Hepatoprotective activity of ethanol extract of *PAVETTA INDICA LINN* leaves

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Abstract

Objective: To investigate the hepatoprotective activity of ethanol extract of *Pavetta Indica Linn* leaves.

Traditionally, the bark of *Pavetta Indica Linn*., in decoction or pulverized, is administered, especially to children, to correct visceral obstructions. The decocted leaves are used externally to alleviate the pains caused by hemorrhoids. The root, pulverized and mixed with the ginger and rice-water, is given in dropsy. A local fomentation with the leaves is useful in relieving the pain of piles.

Paracetamol (PCM) - Hepatotoxin- generates free radicals and raised serum enzyme levels-SGPT, SGOT, AlkPho, S.Alb. It causes necrosis, congested vessels, multifocal area of fatty changes nuclear disintegration, sinusoidal dilation, kuffer cell hyperplasia. The reverse is considered as the index of hepatoprotective activity. To screen hepatoprotective action this study has been taken up.

Materials and Methods: The acute liver damage in albino rats was induced byper oral administration of a single dose of 2000mg/kg b.w. PCMsuspension in 0.5% Carboxy methyl cellulose (CMC) and chronic liver damage by giving the same dose of PCM on the 7th day. The hepatoprotective activity was monitored biochemically by estimating S. transaminase, S. bilirubin and S. Proteinon the 8th day of experiment.

Results: Ethanol extract of *P. Indica* inhibited PCM induced liver toxicity in albino rats at 100mg/kg and 200mg/kg b.w as assessed by the biochemical values.

Conclusion: Ethanol extract of "*P. Indica*" exhibited significant hepatoprotective activity.

Keywords: *Pavetta Indica Linn*, Hepatoprotective activity, Silymarin, Paracetamol, Carboxy methyl cellulose.

Plant material and extract

P. Indica leaves were collected randomly from Imphal east district of Manipur State, and authentication of the plant was done by Department of life sciences, Manipur University, Canchipur, Imphal, Manipur state.

Kiritkar KR et al[1] and Thabrew MI[2] et al reported that the plant leaves were used in the treatment of liver diseases, pain of piles, urinary diseases and fever. Ethanol extract of *P.Indica* leaves was obtained by the extraction procedure as described by Chattopadhyay[3] with slight modification.Powder leaves was defatted with petroleum ether(60-80 c) and Soxhlet extraction with 99.9% ethanol.Further the ethanol extract was distilled and solvent ethanol removed. The residue extract was dried and measured. The yield was 28 gm. The extract thus obtained was used as the study material in the entire study for its hepatoprotective activity in albino rats.Various phytochemical constituents like flavonoids and their glycosides, alkaloids, sterols, phenolics, lignins, terpenoids, coumarins, fatty acids, saponins have been isolated from this plant.[4]

Recent time, focus in plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional system.[5]

Toxicity Testing

Acute oral toxicity study for the test extract of *P. Indica*was carried out using OECD/OCED Guideline 425.[6] The test procedure minimizes the no. of animals required to estimate the oral acute toxicity. Healthy, young adult albino rats (100-200g) were used for this study. 1/10th-1/20th of acute toxicity dose (2000mg) was taken as daily dose in the experimental models.

Hepatoprotective studies

For the study of hepatoprotective activity of ethanol extract of *P. Indica* leaves, the method of Rajasekaran A et al[7] was followed with slight modification.

Singh BN and Saravanan N[8] evaluated the aqueous extract of *P.Indica* leaves against carbon tetrachloride induced hepatotoxicity in rats. It showed a decreased in the serum enzymatic level of ALT, AST, ALP, total bilirubin. The effects produced were comparable to that of a standard hepatoprotective agent. The results indicated that the *P.Indica* leaves possessed significant hepatoprotective activity.

Muthu AK et al[9] clearly indicated the methanolic extract of *P.Indica* showed strong antioxidant activity by inhibiting super oxide anion scavenging activity, nitric oxide radical scavenging activities when compared with standard quercetin and ascorbate.

As experimental animals, rats have been employed extensively because of the size and low cost and being omnivorous, rats resemble man nutritionally. Paracetamol can be administered intragastrical or intraperitoneally.[10,11]
Antihepatotoxic potential of Silymarin against several chemicals were reported by various workers.[12]

Paracetamol overdose may cause severe hepatotoxicity and sometimes even fatal liver failure and centrilobular hepatic necrosis in humans and experimental animals.[13]

Test drug: The ethanol extract of *P. Indica*leaves was suspended in 0.5% Carboxy methyl cellulose sodium (CMC) and given orally at a dose of 100 and 200 mg/kg respectively for 7 days.

Animal: Albino rats of either sex-100-200g.Animals were acclimatized for 10days, feed with standard pellet diet and water ad libitum.

Induction of hepatic injury: The acute liver damage in albino rats was induced byper oral administration of a single dose of 2000mg/kg b.w. PCMsuspension in 0.5% Carboxy methyl cellulose (CMC) and the chronic liver damage by giving the same dose of PCM on the 7th day.

Treatment of animals: 35 healthy albino rats were divided into 5 groups of 6 each forhepatoprotective testing.

a) **GROUP 1** (normal control) served as a control and received normal saline, 5 ml/kg body weight, daily for 7 days.
b) **GROUP 2** (toxic control) constituted the hepatotoxic group and was treated similarly to group 1.
c) **GROUP 3** (Test dose 1) received ethanol extract of *P.Indica* 100 mg/kg body weight per day suspended in 0.5% CMC for 7 days.
d) **GROUP 4** (Test dose 2) received ethanol extract of *P.Indica* 200 mg/kg body weight per day suspended in 0.5% CMC for 7 days.
e) **Group 5** (standard) were given the standard drug, Silymarin 100 mg/kg body weight daily) for 7 days.
f) On the 7thday, paracetamol suspension in 0.5% CMC was given orally, 2 g/kg body weight, to all the Groups except Group 1, which was given CMC.

Preparation of samples for biochemical studies:

After induction of hepatotoxicity, on the 8th day of the experiment the blood was withdrawn from orbital sinus by using capillary tube as mention by Rao KS and Mishra SH[14]for analysis of liver parameters.

All rats were anaesthetized with ether and blood was withdrawn from orbital sinus by using capillary tube.The most efficient method of collecting blood in rats and mice causing least stress to the animals is from the orbital sinus with the help of a capillary tube.[15]Then the blood was kept for 30 minutes without disturbing. The clots were dispersed with glass rod and then centrifuged for 20 minutes at 2000 rpm to separate the serum. The serum of each animal of all groups was estimated for SGPT, SGOT, bilirubin and total protein content.

Lablife chem Master instrument was used for the estimation of different biochemical parameters included in the present study. It is a semi automated chemistry analyser made by DIAGNOVA.SGPT and SGOT were determined by Reitman and Frankel[16] method (using kits from span Diagnostic Ltd.). Malloy and Evelyn method[17]was followed to estimate total bilirubin content and Biuret[18] (manual) method for the measurement of total protein.

Table 1: Effect of ethanol extract of *P. Indica* on serum level of SGPT, SGOT, direct bilirubin, total bilirubin, albumin, globulin & total protein in paracetamol induced hepatotoxicity in albino rats

Treatment	SGOT	SGPT	Direct bilirubin	Total bilirubin	Albumin	Globulin	Total protein
Group 1 CMC	71±2.36	102.67±6.74^{c}	0.53±0.25	0.92±0.5	3.25±0.4	2.317±0.42^{b}	5.53±0.3
Group 2 Toxic control (PCM)	135.17±4.99^{b}	147.5±5.68^{b}	0.98±0.34	2.78±0.6^{a}	1.12±0.38^{b}	1.5±0.18^{a}	2.62±0.36^{b}
Group 3 *P.indica* (100mg/kg) + PCM	101.5±4.7^{a}	123.17±9.1^{a}	0.92±0.41	1.57±0.49	2.52±0.5^{c}	1.78±0.38^{ab}	4.35±0.37^{a}
Group 4 *P.indica* (200mg/kg) + PCM	100.5±6.53^{a}	108.33±7.84^{c}	0.6±0.1	1.17±0.28	2.4±0.41^{ac}	2.37±0.32^{b}	4.6±0.46^{a}

Group 5 Silymarin (100mg/kg) + PCM	74.17±20.83	102.5±5.5	0.97±0.28	1.26±0.31	3.12±0.2	2.62±0.55	5.73±0.6

Mean± SD (n=6). Within a column means marked with different superscript letters are significantly different ($p < 0.001$- 0.05) as analyzed by Tukey-Kramer multiple comparisons test.

Results

The ethanol extract of *P. Indica* was found to be safe and there was no mortality up to 2000mg/ kg body weight p.o. after 14 days. $1/10^{th}$-$1/20^{th}$ of this dose was selected to carry out the hepatoprotective activity studies. Serum enzymes level was very high in rats challenged with PCM. In group 3 and 4, the enzymes activity is significantly lowered when compared to that of group 2 and the values are closer to that of normal control. There is no significant raise in total bilirubin in groups treated with the test extract dose 1 and 2. Also the total protein values are near normal in those rats in which test extract was given.The total bilirubin for normal control group was 1.25±0.31 and total protein level for normal control group was 5.73±0.65 which were in conformity with the finding of Oinam et al.[19]

Discussion

The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effect or in maintaining the normal hepatic physiology, which have been disturbed by a hepatotoxin. The present study shows that ethanol extract of *P. Indica* has remarkably good hepatoprotective effect in acute and chronic studies. The elevation in the plasma levels in group 2 reflects that the liver injury is induced by PCM.

The extract decreased the PCM induced elevated enzymes levels in Group 3 and 4are suggestive of production of the structural integrity of hepatocytes cell membrane or regeneration of damaged liver cells by the extract. Lower serum bilirubin level and near normal protein levelin extract treated groups 3 and 4 indicate the effectiveness of the extract in normal functional status of the liver.

The results of the present investigation infer that ethanol extract of *Pavetta Indica linn* leaves possess fairly good hepatoprotective activity.

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