

Full Length Research Paper

## Evaluation of acute and sub-acute toxicity of ethanol extracts of *Cansjera rheedii* J. Gmelin (Opiliaceae)

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**Acute and subacute toxicity of ethanol (95% v/v) extract of aerial parts of *Cansjera rheedii* J. Gmelin (Opiliaceae) was evaluated in Swiss mice and Wistar albino rats. The acute toxicity studies were conducted as per the OECD guidelines 420, where the limit test dose of 2000 mg/kg used. Observations were made and recorded systemically on 1, 2, 4 and 24 h after dose administration for skin changes, morbidity, aggressivity, sensitivity of the sound and pain, as well as respiratory movement. For the sub acute toxicity, four groups of 6 rats (3 male and 3 female) were received, distilled water (control) 125, 250 and 500 mg/kg of extracts every 24 h orally for 28 days. No significant variation ( $p < 0.05$ ) in the body and organ weights between the control and the treated group was observed after 28 days of treatment. Haematological analysis and clinical blood chemistry revealed no toxic effects of the extract. Pathologically, neither gross abnormalities nor histopathological changes were observed. No mortality was recorded in 28 days.**

**Key words:** *Cansjera rheedii*, acute, subacute, histopathology, biochemical parameters.

### INTRODUCTION

*Cansjera rheedii* J. Gmelin (Family-Opiliaceae) is a climbing shrub, sometimes armed, commonly known as "Kalimanakeera" in Tamil, is generally found in India through Malaya to Hong Kong and North Australia (Gamble, 1981; Matthew, 1991). The aerial part of *C. rheedii* was used for the treatment of post-natal pain (Ravikumar and Vijaya, 2003). The tribes of Nilgiris in Tamil Nadu, India use the plant extract for the treatment of intermittent fever (Hosagoudar and Henry, 1996). Despite the popular use of this plant by the rural communities to treat several diseases, our study was aimed to obtain data on the safety of the extract. The acute and subacute toxicity of the ethanol (95% v/v) extract of the aerial parts of *C. rheedii* (Opiliaceae) in mice and rats were assessed with the hope that the

result would provide information on the safety of this extract prior to the evaluation of its therapeutic efficacy in humans. In subacute toxicity study the effect on biochemical, hematological and histopathological parameters were investigated.

### MATERIALS AND METHODS

#### Chemicals

All the reagents used were of analytical grade obtained from S.D. Fine Chemicals Ltd., Mumbai, India.

#### Plant material

The aerial parts of the plant *C. rheedii* (Opiliaceae) were collected around Auroville; Puducherry, India in the month of June, 2006 and it was identified and authenticated by Auro-Herbarium Sakthi Botanical Survey Department, Auroville, Puducherry. A voucher specimen has been kept in our laboratory for future reference (VS-12). The aerial parts of *C. rheedii* were cut into small pieces; shade

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dried, powdered by a mechanical grinder and was passed through #40 mesh sieve and stored in an airtight container for further use.

#### Preparation of extract

The powdered plant material (1.0 kg) was extracted successively with Petroleum Ether, Chloroform, Ethanol (95% v/v) and water using soxhlet apparatus. The solvents were then removed under reduced pressure. The yield of the ethanol extract was 8.6% w/w. The extracts were dissolved in distilled water and used for the experiment (Mounnissamy et al., 2008).

#### Experimental animals

The Swiss albino mice (20 - 25 g) and male Wistar rats (150 - 200 g) were purchased from Perundurai Medical College, Perundurai, Tamil Nadu and housed in poly-propylene cages at room temperature ( $22 \pm 2^\circ\text{C}$ ) with proper ventilation. Prior to the experiments, animals were fed with standard diet for one week in order to adapt laboratory conditions. They were fasted but allowed free access to water 16 - 18 h prior to administration of the test drug.

#### Acute toxicity

The acute toxicity studies were conducted as per the OECD guidelines 420 (OECD, 2001) where the limit test dose of 2000 mg/kg used (Lipnick et al., 1995; Kulkarni, 1993). Observations were made and recorded systemically 1, 2, 4 and 24 h after dose administration for skin changes, morbidity, aggressivity, sensitivity of the sound and pain, as well as respiratory movement.

#### Subacute toxicity

The plant extract at the dose of 125, 250 and 500 mg/kg body weight were administered orally to 4 groups of six rats respectively to every 24 h for 28 days and control received vehicle at the same volume. The toxic manifestation such as body weight, mortality, food and water intake was monitored. After 28 days all surviving animals were fasted overnight and anaesthetized with ether. The heparinized blood samples were collected for determining hematological parameters and the serum from non-heparinized blood was carefully collected for determining clinical blood chemistry. Animals were sacrificed after blood collection and the internal organs were removed and weighed to determine the relative organ weights and observed for gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histological examination.

#### Biochemical estimations

Blood collected in non-heparinized tubes were than centrifuged at 3000 rpm for 10 min. The serum separated was analyzed for various parameters such as Aspartase amino Transferase (AST) (Reitman and Frankel, 1957), Alanine aminotransferase (ALT) (Reitman and Frankel, 1957), Alkaline Phosphatase (ALP) (Kind and King, 1954), Cholesterol (Hawerof, 1987), Urea (Webster, 1997) and Blood Urea Nitrogen (BUN) (Webster, 1997). Blood glucose (Sasaki et al., 1972) and Gamma Glutamyl transferase (GT) (Rosalki and Raw, 1972).

#### Haematological assay

Blood sample collected in the heparinized tubes were used to estimate white blood cells (WBC), red blood cells (RBC),

hemoglobin content (Hb) (Zijlstra, 1960) and clotting time (Dacie and Lewis, 1991).

#### Histopathological study

Histopathological investigation of the organs was done according to the method described by Lamb (Lamb, 1981). The organ pieces (3 - 5 micro meters thick) were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in benzene to remove absolute embedding was done by passing the cleared samples through three cups containing molten paraffin at  $50^\circ\text{C}$  and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

#### Statistical analysis

The values were expressed as mean  $\pm$  SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values  $< 0.05$  were considered as significant.

## RESULTS

The acute toxicity studies were conducted as per the OECD guidelines 420, where the limit test dose of 2000 mg/kg used. No test substance- related mortality was observed at 2000 mg/kg (Louis and Wallace, 2001). So, testing at higher dose mayn't be necessary and the compound was said to be practically non-toxic (Gosh, 1984). The ethanol (95% v/v) extract of *Cansjera rheedii* at dose of 125, 250 and 500 mg/kg orally for every 24 h for 28 days did not produced in tested animals. No sign of observable toxicity was detected during the experimental period according to the OECD Guidelines up to the dose level of 2000 mg/kg. All the tested Haematological parameters such as Hb, RBC, WBC and clotting time and biochemical parameters such as Liver, Heart, Lung, Spleen, Kidney and Body Weight were within the normal. No abnormalities in histopathological studies were also found.

## DISCUSSION

Generally, the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substances (Raza et al., 2002; Teo et al., 2002). In subacute toxicity study rats treated with 125, 250 and 500 mg/kg doses of ethanol extract of *C. rheedii* had a progressive weight in body and organ gained. The increase in weight was not significantly different from that of the control. The progressive increase in body weight and organ weight at dose of 125, 250 and 500 mg/kg of rats during 28 days of administration of ethanol extract of *C. rheedii* may indicate the improvement in the nutritional state of the animal. The growth response effect could be as a result of increased food and water intake. The calculated

**Table 1.** Effect of oral administration of ethanol extract of *C. rheedii* on body weight (g) and organs (g per 100 g body weight) of rats.

	Control	125 mg/kg	250 mg/kg	500 mg/kg
Liver	7.62 ± 0.01	7.63 ± 0.09	7.88 ± 0.80	7.82 ± 0.08
Heart	0.75 ± 0.04	0.77 ± 0.01	0.76 ± 0.03	0.78 ± 0.02
Lung	1.84 ± 0.01	1.85 ± 0.02	1.87 ± 0.02	1.88 ± 0.01
Spleen	0.83 ± 0.02	0.85 ± 0.01	0.86 ± 0.02	0.83 ± 0.02
Kidney	0.62 ± 0.01	0.64 ± 0.02	0.65 ± 0.01	0.66 ± 0.01
Body weight	199.34 ± 1.64	184.17 ± 1.26	186.15 ± 1.18	187.31 ± 0.79

Values are expressed as mean ± SEM of 6 rats in each group. P < 0.05 was considered significant. No significant difference was observed in any organ.

**Table 2.** Haematological parameters after 28 days oral treatment with the *C. rheedii* ethanol extract.

Parameters	Control	125 mg/kg	250 mg/kg	500 mg/kg
Hb (gm %)	12.43 ± 0.31	12.73 ± 0.18	12.62 ± 0.29	12.86 ± 0.34
RBC (10 <sup>6</sup> /Cu.Mm)	3.59 ± 0.12	3.23 ± 0.86	3.34 ± 0.13	3.85 ± 0.12
Total WBC (10 <sup>3</sup> /Cu.Mm)	8.048 ± 0.74	8.18 ± 0.63	8.24 ± 0.66	8.26 ± 0.52
Clotting time (S)	111.12 ± 0.86	111.82 ± 0.63	112.19 ± 1.06	112.56 ± 1.12

Values are expressed as mean ± SEM of 6 rats in each group. P < 0.05 were considered significant. No significant difference was observed in any parameter

**Table 3.** Effect of treatment with *C. rheedii* ethanol extract on biochemical parameters.

Parameters	Control	125 mg/kg	250 mg/kg	500 mg/kg
Cholesterol (mg %)	77.00 ± 2.11	79.84 ± 1.99	83.14 ± 1.89	81.12 ± 0.91
ALT (U/L)	185.00 ± 2.71	183.84 ± 1.61	180.16 ± 0.84	182.10 ± 0.19
AST (U/L)	202.00 ± 2.28	199.00 ± 2.59	201.16 ± 1.64	200.19 ± 1.21
GT (U/L)	262.52 ± 2.24	261.68 ± 4.28	262.36 ± 2.12	263.74 ± 2.70
ALP (U/L)	379.52 ± 0.90	380.84 ± 0.84	379.52 ± 1.66	381.78 ± 0.92
Glucose (mg %)	73.86 ± 1.74	72.65 ± 0.74	70.34 ± 0.78	69.48 ± 1.62
Urea (mg %)	49.12 ± 0.82	47.36 ± 1.12	52.56 ± 1.56	48.56 ± 1.70
BUN (mg %)	21.76 ± 0.78	22.86 ± 0.48	23.18 ± 0.62	22.36 ± 0.86

Values are expressed as mean ± SEM of 6 rats in each group. P < 0.05 was considered significant. No significant difference was observed in any parameter.

relative weight of the control and treated animal groups varied from one organ to other, no significant differences were noted in the relative weight of other organ (liver, heart, lung, spleen and kidney). However there was no correlation between relative weight of the organs and the various doses of the extract of *C. rheedii* administered (Table 1).

The haematological status (Table 2) after 28 days of oral administration of ethanol extract of *C. rheedii* was also assessed. In general the results showed that the values for the RBC and WBC were slightly increased in groups compared to the control. However no significant variation for RBC, WBC, Hb and clotting time were observed. The small transient of values observed in blood hematology did not show any dose responsiveness. Nevertheless, all values lay within the normal limits

(McIntyre and Rosaki, 1987). *C. rheedii* not showed any significant reduction of normal blood glucose.

The ethanol extract of *C. rheedii* did not induce any damage to the kidney and liver as examined by clinical blood chemistry (Table 3). ALT and AST are two liver enzymes that are associated to the hepatocellular damage. Although both AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, only ALT is remarkably specific for liver function since AST is mostly present in the myocardium, skeletal muscle, brain and kidneys (Sacher and Mepherson, 1991). A slight but not significant change was noted on ALP activities in the rat. No significant changes in ALT, AST and ALP activities in the serum of rats. In other parameters like total Cholesterol, Urea and Blood Urea Nitrogen (BUN) there was no significant

changes observed.

In general with liver disease serum levels of AST and ALT rise and fall at the same time (Haweroft, 1987). A mild elevation of AST level has been shown to be associated with liver injury or myocardial infarction. The higher the activity of AST has been observed in larger infarction size (Feldman and Zinkl, 2000). A typical myocardial infarction gives an AST/ALT ratio greater than 1 while an AST/ALT ratio less than 1 is a result of release of ALT from the affected liver (Sacher and Mepherson, 1991), AST /ALT of more than 2 indicates alcoholic hepatitis or cirrhosis (Sacher and Mepherson, 1991). These results indicated that the ethanol extract of *C. rheedii* when taken for long periods of time might not cause liver disease. The GT level increase gradually at doses of 125, 250 and 500 mg/kg of body weight compared to the control after 28 days of administration. This increased level of GT is not statistically significant. These values are within the normal range (Barry, 1995). Furthermore, gross examination of internal organs of all the rats revealed no detectable abnormalities.

In conclusion, this study presents strong evidence of the nontoxic effect of the ethanol extract of *C. rheedii*. These results showed that the use of extract of *C. rheedii* is safe and explained the extensive utilization of the plant in traditional medicine.

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## REFERENCES

- Barry SL (1995). Animal Clinical Pathology. In: Micheal JD, Mannfred AH, eds. CRC Handbook of Toxicology, CRC Press, Inc. U.S.A. pp. 517-537.
- Dacie JV, Lewis S (1991). Practical Haematology, 7th ed., Churchill Livingstone, New York pp.321-327.
- Feldman BV, Zinkl JG (2000). Schalm's Veterinary Hematology, 5<sup>th</sup> ed. Philadelphia, Lea Febiger pp. 1210-1218.
- Gamble JS (1981). Flora of Presidency of Madras, Botanical Survey of India, Calcutta pp.137-138.
- Gosh MN (1984). Toxicity studies in Fundamentals of Experimental Pharmacology, 2nd Edition, Scientific Book Agencies, Calcutta pp. 154-158.
- Haweroft DM (1987). Diagnostic enzymology analytical chemistry by open learning Published by permission of the controller of her majesty's stationary office pp. 186-221.
- Hosagoudar VB, Henry AN (1996). Ethanobotany of tribes irular, kurumban and paniyan of Nilgiris in Tamil Nadu, Southern India. J. Econ. Taxon. Bot. 12(12): 272-283.
- Kind PRN, King EJ (1954). Estimation of plasma phosphatase by determination of hydrolyzed phenol with antipyrine, J. Clin. Path. 7: 322-330.
- Kulkarni S (1993). Experimental pharmacology, India pp. 168-172.
- Lamb GM (1981). Manual of veterinary techniques in Kenya. Kenya: Ciba-Geigy pp. 100-104.
- Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, Chu I, Goddard M, Segal L, Springer JA, Myers RC (1995). Comparison of the Up-and-Down, Conventional LD50 and Fixed Dose Acute Toxicity procedures. Fd. Chem. Toxicol. 33: 223-231.
- Louis and Wallace CD, Wallace HA (2001). In principles and method of toxicology, 4th edition, Taylor and Francis, Philadelphia pp. 871-873.
- Matthew KM (1991). An Excursion flora of Central Tamil Nadu, India, Oxford and IBH Publications, New Delhi pp. 647-648.
- McIntyre N, Rosaki S (1987). Investigations biochimiques des affections Hépatiques. Pharmazie 12(3): 294-309.
- Mounnissamy VM, Kavimani S, Balu V, Darlin QS (2008). Preliminary Phytochemical screening of *Cansjera rheedii* J. Gmelin (Opiliaceae), Int. J. Pharmacol. Biol. Sci. 2(3): 157-160.
- OECD (2001). The OECD guideline for testing of chemical. The organization of Economic co-operation development, Paris pp 1-14.
- Ravikumar R, Vijaya SR (2003). Ethanobotany of Malayali tribes in Melpattu village, Javvadhu hills of Eastern Ghats, Tiruvannamalai district, Tamil Nadu. J. Econ. Taxon. Bot. 27(3): 715-726.
- Raza M, Al-Shabanath OA, El-Hadiyah TM, Al-Majed AA (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice, Scientia Pharmaceutica 70: 135-145.
- Reitman S, Frankel S (1957). A colorimetric method for determination of serum glucose oxaloacetate and glutamic pyruvate transaminase, AMJ Clin. Path. 28: 53-56.
- Rosalki SB, Raw D (1972). Serum gamma-glutamyl transpeptidase activity in alcoholism. Clin Chem Acta. 39: 41-47.
- Sacher RA, Mepherson RA (1991). Widmann's clinical interpretation of laboratory test, U.S.A., Pennsylvania. 3rd edition pp. 416-443.
- Sasaki T, Matsy S, Sonae A (1972). Effect of acetic acid concentration on the color reaction in the O-toluidine boric acid method for blood glucose estimation. Rinshobokagaku 1: 346-353.
- Teo S, Strlig D, Thomas S, Hoberman A, Kiorpes A, Khetani V (2002). A 90-days oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-Dawley rats, Toxicol. 79: 183-196.
- Webster D (1997). Bio-chemical parameters and its assay, Clin. Chem. 23: 663-665.
- Zijlstra NC (1960). Hematological assay methods, Clin. Chem. Acta. 5: 719-721.