline phosphatase, ALP)、总胆红素以及总蛋白并进行肝脏组织病理学检测。检测大鼠肝组织匀浆中的氧化应激标记物如脂质过氧化反应 (lipid peroxidation, LPO)、还原型谷胱甘肽 (reduced glutathione, GSH)、超氧化物歧化酶 (superoxide dismutase, SOD)、过氧化氢酶 (catalase, CAT) 以及谷胱甘肽过氧化物酶 (glutathione peroxidase, GPX) 以衡量西印度醋栗的体外抗氧化作用。此外，本实验还记录了伊维巴诱导的给药后小鼠睡眠时间，使用 2,2-二苯基-1-硫基肼 (2,2-diphenyl-1-picrylhydrazil, DPPH) 法测定小鼠亚药后的自由基清除率。
结果：与对照组相比，西印度醋栗果实的乙醇提取物及水飞蓟素均显著降低了模型大鼠血清 AST、ALT 和 ALP 水平及 LPO 并升高了 TP、GSH、SOD、CAT 和 GPX 的水平 (P<0.01 或 P<0.05)。大鼠肝脏组织的组织病理学切片结果也证实了以上实验结果。在小鼠实验中，与对照组相比，西印度醋栗果实的乙醇提取物显著缩短了伊维巴诱导的小鼠睡眠时间 (P<0.01)，具有较高的 DPPH 清除率。
结论：这研究的实验结果证实了西印度醋栗果实对四氯化碳引起的大鼠急性过氧化肝损伤的保护作用，机制可能与其抗氧化作用及自由基清除功能有关。
关键词：叶下珠属；植物提取物；四氯化碳；氧化性应激；肝功能实验；动物实验；大鼠；小鼠