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

Anbarasu C[1*], Rajkapoor B[2], Bhat KS[3], John Giridharan[4], A Arul Amuthan[4], Satish K[4]

[1]*Department of Pharmacology, Karpagam University, Eachanari Post-641 021, Coimbatore, Tamil Nadu, India*
[2]*Department of Pharmacology, Dayananda Sagar College of Pharmacy, Bangalore-560 078, India*
[3]*Strides Arcolab, Bangalore-560 076, India*
[4]*Regulatory Affairs, Strides Arcolab, Bangalore-560 076, India*

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ABSTRACT

Objective: To evaluate the protective effect of *Pisonia aculeata* (*P. aculeata*) on thioacetamide induced hepatotoxicity in rats. **Methods:** Male Wistar rats were administered 250 or 500 mg/kg p.o. of *P. aculeata* extract for 21 days and simultaneously administered thioacetamide (TAA) 50 mg/kg bw s.c. 1 h after the respective assigned treatments every 72 h. 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:** TAA induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST). Treatment of rats with different doses of plant extract (250 and 500 mg/kg) significantly ($P<0.001$) altered serum marker enzymes and antioxidant levels to near normal against TAA treated rats. The activity of the extract at a dose of 300 mg/kg was comparable to the standard drug, silymarin (50 mg/kg, p.o.). **Conclusions:** It can be concluded that *P. aculeata* extract possesses a remarkable hepatoprotective and antioxidant activity against TAA induced hepatotoxicity. More research is required to derive an optimal therapeutic dose.

1. Introduction

The liver is the most important organ in the body. It plays a pivotal role in regulating various physiological processes. It helps in the maintenance, performance and regulating homeostasis of the body. It is involved in almost all the biochemical pathways to growth, fighting against disease, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins[1].

The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction[2]. Liver diseases have become one of the major causes of morbidity and mortality all over the world. Among them, drug induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge[3].

In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are in rise. Jaundice and hepatitis are two major hepatic disorders that account for the high death rate[4]. There are potent indigenous herbal medicines available for the treatment of liver disorders in various parts of the world and most of them have not yet scientifically been validated. If they are conducted, it could lead to the development of cost effective drugs[5].

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or another for this purpose. Liver protective plants contain a

*Corresponding author: Mr. Anbarasu C, Department of Pharmacology, Karpagam University, Eachanari Post-641 021, Coimbatore, Tamil Nadu, India.
Tel: 91-42-2261-1146, 91-9886249910
Fax: 91-42-2261-1146
E-mail: anbukings@gmail.com

variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes[6]. Flavonoids are phenolic compounds widely distributed in plants, and have been reported to exert multiple biological effects, including antioxidant and free radical scavenging abilities[7]. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders[8] and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs.

Pisonia aculeata (*P. aculeata*) Linn. is a large scandent shrub, which holds an important place in folklore medicine. It is extensively used by native medical practitioners and tribes for treating swelling, rheumatic pains, jaundice and tumors[9,10]. Preliminary phytochemical screening of the extracts reveals the presence of alkaloids, phenolic compounds, tannins, saponnins 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 *P. aculeata* against thioacetamide (TAA) induced hepatotoxicity in rats.

2. Materials and methods

2.1. Plant material and extraction

The plants *P. aculeata* were collected in July 2010 from the Tirunelveli District, 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.

2.2. 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.

2.3. Chemicals

TAA was obtained from Micro labs, Hosur, 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 and purchased from local firms.

2.4. Acute toxicity study

The acute toxicity of the extract of *P. aculeata* was evaluated in mice using the up and down procedure (OECD, 2001). Mice received alcohol extract at various doses (500-2 000 mg/kg) orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h.

2.5. Experimental design

Rats were divided into five groups, each group consisting of six animals. Rats in group I (control group) received the vehicle *viz.* normal saline (2 mL/kg). Rats in group II received TAA 50 mg/kg bw s.c., every 72 h for 21 days. Rats in group III received silymarin 50 mg/kg p.o. for 21 days and simultaneously administered TAA 50 mg/kg b.w. s.c. 1 h after the respective assigned treatments every 72 h. Rats in group IV received methanol extract of *P. aculeata* 250 mg/kg p.o. for 21 days and simultaneously administered TAA 50 mg/kg bw s.c. every 72 h. Rats in group V received methanol extract of *P. aculeata* 500 mg/kg p.o. for 21 days and simultaneously administered TAA 50 mg/kg bw s.c. every 72 h.

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

2.6. Assessment of liver function

Biochemical parameters *i.e.*, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), γ-glutamate transpeptidase (GGTP), total bilirubin and total protein were analyzed according to the reported 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),

superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST). A portion of liver was fixed in 10% formalin for histopathological studies.

2.7. Histopathological studies

Liver slices fixed for 12 h in Bouin's solution were processed for paraffin embedding following standard micro techniques. Sections of liver (5 μm) grained with alum haematoxylin and eosin were observed microscopically for histopathological changes.

2.8. 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.

3. Results

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

Analysis of LPO levels by thiobarbituric acid reaction showed a significant (P<0.001) increase in the TAA 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 to that of standard drug silymarin (Table 2). TAA treatment caused a significant (P<0.001) decrease in the level of SOD, catalase, GPx and GSH in liver tissue when compared with control group. Treatment of *P. aculeata* at the doses of 250 and 500 mg/kg resulted in a significant increase of SOD, catalase, GPx and GSH when compared to TAA treated rats. The liver of silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to TAA treated rats.

Morphological observations showed an increased size and enlargement of the liver in TAA treated groups. These changes were reversed by treatment with silymarin and also *P. aculeata* at the doses tested (Table 3).

Table 3
Effect of *P. aculeata* on liver weight variation of TAA-induced hepatotoxicity rats (mean±SEM) (n=6).

Treatments	Dose (mg/kg)	Liver weight per 100 g bw (g)
Normal (normal saline)	2 mL/kg	4.13±0.17
TAA	50	6.65±0.26[a]
Silymarin + TAA	50	4.79±0.13
P. aculeata extract + TAA	250	5.48±0.07[a,c]
	500	5.08±0.34[b,d]

[a]: P<0.001, [b]: P<0.05 compared with normal; [c]: P< 0.01, [d]: P<0.001 compared with TAA.

Histopathological studies showed that TAA produced extensive vascular degenerative changes and centrilobular necrosis in hepatocytes (Figure 1). Treatment with different doses of *P. aculeata* extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control. All these results indicated a hepatoprotective potential of the extract.

Table 1
Effect of *P. aculeata* on biochemical parameters in TAA-induced hepatotoxicity rats (mean±SEM) (n=6).

Treatments	Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg%)	Total protein (mg%)	GGTP (U/L)
Normal (normal saline)	2 mL/kg	132.70±1.18	76.25±1.78	196.50±2.45	0.80±0.05	8.13±0.46	75.34±1.05
TAA	50	264.20±2.38[a]	182.60±1.27[a]	402.80±4.84[a]	1.30±0.04[a]	6.15±0.14[b]	169.50±2.14[a]
Silymarin + TAA	50	163.50±1.14[c]	96.50±2.38[a,c]	205.70±2.14[c]	0.86±0.07[c]	8.01±0.25[c]	96.50±1.12[a,c]
P. aculeata extract + TAA	250	213.30±1.47[a,c]	136.90±1.84[a,c]	308.54±3.42[a,c]	1.12±0.03	7.30±0.12[b]	129.40±1.26[a,c]
	500	188.60±1.26[a,c]	117.50±2.63[a,c]	227.40±2.10[a,c]	0.93±0.02[c]	7.90±0.09[c]	103.60±1.54[a,c]

[a]: P<0.001 compared with normal; [b]: P<0.05, [c]: P<0.001 compared with TAA.

Table 2
Effect of *P. aculeata* on antioxidants level in TAA-induced hepatotoxicity in rats (mean±SEM) (n=6).

Treatments	Dose (mg/kg)	LPO (A)	SOD (B)	Catalase (C)	GPx (D)	GST (E)
Control (normal saline)	–	10.35±0.92	46.70±1.12	78.00±3.10	28.45±1.16	2.04±0.06
TAA	50	21.63±1.16[a]	19.30±1.17[a]	31.00±2.36[a]	10.65±1.45[a]	1.16±0.07[a]
Silymarin + TAA	50	12.65±0.87[c]	39.70±1.23[a,d]	67.00±2.97[b,d]	26.42±1.25	1.95±0.05[d]
P. aculeata extract + TAA	250	17.03±1.15[b]	29.60±0.64[b,d]	42.00±2.08[a,e]	16.72±1.18[d,e]	1.49±0.04[a,f]
	500	13.86±1.32[c]	35.90±1.10[b,d]	59.00±2.14[a,d]	21.85±1.36[c,d]	1.86±0.03[d]

[a]: P<0.001, [b]: P<0.05, [c]: P<0.01 compared with control; [d]: P<0.001, [e]: P<0.05, [f]: P<0.01 compared with TAA. A: μ moles of MDA/ min/mg protein; B: units/min/mg protein; C: μ mole of H_2O_2 consumed/min/mg protein; D: μ moles of GSH oxidized/min/mg protein; E: μ moles of CDNB conjugation formed/min/mg protein.

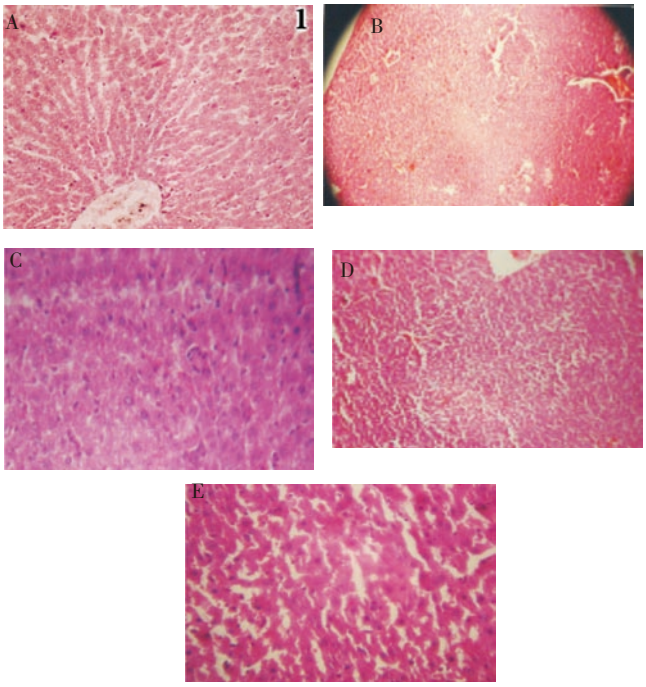

Figure 1. Effect of *P. aculeata* on TAA-induced hepatotoxicity in rats (histopathology).
A: Normal control, histology of the liver sections of normal control animals showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein; B: Disease control (TAA treated), the liver sections of TAA treated animals showed hepatic cells with severe toxicity characterized by centrilobular necrosis along with various gradation of fatty changes comprising of tiny to large sized vacuoles (fatty droplets, thereby thioacetamide showed cumulative type of toxicity; C: Silymarin (50 mg/kg) + TAA, silymarin (50 mg/kg per day, p.o. for 10 days) also reversed thioacetamide toxicity; D: *P. aculeata* low dose + TAA, E: *P. aculeata* high dose + TAA, *P. aculeata* treatment (250 and 500 mL/kg per day, p.o. for 10 days) appeared to significantly reverse TAA toxicity as revealed by normal central vein, hepatic cells with well-preserved cytoplasm along with prominent nucleus and nucleolus.

4. Discussion

Liver is one of the vital organs of the animal body and plays a central role in transforming and clearing the chemicals, but it is susceptible to the toxicity from these agents. Certain medicinal agents, like paracetamol, when taken in overdoses or sometimes even within therapeutic ranges, may damage the liver. Other chemical agents, such as those used in laboratories and industries, natural chemicals (*e.g.* microcystins) and herbal remedies can also induce hepatotoxins. More than 900 drugs have been implicated in causing liver injury and it is one of the most common reasons for a drug to be withdrawn from the market[11].

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. About 75%-80% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen *via* portal veins which bring drugs and xenobiotics in concentrated form[12]. Injury to hepatocyte and bile duct cells leads to accumulation of bile acid inside the liver, which promotes further liver damage[13].

Administration of TAA at doses of 100-300 mg/kg, i.p. results in the hepatic damage in animals[14,15]. The mechanism behind its toxicity is thought to be associated with its toxic metabolite (s-oxide). It interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury. It reduces the number of viable hepatocytes as well as rate of oxygen consumption and also decreases the volume of bile and its content, *i.e.*, bile salts, cholic acid and deoxycholic acid[13].

In the assessment of liver damage by TAA, the enhanced activities of these serum marker enzymes observed in TAA treated rats in our study correspond to the extensive liver damage induced by TAA. Results indicate that *P. aculeata* leaves extract administration could blunt TAA-induced increase in activities of different marker enzymes of heptocellular injury, *viz.* AST, ALT, ALP, total bilirubin, GGTP and total protein, suggesting that *P. aculeata* possibly has a protective influence against TAA-induced heptocellular injury and degenerative changes.

Excessive production of free radicals resulted in the oxidative stress, which leads to damage of macromolecules *e.g.* lipids, and can induce lipid peroxidation *in-vivo*[16-18]. In our study, TAA treatment produced the elevation in the levels of LPO. Treatment of the rats with *P. aculeata* significantly reduced the elevated levels of LPO. These results suggest that the hepatoprotective action of *P. aculeata* might be due to the presence of antioxidants like flavonoids. 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 is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. Catalase decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals[19]. Therefore, reduction in the activity of catalase 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 catalase as produced by silymarin, the standard hepatoprotective drug.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for GPx[20].

Decreased level of GSH is associated with an enhanced lipid peroxidation in TAA treated rats. Administration of *P. aculeata* significantly ($P<0.001$) increased the level of GPx in a dose dependent manner. *P. aculeata* inhibited significantly lipid peroxidation and recovered the decreased hepatic GSH level induced by TAA towards normalization. Administration of *P. aculeata* to TAA treated rats, maintained the level of non-enzymic antioxidants to near normal, by the possible role of TAA in improving the GSH status.

The inhibitory effect of TAA on cytochrome P450 and Cyt c reductase levels was also compensated by the extract through maintenance of its normal level. The role of the *P. aculeata* extract in the protection of TAA mediated loss in cytochrome P450 content may be considered as an indication of improved protein synthesis in hepatic tissue[21].

P. aculeata extract which decreases the activity of GST, which metabolizes toxic compounds to non-toxic compounds, means they have an increasing protective activity of the liver. In TAA intoxicated rats, the activity of GST increased drastically compared to that of normal group. The activity of GST recovered significantly ($P<0.001$) at 250 and 500 mg/kg of *P. aculeata* compared to that of TAA group. In contrast, the GST activity at 500 mg/kg is almost similar to the activity shown by silymarin, a potent hepatoprotective agent. The present study demonstrates that the hepatoprotective effect of *P. aculeata* against TAA-induced hepatotoxicity is due to multiple mechanisms.

In conclusion, we determined that TAA could increase the liver enzyme levels and affect some hepatospecific biochemical parameters. Increase in these parameters may occur due to peroxidation reactions, arising in TAA drug administration and these reactions may inflict oxidative injury to cellular components. In the light of these results, *P. aculeata* extract may play a role in the prevention of hepatic cellular injury produced by TAA. Preliminary phytochemical studies reveal the presence of flavonoids in methanolic extract of *P. aculeata*. The observed antioxidant and hepatoprotective activity of *P. aculeata* may be due to the presence of flavonoids. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

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