

THE EFFECT OF *Pavetta indica* IN CCl₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The objective of present study was to investigate the effect of aqueous leaf extract of *Pavetta indica* on carbon tetra chloride (CCl₄) induced hepatotoxicity in rats. Liver damage was induced by intraperitoneal administration of CCl₄ (0.4 ml/kg body weight) for 3 days. Rats were treated with aqueous leaf extract of *Pavetta indica* (10 mg/kg body weight) by oral administration. The protective activity has been measured by using biochemical parameters such as aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein and bilirubin in treatment rats. The CCl₄ plus aqueous leaf extract of *Pavetta indica* treated rats showed significant reduction of above serum enzyme activities when compare to control rats. Histopathology findings are also in agreement with the biochemical findings. Results indicate the hepatoprotective properties of *Pavetta indica* against CCl₄ induced hepatotoxicity in rats.

Keywords: *Pavetta indica*; carbontetrachloride; liver.

INTRODUCTION

Liver is vital organ present in vertebrates and some others animals involved in the maintenance of metabolic function including detoxification, glycogen storages, protein synthesis and production of biochemical's necessary for digestion.^{1,2} Liver damage is a widespread disease which can be caused by reactive oxygen species (ROS), and is characterized by a progression from steatosis to chronic hepatitis, cirrhosis and hepatocellular carcinoma.^{3,4} Several compounds, such as carbon tetrachloride (CCl₄), acetaminophen, bromo benzene, ethanol and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver diseases.⁵ CCl₄ is a classical hepatotoxin that causes rapid liver damage progressing from steatosis to centrilobular necrosis.⁶ In view of server undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and evaluate scientific basis for the traditional herbal medicines which are claimed to *Pavetta indica* is an herbal drugs traditionally used for hepatoprotective.^{7,8} It belongs to the family, Rubiaceae. The plant is widely distributed in north-eastern region of India especially Manipur and Assam. The leaves are elliptic-oblong to elliptic-lanceolate, 6-15 cm long, and pointed at both ends.⁹ The flowers are white, rather fragrant and borne in considerable numbers in hairy terminal panicles which are 6-10 cm long.¹⁰ The sepals are very small and toothed. The flower-tube is slender and about 1.5 cm long; with obtuse petals about half the length of the tube.¹¹ Therefore the present study was planned to evaluate the hepatoprotective efficacy of aqueous leaf extract of *Pavette indica* against CCl₄ induced

liver injury.

MATERIAL AND METHODS

Collection of plant material

The plants leaves were collected from a village called Sunusiphai, Bisnupur district, Manipur, India and leaves were dried in room temperature for 5 days and made to fine powder.

Plant Authentication

The plant was authenticated by professor N Ibotombi, Research Botanist, Manipur University, Manipur, India.

Extract preparation

Dried plant powder were weighted and dissolved in distilled water. It was then boiled at 60°C in water bath for 20 min. After heating the pellet was filtered with the use of filter paper in a new beaker and supernatant was made to evaporate. After the complete evaporation the resulting pellet was dissolved in known amount of distilled water and given to experimental animals.

Animals

Male adult albino Wistar rats, 6-7 weeks old (weighing 120-150 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained in an air-conditioned room (23 ± 2°C) with a 12 h light/12 h dark cycle. Commercial diet purchased from animal house and tape water was supplied. All the experimental studies were conducted in the Department of Biochemistry, Faculty of Science, Annamalai University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH 1985); the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA,

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Experimental design

The experiment was carried out after obtaining Clearance from institutional Animal Committee. The animal was randomly divided into four groups (6 rats per group). Group 1 served as normal control and given with 2ml NaCl for 3 days. Group 2 rats were given with the plant extract at a dose of 10mg/kg body weight orally for 3 days. The rats in Group 3 were given intraperitoneally with a dose of CCl₄ (0.4, ml/kg of body weight) for 3 days. And Group 4 were given intraperitoneally with CCl₄ (0.4ml/kg of body weight) and plant extract of 10mg/kg body weight orally for 3 days.¹² On the 4th day, the rats were sacrifice and the blood was collected from abdominal artery and kept at 37°C in the incubator for 30 min afterwards, it was centrifuged at 2000 rpm for 15 min to isolate the supernatant serum, which was used for the biochemical estimations. All the biochemical parameters were done by commercially available reagent kits and analysed in ERBA Smart lab, fully automated biochemistry analyzer.

Histopathological studies

For histopathological studies, tissue slices of liver and kidneys were fixed for 48 h in 10% formalin. 5µm sections were done, stained with haematoxylin-eosin and examined under light microscope.

Table 1. Effect of *Pavetta indica* on the activities of hepatic marker enzymes and bilirubin in the serum of control and CCl₄-administered rats

Parameters	Control	<i>Pavetta indica</i>	CCl ₄	CCl ₄ + <i>Pavetta indica</i>
AST IU/L	183.5 ± 5.09 ^a	181.5 ± 9.67 ^a	307.5 ± 34.5 ^b	214.5 ± 2.72 ^c
ALT IU/L	42.5 ± 1.70 ^a	43.5 ± 1.87 ^a	300.66 ± 108.06 ^b	77.33 ± 2.68 ^c
ALP IU/L	339.66 ± 24.70 ^a	342.33 ± 18.49 ^a	620.16 ± 59.90 ^b	377.8 ± 5.89 ^c
Bilirubin mg/dl	0.29 ± 0.08 ^a	0.310 ± 0.02 ^a	0.43 ± 0.026 ^b	0.31 ± 0.022 ^a

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript differ significantly at p < 0.05 (DMRT).

Table 2. Effect of *Pavetta indica* on serum proteins of control and CCl₄-administered rats

Parameters	Control	<i>Pavetta indica</i>	CCl ₄	CCl ₄ + <i>Pavetta Indica</i>
Total protein(g/dl)	3.5 ± 0.08 ^a	3.6 ± 0.08 ^b	3.15 ± 0.05 ^c	3.4 ± 0.08 ^a
Albumin (g/d)	1.55 ± 0.05 ^{ac}	1.86 ± 0.05 ^a	1.5 ± 0.08 ^b	1.53 ± 0.07 ^c
Globulin (g/dl)	1.95 ± 0.05 ^a	1.53 ± 0.09 ^a	1.65 ± 0.095 ^a	1.86 ± 0.047 ^a
A/G(g/dl)	1.25 ± 0.04 ^a	1.21 ± 0.07 ^a	1.075 ± 0.09 ^b	1.21 ± 0.07 ^a

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript differ significantly at p < 0.05 (DMRT).

The serum levels of total protein, albumin, globulin and A/G ratio in differentially treated rats group are given in table 2. The serum levels of total protein in control group and group 4 (CCl₄ + *Pavetta indica*) were significantly similar to each other. Total protein level decrease in CCl₄ group and increase in Group 2. The albumin level of CCl₄ group slightly decreased when compare to normal. Globulin level slightly but not significantly increased in CCl₄ group when compared to normal. A/G ratios were significant to each other except CCl₄ group.

Liver of the control animal showing the central vein and hepatocytes arranged in the form of cords (Figure 1A). Liver tissue from a control rat treated with *Pavetta indica* showing normal histology (Figure 1B). In CCl₄ administered rats, feathery degeneration, micro- and macrocellular fatty changes, periportal fibrosis and vascular congestion were observed in the liver (Figure 1C). The CCl₄ + *Pavetta indica* treated rat liver has normal histology however; the central vein shows congestion (Figure 1D).

Kidney of the control animal shows normal tubules and glomeruli (Figure 2A). Kidney of control rats treated with *Pavetta indica* shows normal histology (Figure 2B). CCl₄ administered rat kidney shows haemorrhage in the tubular region and fatty infiltration in the parenchyma

Statistical analysis

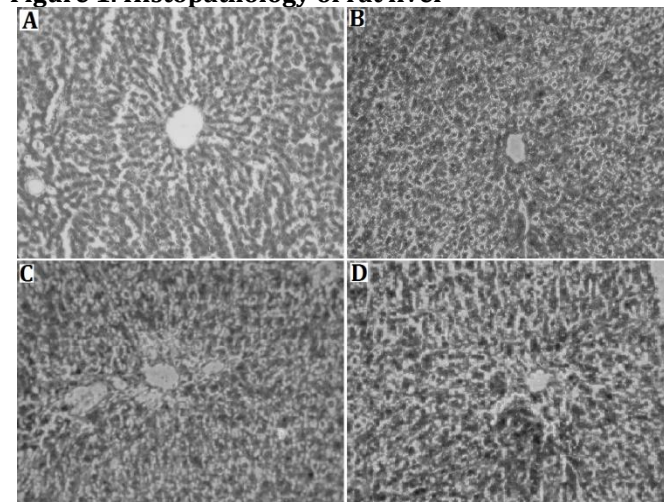
The data values were expressed as mean ± S.D. The effect of *Pavetta indica* in treatment were evaluated, statistically using the one way analysis of variance (One-way ANOVA) followed by the Duncan's test for multiple comparison treatments. Statistical significant was set at the p < 0.05 levels.

RESULTS AND DISCUSSION

The change of serum AST, ALT, ALP and bilirubin level are shown in normal and experimental animals are shown table 1. The estimated values of serum AST, ALT, ALP and bilirubin in the rats control 183.5 ± 5.09, 42.5 ± 1.7, 339.66 ± 24.7, and 0.29 ± 0.08 respectively. The enzyme activities and level of bilirubin were significantly elevated to 307.5 ± 34.5, 300.66 ± 108.06, 620.16 ± 59.9 and 0.43 ± 0.026 respectively in CCl₄ group. Treatment of animals with *Pavetta indica* decreased the serum AST, ALT, ALP activities and bilirubin level to 214.5 ± 2.72, 77.33 ± 2.68, 377.8 ± 5.89, and 0.31 ± 0.022 respectively which was significantly lower than the values of the toxic control group values (p < 0.05). In the *Pavetta indica* alone given control, the AST, ALT, ALP and bilirubin values were 181.5 ± 9.67, 43.5 ± 1.87, 342.33 ± 18.49 and 0.310 ± 0.02 respectively, which was not significant with the normal levels showing non toxicity of the leaves.

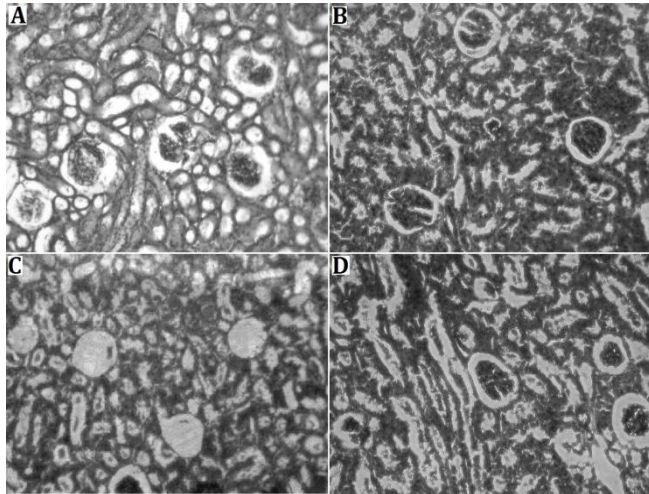
(Figure 2C). CCl₄ + *Pavetta indica* treated rat kidney shows tubules containing fat vacuoles and normal glomeruli (Figure 2D).

Figure 1. Histopathology of rat liver



A: Control animal liver showing the central vein and hepatocytes arranged in the form of cords (H&E x 20). **B:** Liver tissue from a control rat treated with *Pavetta indica* showing normal histology (H&E x 20). **C:** CCl₄-administered rat liver: Feathery degeneration, micro- and macrocellular fatty changes, periportal fibrosis and vascular congestion in the liver (H&E x 20). **D:** CCl₄ + *Pavetta indica* treated rat liver: Normal histology was observed; however, the central vein shows congestion (H&E x 20).

Figure 2. Representative photomicrographs of histopathological changes due to *Pavetta indica* treatment in the kidney of control and experimental rats.



A: Control animal kidney showing normal tubules and glomeruli (H&E x 20). **B:** Kidney of control rats treated with *Pavetta indica* showing normal histology (H&E x 20). **C:** CCl₄- administered rat kidney showing haemorrhage in the tubular region and fatty infiltration in the parenchyma (H&E x 20). **D:** CCl₄ Ethanol + *Pavetta indica* treated rat kidney showing tubules containing fat vacuoles and normal glomeruli (H&E x 20).

Carbontetrachloride is the main classical hepatotoxin that causes rapid liver damage progressing from steatosis to centrilobular necrosis.¹³ The mechanism of liver injury induced by CCl₄ is thought to involve free radicals (CCl₃^{*}) and lipid peroxidation.¹⁴ The covalently binding free radicals to cell component initiates the secretion of lipoprotein and thus steatosis, whereas reaction with oxygen, to form CCl₃^{*} which initiates lipid peroxidation¹⁵. Severe hepatocytes membrane damages cause during CCl₄ toxicity. The increase level of serum bilirubin, AST, ALP,

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