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Effect of Ethanol Extract of *Cansjera rheedii* J. Gmelin (Opiliaceae) on Hepatotoxicity

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Abstract: The hepatoprotective activity of ethanol (95%) extract of (250 mg kg⁻¹) *Cansjera rheedii* J. Gmelin whole plant was evaluated against paracetamol induced hepatotoxicity by evaluating biochemical parameters such as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total bilirubin, Total Protein and Gamma Glutamyl Transpeptidase (GGTP). A 10% of liver homogenate was used for estimation of enzyme such as Superoxide Dismutase (SOD), Glutathione S-Transferase (GST), Lipid Peroxidase (LPO) and Glutathione Peroxidase (GPx) for antioxidant study. Treatment of rats with ethanol extract significantly (p<0.001) altered serum marker enzymes and antioxidants level near to normal against paracetamol intoxicated rats. Silymarin (50 mg kg⁻¹, p.o.) used as control.

Key words: *Cansjera rheedii*, paracetamol, hepatoprotective activity

INTRODUCTION

In traditional medicines, various herbal preparations are being used for treating liver disorders. In the absence of an effective treatment in modern medicine, efforts are being made to find out suitable herbal drugs. In previous work we have reported the hepatoprotective activity of *Moringa oleifera* (Ruckumani *et al.*, 1998), *Casaria esculenta* (Jayakar *et al.*, 1999), *Orthosiphon thymiflorus* (Kavimani and Thangadurai, 1998) and *Carica papaya* (Raj Kapoor *et al.*, 2002) against paracetamol induced hepatotoxicity.

Cansjera rheedii (Family: Opiliaceae) is a climbing shrubs, sometimes armed, commonly known as Kalimanakeerai in Tamil is generally found in India through Malaya to Hong Kong and North Australia (Gamble, 1981; Matthew, 1991). No reports were available regarding its pharmacological activity. But whole plant of *Cansjera rheedii* was used by the tribes of Auroville village near Puducherry for various liver disorders. So, the present study was undertaken to establish a scientific evidence for its hepatoprotective activity.

MATERIALS AND METHODS

Materials

The whole plant of *Cansjera rheedii* (Opiliaceae) were collected in and around auroville, puducherry in the month of June 2006 and it was identified and authenticated by Auro Herbarium

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Sakthi Botanical Survey Department, Auroville. A voucher specimen has been kept in our laboratory for future reference (VS-12). The whole plant of *Cansjera rheedii* were cut into small pieces, shade dried and powdered. The coarse powder was subjected to continue hot extraction in a soxhlet by using ethanol (95%v/v). The ethanol was removed by distillation under reduced pressure. This extract was suspended in 5% gum acacia and used for the experiment. The LD₅₀ value of ethanol extract of *C. rheedii* was determined by using OCED (2001).

Animals

Adult male Wister rats were procured from Kings Institute, Chennai, India. They were fed on commercial diet (Hindustan lever Ltd., Bangalore) and water *ad libitum*. All the animals acclimatized for a week before use. The room temperature was maintained at 25±1°C. Four groups (I-IV) comprising each of six animals weighing between 150-175 g were selected. Group 1 served as control and received 2 mL kg⁻¹ of normal saline daily for 10 days orally. Groups 2 rats were similarly treated as group 1. Groups 3 treated with ethanol extract of *Cansjera rheedii* at a dose of 250 mg kg⁻¹ and Group 4 were treated with silymarin 50 mg kg⁻¹ for 10 days. On the 10th day, paracetamol suspension was given by oral route in a dose of 750 mg kg⁻¹ (Kapur *et al.*, 1994; Hiroshini *et al.*, 1987) to all rats of Group 2, 3 and 4.

After 36 h of the last dose, all the rats were sacrificed under light ether anesthesia; blood was collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and used for the estimation of biochemical parameters such as SGPT (Reitman and Frankel, 1975), SGOT, ALP (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937), total protein (Lowry *et al.*, 1951) and GGTP (Szazsi, 1969). After weighing the ratio of wet liver weight per 100 g of animal body weight, a 10% of liver homogenate was used for antioxidants studies such as catalase (Devasagayam and Tarachand, 1984), SOD (Marklund and Marklund, 1997), GST (Sinha, 1972), LPO (Rotruck *et al.*, 1973) and Gpx (Habig *et al.*, 1974).

The values were expressed as mean±SEM. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test and data of liver weight variation were analyzed using Student's t-test. p-values <0.05 were considered significant.

RESULTS AND DISCUSSION

Ethanol extract of *Cansjera rheedii* did not produce any mortality up to the dose of 2000 mg kg⁻¹. The results of biochemical parameters revealed the elevation of all the enzyme level in paracetamol treated group indicating that paracetamol induces damage to the liver (Table 1). Both transaminases rich in liver tissues were increased in patients with acute hepatic diseases; SGPT, which is slightly elevated in cardiac necrosis, is a more specific indicator of liver disease (Rodwell *et al.*, 1983). A significant reduction was (p<0.001) observed in SGPT (from 176 to 96 IU), alanine aminotransferase (SGOT) (from 227 to 184 IU), Alkaline phosphatase (ALP) (from 578 to 381 IU), total bilirubin and GGTP (from 62 to 31 IU) levels in the group treated with ethanol extract of *C. rheedii*. The enzyme levels were almost restored to the normal.

Thiobarbuturic acid reactive substance LPO level are significantly (p<0.001) increased (from 7.85 to 17.17 µmole) in the paracetamol control when compared with normal rats. Treatment with ethanol extract of the plant significantly (p<0.001) prevented increase in LPO levels and brought them to near normal level. Paracetamol treatment caused significant (p<0.001) decrease in the level of SOD, catalase, GPx and GST in liver tissue when compared with control group (Table 2). After treatment with ethanol extract of *C. rheedii* increase SOD (from 7.05 to 14.24 µmole), catalase (from 26.17 to 46.85 µmole), Gpx (from 16.82 to 37.79 µmole) and GST (from 0.09 to 0.10 µmole) levels

Table 1: Effect of ethanol extract of *C. rheedii* on paracetamol induced hepatotoxicity in rats

Treatments	Dose (mg kg ⁻¹)	SGOT (U L ⁻¹)	SGPT (U L ⁻¹)	ALP (U L ⁻¹)	Total billurubin (mg %)	Total protein (mg %)	GGTP (U L ⁻¹)
Control	-	157.66±4.6	74.20±2.92	188.40±3.16	0.80±0.005	8.13±1.40	26.01±1.10
Paracetamol	750	227.50±6.8*	176.00±4.7*	578.00±8.9*	1.10±0.08**	6.35±0.35	62.10±2.48*
Silymarin	50	151.40±6.63****	89.20±3.6****	228.40±5.42**	0.72±0.03*	8.12±0.56	35.30±1.78***
Ethanol extract	250	184.25±8.37*	96.75±5.1***	381.75±8.76****	0.65±0.06****	8.90±0.60	31.50±3.61***

N = 6 animals in each group. Values are expressed as Mean±SEM; *: p<0.001; **: p<0.01; ***: p<0.05 vs Control. ****: p<0.001 vs Paracetamol; Data were analyzed by ANOVA followed by tukey multiple comparison test

Table 2: Effect of ethanol extract of *C. rheedii* on Antioxidants level in paracetamol induced hepatotoxicity rats

Treatments	Dose (mg kg ⁻¹)	SOD	Catalase	LPO	GPX	GST
Control	-	14.61±1.68	51.29±1.67	7.85±0.92	38.75±1.96	0.38±0.05
Paracetamol	750	07.05±1.14*	26.17±2.10*	17.17±1.14*	16.82±1.30*	0.09±0.02*
Silymarin	50	15.36±1.58***	48.29±1.92***	11.01±0.87***	33.14±1.45***	0.25±0.03***
Ethanol extract	250	14.24±1.30***	46.85±1.97**	5.63±0.75***	37.79±3.88***	0.10±0.01*

N = 6 animals in a group; Values are expressed as Mean±SEM; *: p<0.001, **: p<0.01 vs control, ***: p<0.001 vs paracetamol. Data were analysed by using one way ANOVA followed by Tukey multiple comparison test. LPO = μ moles of MDA/min/mg protein; SOD = Units min⁻¹ mg⁻¹ protein; CAT= μ mole of H₂O₂ consumed min⁻¹ mg⁻¹ protein; Gpx = μ moles of GSH oxidized min⁻¹ mg⁻¹ protein; GST= μ moles of CDNB conjugation formed min⁻¹ mg⁻¹ protein

Table 3: Effect of ethanol extract of *Cansjeera rheedii* on liver weight variation

Treatments	Dose (mg kg ⁻¹)	Liver weight/100 g of body weight (g)
Control	-	4.26±0.53
Paracetamol	750	5.89±0.17
Silymarin	50	3.97±0.42*
Ethanol extract	250	4.83±0.46**

N = 6 animals in a group, Values are expressed as Mean±SEM; **: p<0.001; *: p<0.05 vs paracetamol; Data were analysed by using Student's t-test

were observed when compared with paracetamol treated rats. The size of the liver was pale reddish brown and enlarged in paracetamol intoxicated rats but it was normal in drug treated groups. A significant reduction (p<0.001) in liver weight supports this finding (Table 3).

Paracetamol is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of paracetamol are largely due to its active metabolite N-acetyl parabenzoquinamine (NAPQI). Induction of cytochrome P-450 depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity. Both transaminases rich in liver tissues were increased in patients with acute hepatic diseases, SGPT which is slightly elevated in cardiac necrosis, is a more specific indicator of liver disease (Dahlin *et al.*, 1984).

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology, which has been disturbed by a hepatotoxin. The extract decreased the paracetamol induced elevated levels of the enzymes in group 4, indicated the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts. The increases in LPO level in liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *C. rheedii* significantly reverse these changes. Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index of liver injury (Curtis and Mortiz, 1972; Sheeja and Kuttan, 2005). 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 hence diminishing the toxic effect caused by this radical.

In the present study it was observed that, the *C. rheedii* causes a significant increase in the hepatic SOD activity. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Chance and Greenstein, 1992). Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver. Their functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPx) (Prakash *et al.*, 2001). In the present study the decreased level of GST (from 0.1 to 0.09 μmole) to has been associated with an enhanced lipid peroxidation in paracetamol treated rats (from 17.17 to 5.63 μmole). Administration of *C. rheedii* significantly ($p < 0.001$) increased the level of GPx from (16.82 to 37.79 μmole) and GST from (0.09 to 0.10 μmole).

Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of *C. rheedii* on paracetamol-induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation process and increasing SOD, catalase, GPx and GST levels. The hepatoprotective effect may be mediated through reported antioxidant and free radicals scavenging action.

Decrease in serum bilirubin after treatment with the extract indicated the effectiveness of the extract in normalizing the functional status of the liver. So, the result of present investigation indicates that the ethanol extract of *C. rheedii* possess good hepatoprotective activity. The hepatoprotective mechanisms of this herbal drug as well as active principles are not known. Further investigations are required to characterise the active hepatoprotective principle and its mechanism of action.

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