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Original Research Article

Hepatoprotective activity of ethanol extract of *Pavetta Indica Linn* leaves

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ABSTRACT

Background: 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) toxicity generates free radicals and raised serum enzyme levels-SGPT, SGOT, Alkaline Phosphatase and S. Albumin. 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. The present study is being taken up to screen hepatoprotective action of *P. Indica Linn*.
Methods: The acute liver damage in albino rats was induced by per oral administration of a single dose of 2000mg/kg b.w. PCM suspension 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. Protein on 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.
Conclusions: Ethanol extract of "*P. Indica*" exhibited significant hepatoprotective activity.

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

INTRODUCTION

Since time immemorial, people have been using plants and herbs as medicines for the treatment of many kinds of diseases. The Rig Veda, dating 3500 B.C to 1800 B.C seems to be the earliest record available on medicinal plants. The world health organization (WHO) estimated that 80% of the population of the developing countries relies on traditional medicines, mostly of plants origin and local health practitioner for their primary health care needs.[1] Plants continue to serve as possible sources of new drugs and chemicals derived from various parts of plants.[2]

Plant derived natural products such as flavonoids, terpenoids, saponins and sterols have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity. Due to the absence of reliable drugs for the management of liver ailments in the modern medicine, plants and natural products are providing to be hepatoprotectants as evident from voluminous published work on their hepatoprotective potentials.

The scientific study of the plants for various medicinal properties should be an important aspect for the scientific validation of folklore claims of the inhabitants with regard

to the utility of the plants as well as this can be a new source of herbal drugs.[3] A variety of plants have been studied which support liver functions and are used to treat diseases of the liver, but still there is a need toexplore more hepatoprotective plants. Keeping this in view, the present study has been undertaken to investigate hepatoprotective activity of ethanol extract of *Pavetta indica Linn.* on paracetamol induced liver damage in rats.

Objective of the study was to investigate the hepatoprotective activity of ethanol extract of *Pavetta Indica Linn* leaves.

METHODS

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, and Thabrew MI et al, reported that the plant leaves were used in the treatment of liver diseases, pain of piles, urinary diseases and fever.[4,5] Ethanol extract of *P. Indica* leaves was obtained by the extraction procedure as described by Chattopadhyay with slight modification.[6] 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.[7]

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.[8]

Toxicity testing

Acute oral toxicity study for the test extract of *P. Indica* was carried out using OECD/OCED Guideline 425.[9] 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. $1/10^{th}$-$1/20^{th}$ 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, was followed with slight modification.[10]

Singh BN and Saravanan N evaluated the aqueous extract of *P. Indica* leaves against carbon tetrachloride induced hepatotoxicity in rats.[11] 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, 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.[12]

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.[13,14]

Antihepatotoxic potential of Silymarin against several chemicals were reported by various workers.[15]

Paracetamol overdose may cause severe hepatotoxicity and sometimes even fatal liver failure and centrilobular hepatic necrosis in humans and experimental animals.[16]

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 200mg/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 7^{th} day.

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

1. Group 1 (normal control) served as a control and received normal saline, 5ml/kg body weight, daily for 7 days.
2. Group 2 (toxic control) constituted the hepatotoxic group and was treated similarly to group 1.
3. Group 3 (Test dose 1) received ethanol extract of *P. Indica* 100mg/kg body weight per day suspended in 0.5% CMC for 7 days.

4. 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.
5. Group 5 (standard) were given the standard drug, Silymarin 100mg/kg body weight daily) for 7 days.
6. On the 7thday, paracetamol suspension in 0.5% CMC was given orally, 2g/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 SHfor analysis of liver parameters.[17]

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.[18]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 method (using kits from span Diagnostic Ltd.).[19] Malloy and Evelyn method was followed to estimate total bilirubin content and Biuret (manual) method for the measurement of total protein.[20,21]

Table 1: Effect of ethanol extract of *P. Indica* on serum level of SGPT, SGOT, direct bilirubin, total bilirubin, albumin, globulin and 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. 1/10th-1/20th 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.[22]

DISCUSSION

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.In the present study, normal control group (mean±SEM U/L) level of AST and ALT were 74.17±20.84 and 102.50±5.51 respectively which were in conformity with the findings of Palanivel M et al, and Anbarasu et al, and 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 Anbarasu et al.[23,24]

There was significant (P<0.001) rise in AST (135.17±5.00) and ALT (147.50±5.68) levels after 24 hours of Paracetamol administration in toxic control group when compared to normal control (71.00±2.37; 102.67±6.74) group which was in conformity with the finding of Rao KS and Mishra SH.[25] The serum total bilirubin level in toxic control (2.78±0.60) is significantly high (P<0.001) when compared to normal control group (0.92±0.51) which was in conformity with the finding of Shenoy KA et al.[26] The serum total protein level in toxic control group (2.62±0.37) was significantly reduced (P<0.001) when compared to normal control group (5.53±0.31) which was in conformity with the finding of Venukumar and Latha MS.[27]

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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