Protective effect of *Pisonia aculeata* on paracetamol induced hepatotoxicity in rats

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**Summary**

**Objective:** *Pisonia aculeata*, is traditionally used in treatment of liver disorder and thought to have a protective effect which may be beneficial to reduce symptoms of hepatotoxicity. The current study evaluated the scientific merit of these anecdotal claims in an *in vivo* model.

**Method:** Male Wistar rats were administered 250 or 500 mg/kg of *Pisonia aculeata* extract for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h by daily oral gavage. At the end of all experimental methods, all the animals were sacrificed by cervical decapitation. Blood samples were collected. Serum was separated and analyzed for various biochemical parameters.

**Results:** The plant extract showed a remarkable hepatoprotective and antioxidant activity against paracetamol induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. Paracetamol induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxides (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST).

**Conclusion:** Treatment of rats with different doses of plant extract significantly altered serum marker enzymes and antioxidant levels to near normal. The efficacy of the extract at dose of 300 mg/kg was comparable to the standard drug silymarin (50 mg/kg, p.o.). Data indicates a positive effect. More research is required to derive an optimal therapeutic dose.

**Key words:** Antioxidants; Histopathology; Lipid peroxidation; Paracetamol; *Pisonia aculeata*

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**Introduction**

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits [1]. Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders. Antioxidants play an important role in inhibiting scavenging free radicals and thus providing protection against infections and degenerative diseases [2].

*Pisonia aculeata* (Nyctaginaceae) is nearly pantropical, found in the new world tropics and is considered introduced in Africa, Asia, and the Phillipines [3]. *Pisonia aculeata* is a large scendent shrub distributed throughout India. The leaves and bark are used by the tribes and native medical practitioners to treat various ailments including liver disorders, inflammation, swelling, cough and tumours [4]. Preliminary phytochemical screening of the extracts reveals the presence of alkaloids, phenolic compounds, tannins, saponins and flavonoids. Hence they have been selected for phytochemical screening and evaluation of antioxidant and hepatoprotective properties. The present study is aimed to evaluate the hepatoprotective and antioxidant activity of methanol extract of the leaves of *Pisonia aculeata* against paracetamol induced hepatotoxicity in rats.

**Materials and methods**

**Plant material and extraction**

The plants *Pisonia aculeata* were collected in the month of July 2010 from the Tirunelveli Dist, Tamilnadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Coimbatore, Tamilnadu. A voucher specimen
has been kept in our laboratory for future reference. The plants were dried in the shade and pulverized. The powder was treated with petroleum ether for dewaxing as well as to remove chlorophyll. The powder was then packed into soxhlet apparatus and subjected to hot continuous percolation using methanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a vacuum desiccator (yield 4.1% w/w) and then suspended in 5% gum acacia for hepatoprotective studies.

**Animals**

Male Wistar rats (125-150 g) and Swiss albino mice (20-25 g) were procured from Sri Venkateswara Enterprises, Bangalore, India. They were housed in microloan boxes with standard laboratory diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance.

**Chemicals**

Paracetamol was purchased from Lupin Ltd., Mumbai, India. 1-chloro2,4-dinitro benzoic acid (CDNB), 5,5-dithio-bis-2-nitro benzoic acid (DTNB) and reduced glutathione (GSH) were supplied by Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Thiobarbituric acid was purchased from E-Merck, India. All other chemicals used were of analytical grade.

**Experimental design**

Rats were divided into five groups, each group consisting of six animals.

**Group I:** Controls received the vehicle viz. normal saline (2 ml/kg).

**Group II:** Received paracetamol (750 mg/kg, p.o.) [5] at every 72 h for 21 days.

**Group III:** Received silymarin 50 mg/kg (p.o.) for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.

**Group IV:** Received methanol extract of *P. aculeata* 250 mg/kg p.o. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.

**Group V:** Received methanol extract of *P. aculeata* 500 mg/kg p.o. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.

At the end of all experimental methods, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

**Assessment of liver function**

Biochemical parameters, i.e. aspartate amino transferase (AST) [6], alanine amino transferase (ALT) [6], alkaline phosphatase (ALP) [7], γ-glutamate transpeptidase (GGTP) [7], total bilirubin [9], and total protein [10] were analyzed according to the referred methods. The liver was removed, weighed and morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO) [11] superoxide dismutase (SOD) [12], catalase [13], glutathione peroxidise (GPx) [14], and glutathione S-transferase (GST) [15]. A portion of liver was fixed in 10% formalin for histopathological studies.

**Histopathological studies**

Liver slices fixed for 12 hr in Bouin’s solution were processed for paraffin embedding following standard micro techniques [16]. 5 μm sections of liver grained with alun haematoxylin and eosin, were observed microscopically for histopathological changes.

**Statistical analysis**

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test and data on liver weight variations were analyzed using Student’s ‘t’ test. P values less than 0.05 were considered significant.

**Results**

The effects of *P. aculeata* on biochemical parameters are presented in Table 1. The levels of serum AST, ALT, ALP, total bilirubin, GGTP were markedly elevated and that of protein decreased in paracetamol treated animals, indicating liver damage. Administration of *P. aculeata* extract at the doses of 250 and 500 mg/kg remarkably prevented paracetamol induced hepatotoxicity in a dose dependent manner.

Analysis of LPO levels by thiobarbituric acid reaction showed a significant (P<0.001) increase in the paracetamol treated rats. Treatment with *P. aculeata* (250 and 500 mg/kg) significantly (P<0.001) prevented the increase in LPO level which was brought to near normal. The effect of *P. aculeata* was comparable with that of standard drug silymarin (Table 2).

Paracetamol treatment caused a significant (P<0.001) decrease in the activities of SOD, catalase, GPx and GST in liver tissue when compared with control group (Table 2). The treatment of *P. aculeata* at the doses of 250 and 500 mg/kg resulted in a significant increase in the activities of SOD, catalase, GPx and GST when compared to paracetamol treated rats. The liver of
Table 1. Effect of *Pisonia aculeata* on biochemical parameters in paracetamol induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>GGTP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>132.7±1.18</td>
<td>76.25±1.78</td>
<td>76.25±1.78</td>
<td>0.8±0.05</td>
<td>8.13±0.46</td>
<td>75.34±1.05</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>269.7±2.45</td>
<td>194.8±43</td>
<td>468.5±17a</td>
<td>1.4±0.06(b)</td>
<td>6.09±0.32(b)</td>
<td>179.6±18(a)</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>178.4±2.17</td>
<td>96.8±1.93</td>
<td>226.2±2.67</td>
<td>0.79±0.02(ad)</td>
<td>8.09±0.47(e)</td>
<td>89.3±1.1(ad)</td>
</tr>
<tr>
<td><em>P. aculeata</em> extract</td>
<td>500</td>
<td>193.6±1.32</td>
<td>110.2±1.24</td>
<td>236.19±2.05</td>
<td>0.92±0.04(d)</td>
<td>7.42±0.14</td>
<td>96.4±1.67(ad)</td>
</tr>
</tbody>
</table>

N=6; Values are expressed as mean ± SEM; \(P<0.001\), \(P<0.05\) vs. control; \(P<0.05\), \(P<0.001\) vs. paracetamol group.

Table 2. Effect of *Pisonia aculeata* on lipid peroxidation levels and antioxidant enzyme activities in paracetamol induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>LPO</th>
<th>SOD</th>
<th>Catalase</th>
<th>GPx</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>7.85±0.92</td>
<td>46.7±1.12</td>
<td>78.0±3.1</td>
<td>28.45±1.16</td>
<td>2.04±0.06</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>19.8±2.1(a)</td>
<td>15.6±1.17(a)</td>
<td>32±2.36(a)</td>
<td>11.18±1.45(a)</td>
<td>1.16±0.07(a)</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>10.01±0.87(bd)</td>
<td>36.1±1.27(ace)</td>
<td>59±1.87(ad)</td>
<td>26.42±1.25(e)</td>
<td>1.92±0.05(e)</td>
</tr>
<tr>
<td><em>P. aculeata</em> extract</td>
<td>500</td>
<td>15.9±1.14(b)</td>
<td>25.6±0.37(a)</td>
<td>37±1.35(a)</td>
<td>16.64±1.18(ace)</td>
<td>1.36±0.05(a)</td>
</tr>
</tbody>
</table>

N=6; Each value is expressed as mean ± SEM; \(P<0.001\), \(P<0.01\), \(P<0.05\) vs. control; \(P<0.001\), \(P<0.01\) vs. paracetamol group.

Units: LPO = \(\mu\) moles of MDA/min/mg protein; SOD = Units/min/mg protein; CAT = \(\mu\) mole of H\(_2\)O\(_2\) consumed/min/mg protein; GPx = \(\mu\) moles of GSH oxidized/min/mg protein; GST = \(\mu\) moles of chloro-dinitro benzene (CDNB) conjugation formed/min/mg protein.

silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to paracetamol treated rats. Morphological observations showed an increased size and enlargement of the liver in paracetamol treated groups. These changes were reversed by treatment with silymarin and also *P. aculeata* at the doses tested (Table 3).

Histopathological studies showed paracetamol to produce extensive vascular degenerative changes and centrilobular necrosis in liver. Treatment with different doses of *P. aculeata* extract resulted in mild degenerative changes and prevented formation of centrilobular necrosis when compared with control (Fig.1). All these results indicate that *P. aculeata* extract has hepatoprotective effect against paracetamol induced hepatotoxicity.

Table 3. Effect of *Pisonia aculeata* on comparison of liver weight in all groups

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Dose (mg/kg)</th>
<th>Liver wt per 100 g body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>2 ml/kg</td>
<td>4.32±0.18</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>7.16 ± 0.14(ae)</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>4.98 ± 0.1(be)</td>
</tr>
<tr>
<td><em>P. aculeata</em> extract</td>
<td>250</td>
<td>6.23 ± 0.06(a)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.64 ± 0.12(ae)</td>
</tr>
</tbody>
</table>

N=6; values are expressed as mean ± SEM; \(P<0.001\), \(P<0.05\) vs. control; \(P<0.001\) vs. paracetamol.

Discussion

Paracetamol, a widely used antipyretic- analgesic drug, produces acute hepatic damage on accidental overdosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, N-acetyl–p–benzoquinamine (NAPQI) [17], which is normally conjugated with glutathione and excreted in urine. Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction [18], and development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in N-acetyl-p-aminophenol (APAP) bioactivation to NAPQI. P450 2E1 (CYP2E1) have been suggested to be primary enzymes for paracetamol bioactivation in liver microsomes [19]. Studies demonstrated that paracetamol induced hepatotoxicity can be modulated by substances that influence P450 activity [20].

In the assessment of liver damage by paracetamol the determination of levels of enzymes such as AST, ALT is largely used. Necrosis or membrane damage releases the intracellular enzymes into circulation and hence it can be measured in the serum. Increased levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and

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glutamate and is released in a similar manner. Therefore ALT is more specific to the liver and is a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [21]. Serum ALP, bilirubin and total protein levels are also related to the function of the hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [22]. Administration of Paracetamol caused a significant elevation of enzyme levels such as AST, ALT, ALP, GGTP, total bilirubin and decrease in total protein when compared to control. There was a significant restoration of these enzyme levels on administration of the P.aculeata extract in a dose dependent manner and also by silymarin at a dose of 50 mg/kg (Table 1). The reversal of increased serum enzymes in paracetamol induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [23].

The increase in LPO levels in liver induced by paracetamol suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. As mentioned above, one of the phenomena often observed in combination with oxidative stress is lipid peroxidation. Reactive oxygen species (hydrogen peroxide, superoxide anions, and hydroxyl radicals) are required for its initiation as NAPQI is expected to be incapable of initiating a radical hydrogen abstraction from lipid molecules. However, reduction of NAPQI, which could occur in the presence of flavoproteins, followed by reoxidation by oxygen could give rise to superoxide anions with a consequent formation of reactive reduced oxygen species. Even protein bound NAPQI was suggested to be liable to one electron reduction. LPO has been regarded to be an important initiation event in the toxicity mechanism of paracetamol [24]. Treatment of the rats with P.aculeata significantly reduced the elevated levels of LPO on dose dependent manner (Fig.2).

Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage [25]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. P.aculeata causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the
highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [26]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. A higher dose (500 mg/kg) increases the level of CAT as produced by silymarin, the standard hepatoprotective drug.

Glutathione (GSH) is one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Decreased level of GSH is associated with an enhanced lipid peroxidation in paracetamol treated rats. Administration of Aculeata significantly increased the level of GPx and GST in a dose dependent manner.

Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of P. aculeata on paracetamol induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action. Preliminary phytochemical studies reveal the presence of flavonoids in methanolic extract of P. aculeata. Flavonoids are known as hepatoprotectives [27-29]; the observed antioxidant and hepatoprotective activity of P. aculeata may be due to the presence of flavonoids. Further studies to characterise the active principles and to elucidate the mechanism are in progress.

References

Anbarasu et al: P.aculeata against paracetamol-induced hepatotoxicity


