



Toxicity assessment of the medicinal plant *Cyathula prostrata*

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ABSTRACT

Objectives: To evaluate the potential toxicity of methanolic extracts of the medicinal plant *Cyathula prostrata*.

Methodology and results: Methanolic extract of *C. prostrata* (MECP) were evaluated at rates of 50, 100, 150, 200 and 250 mg/kg/bw to assess its impact on biochemical indices of Swiss albino mice. Hematological profile, biochemical assays, activities of liver marker enzymes, antioxidant potential and histopathological examinations were compared between control and experimental animals. The changes in biochemical parameters were statistically insignificant. The treated mice showed conspicuous toxic symptoms only after 150 mg/kg. These observations were supported by histopathological examination of liver and spleen sections.

Conclusion and application of findings: These results demonstrate that the methanolic extract of *Cyathula prostrata* is not toxic and therefore it may be used safely in clinical trials.

Key words: *Cyathula prostrata*, marker enzymes, antioxidant, hematology, histopathology.

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INTRODUCTION

About 80% of the world population, mainly in the developing countries, depends on herbal medicine for primary health care. These medicines are more culturally acceptable, have better compatibility with the human body and lesser side effects (Kamboj, 2000). India has a rich diversity of medicinal plants and a number of plant extracts are used against diseases in various medicinal applications, e.g. Ayurveda, Unani, and Siddha. However, in spite of widespread use, only a few of these plants have been scientifically explored (Malaya, 2004).

Upon administration of a chemical substance to a biological system, different types of interactions can occur resulting into a series of dose-related responses. In most cases these

responses are desired and useful, but there are a number of other effects which are not advantageous, and which could be harmful to the patients (Shetty, 2007). Toxicology is the aspect of pharmacology that deals with the adverse effect of bioactive substances on living creatures along with their diagnosis and clinical use (Bytul, 2007). In order to develop and establish the safety and efficacy level of a new drug, necessary toxicological studies are conducted on animals, e.g. mice, rat, guinea pigs, dog, monkey, usually under varying conditions and drug levels (Abdullahil, 2007).

Cyathula prostrata is an annual herb usually found in moist ecologies. The ascending

branch ends in inflorescence, its stems and branches are greenish red, leaves are simple, opposite, exstipulate and short petioled (Ayurvedic Medicinal Plants). The Malays use the plant as a source of medicine for external or internal ailments. As a decoction it is administered for coughs while a decoction of the roots is used for dysentery. The Kuro people use the ashes of the

burnt plant mixed with water to smear on the body for crawl-crawl, scabies, and other skin diseases. In Cameroon the plant is used to treat articular rheumatism and dysentery (www.bpi.da.gov.ph). To determine the likely effects of acute overdose in man, we carried out an acute toxicity study of methanolic extract of *Cyathula prostrata* on Swiss albino mice.

MATERIALS AND METHODS

Chemicals: All the chemicals, solvents and reagents were of analytical reagent grade.

Plant material: The plant *Cyathula prostrata* (Family: Amaranthaceae) was collected in the month of December 2007 from Thrissur, Kerala. Taxonomic authentication was done by Dr.V.S.Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India. The dried powder material of the leaves of *Cyathula prostrata* was extracted with methanol in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried under vacuum.

Toxicity assessment: Ten-week-old female Swiss Albino strain mice weighing 20 ± 2 g were used for the study. The mice were procured from the Small Animal's breeding centre of Kerala Agricultural University, Mannuthy, Thrissur. The mice were grouped and housed in polyacrylic cages with not more than twelve animals per cage, and maintained at temperature of 25 ± 2 °C; relative humidity of $55 \pm 5\%$, 14 /10 h, dark/light cycle, with free access to feed and water (*ad libitum*). The mice were acclimatized to laboratory conditions for 10 days before commencement of the experiment. The animal care and handling was done according to the regulations of Council Directive CPCSEA No: 659/02/a about Good Laboratory Practice (GLP) on animal experimentation. The animals were allowed to fast by withdrawing food and water for 18 h and divided into six groups of eight individuals (one control and five groups for the test substance).

Briefly, the powder (MECP) was dissolved in aqueous solution at different concentration. The test substance was administered orally. Weight control and observations were continued for 14 days. After treatment, the animals were deprived of food, but not water, overnight and then euthanized under chloroform anesthesia. Cardiac blood samples were collected and centrifuged at 3000 rpm for 20 min. Several hematology

and blood biochemistry variables were determined as described below.

Experimental design: After the adaptation period, the animals were divided into six groups with eight animals in each group. Mice in group I served as normal control while those in group II, III, IV, V and VI were fed orally with methanolic extract of *Cyathula prostrate* at 50, 100, 150, 200 and 250 mg/kg/BW, respectively, for 14 days.

Biochemical analysis: After the experimental period, animals in different groups were sacrificed by cervical dislocation. Blood was collected in two different tubes from an incision made in the jugular veins. One tube had anticoagulant (for plasma separation) and the other tube without anticoagulant (to separate serum for various biochemical estimations). The liver was dissected out, washed in ice-cold saline, blotted dry, and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer, pH 7.4 and used for the biochemical analyses. Several hematology and blood biochemistry variables were determined.

Hematology: Hemoglobin content, red blood cell (RBC) and white blood cell (WBC) counts were measured from freely flowing blood (Ghai, 1990).

Biochemical parameters: Protein was determined by the method of Lowry et al. 1957 using Bovine Serum Albumin (BSA) as standard, at 660 nm. Urea content in serum was estimated by the method of Seaton and Ali, 1984.

Hepatospecific markers: Activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman and Frankel, 1957. 0.2 ml of sample with 1 ml of substrate (aspartate and α -ketoglutarate for AST; alanine and α -keto glutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 minutes for ALT. 1 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and

absorbance was read at 540 nm. Activities expressed as IU/L.

Estimation of lipid peroxidation: Lipid peroxidation in liver was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Nichans and Samuelson, 1968. In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA - TCA - HCl reagent (TBA 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank.

Assay of enzymic antioxidants: Superoxide dismutase (SOD) activity was determined at 560nm by the modified method of Das *et al*, (1984). Catalase (CAT) was assayed colorimetrically as described by Sinha, 1972 using dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The intensity was measured at 620

nm and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity.

Macroscopic and microscopic examination of tissues: A part of the dissected liver and a part of the spleen from all the groups were cleared off of the surrounding tissues and kept in 10% buffered neutral formalin, dehydrated in alcohol, and then embedded in paraffin. The paraffin blocks were sectioned at 5- μ m intervals and stained with haematoxylin-eosin for histological examinations. The slides were coded and examined in a single-blind fashion by a veterinary pathologist.

Statistical analysis: The results are reported as mean \pm SD. For hematology, blood biochemistry and biochemical data, the significance of differences was assessed using computer Agres software.

RESULTS AND DISCUSSION

An acute toxicity test was performed in mice at different concentration of MECP in order to establish the approximate oral LD50. No deaths occurred during the two weeks experimental period, in both control and experimental groups.

Upon examination of the hematological profile (Table 1), Hb, RBC and WBC were found to be near normal in group II and III (treated with 50 and 100 mg/kg/BW) compared to the control group. However, there were mild changes in the rest of the groups. The results indicate the non-toxic nature of the extract and between the two doses, 100 mg/kg BW was found to be more effective. Significant increases in AST and ALT

were observed from Group IV to Group VI (150-250 mg/kg/BW) as compared to normal control animals (Group I) (Table 2). Serum AST and ALT, are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage (Sallie, 1991). Methanolic extracts of *C. prostrata* at doses of 50mg/Kg/BW and 100 mg/Kg/BW (Group I-III) did not show any effect on levels of AST and ALT concentrations as compared to normal control animals (Group I). Leakage of enzymes into the surrounding confirms the toxicity of the dosage above 150 mg/kg/BW.

Table 1: Effect of the methanol extract of *Cyathula prostrata* leaves on haematological profile of experimental Swiss Albino mice.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Hemoglobin (g %)	12.6 \pm 0.09	11.3 \pm 0.08a	12.4 \pm 0.09b	13.1 \pm 0.11c	13.45 \pm 0.11d	14.11 \pm 0.10e
RBC (X10 ⁹ / μ l)	5.9 \pm 0.04	5.1 \pm 0.03a	5.8 \pm 0.04b	6.0 \pm 0.05c	6.2 \pm 0.04d	6.6 \pm 0.05e
WBC (X10 ⁶ / μ l)	5.1 \pm 0.03	4.8 \pm 0.02a	5.3 \pm 0.04b	5.8 \pm 0.05c	6.1 \pm 0.04d	6.5 \pm 0.05e

Values are mean \pm SD (n=6). Mice in Group I, II, III, IV, V and VI received 0 (control), 50, 100, 150, 200 and 250 mg/kg/BW of the extract, respectively. RBC = red blood cells; WBC = white blood cells. Means followed by different letters across the rows are significantly different.

There was no significant difference in the level of protein and urea between groups I –III, but a significant variation was observed from Group IV-VI (150-250 mg/kg BW) (Table 3), with protein and urea levels increasing as the dose of extract applied increased.

The site-specific oxidative damage of some of the susceptible amino acids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis (Uday *et al.*, 1999). The elevation in the levels of urea is considered as a significant marker of

renal dysfunction. These results indicate that dosage above 150 mg/kg/BW. methanolic extracts of *C. prostrata* may be toxic at

Table 2: Effect of the methanol extract of *Cyathula prostrata* leaves on liver marker enzymes in serum and liver of experimental Swiss Albino mice.

Parameters		Group I	Group II	Group III	Group IV	Group V	Group VI
SGPT (U/I)	Serum	11.26±0.12	9.32±0.07a	11.44±0.08b	11.94±0.08c	12.32±0.10d	13.32±0.10e
	Liver	68.1± 0.62	58.2±0.51a	67.3±0.56b	69.6±0.59c	70.9±0.66d	75.2±0.68e
SGOT (U/I)	Serum	28.23±0.25	23.26±0.21a	27.25±0.20b	29.31±0.19c	32.01±0.21d	35.26±0.21e
	Liver	40.3± 0.33	32.3±0.28a	39.6±0.31b	42.3±0.31c	44.6±0.34d	46.1±0.38e

Values are mean ± SD (n=8). Mice in Group I, II, III, IV, V and VI received 0 (control), 50, 100, 150, 200 and 250 mg/kg/BW of the extract, respectively. SGPT = serum glutamate pyruvate transaminase; SGOT = serum glutamate oxaloacetate transaminase.

Table 3: Effect of the methanol extract of *Cyathula prostrata* leaves on protein and urea levels (mg/dl) in experimental Swiss albino mice.

Parameters		Group I	Group II	Group III	Group IV	Group V	Group VI
Protein	Serum	6.24±0.05	5.23±0.05a	6.34±0.04b	6.68±0.07c	6.75±0.06d	8.32±0.07e
	Liver	9.16±0.07	6.34±0.05a	8.82±0.06b	9.74±0.07c	10.02±0.07d	14.06±0.06e
Urea	Serum	25.3±0.21	18.6±0.16a	24.4±0.18b	26.6±0.18c	28.3±0.21d	31.5±0.26e

Values are mean ± SD (n=6). Mice in Group I, II, III, IV, V and VI received 0 (control), 50, 100, 150, 200 and 250 mg/kg/BW of the extract, respectively.

Table 4: Effect of the methanol extract of *Cyathula prostrata* leaves (MECP) on superoxide dismutase (SOD) and catalase (CAT) (U/mg protein) in liver of experimental Swiss albino mice.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
SOD	5.1±0.09a	4.3±0.06b	4.9±0.05c	5.4±0.06d	5.9±0.08e	6.2±0.09f
CAT	28.4±0.24a	23.5±0.26b	27.1±0.28c	29.2±0.24d	30.5±0.31e	35.2±0.29f

Values are expressed as mean ± SD (n=6). Mice in Group I, II, III, IV, V and VI received 0 (control), 50, 100, 150, 200 and 250 mg/kg/BW of the extract, respectively.

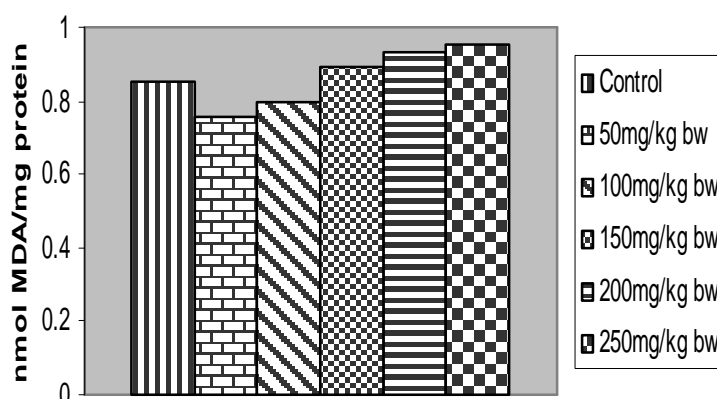


Figure 1: Effect of methanolic extract of *Cyathula prostrata* leaves (MECP) on lipid peroxidation in liver of experimental Swiss albino mice.

Marked increase in levels of LPO were seen from group IV to group VI (150-250 mg/kg/BW) when compared with control animals (group I) (Fig. 1). No significant differences were noted between groups II & III and the control (group I). Membrane lipids succumb easily to deleterious actions of reactive oxygen species (Reiter, 1995), and the measurement of lipid peroxidation is a convenient method to monitor oxidative damage (Viani, 1991). Under normal physiological conditions, low concentrations of lipid peroxides are found in tissues. Free radicals react with lipids causing peroxidation, which further enhances lipid peroxidation. An increase in lipid peroxidation indicates serious damage to cell membranes affecting their fluidity as well as inhibiting several enzymes and cell function (Girotti, 1985).

Marked increases in levels of LPO were seen from Group IV to Group VI (150-250 mg/kg/BW) (Table 4). There were no significant differences between groups II and III and the normal control (group I). The enzymic antioxidants are the natural defense systems against lipid peroxidation. SOD and CAT enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and therefore protect the cellular constituents from oxidative damage (Scott, 1991). These results further show that dosage below 150 mg/kg/BW is non toxic.

Histological examination of the liver and spleen tissues under a light microscope was done to

observe the effects of MECP on the structural integrity of the cells. The liver and spleen of control animals (Fig. 2 and 3) showed normal histological architecture. In all the groups the hepatocytes were found to be normal, with normal triads, central vein and hepatic sinusoids. Fibrosis and regenerative activity were not observed. Inflammations were not observed in Group I-IV. However, in group V (200 mg/kg/BW) mild severity were seen in lymphocytes around the central veins.

The lymphoid areas of the spleen were found to be normal, mild congestions were seen in all the groups and moderate congestions were seen in Group III (150 mg/kg/BW). Blood vessels were observed to be normal. From the histopathological examination it could be clearly seen that the dosage above 150 mg/kg/BW may be toxic.

Based on this study, we conclude that the medicinal herb *Cyathula prostrata* can be administered at a dose range of 100 mg/kg/BW without any side effects. Since, the toxicity studies in experimental animals cannot always be totally extrapolated to humans, and a reasonable estimate of the self-administered dose is difficult to make such as that applied during traditional use of this plant, additional clinical toxicological evaluations need to be performed to define a safe dose and protect the population from possible toxic effects of the plant.

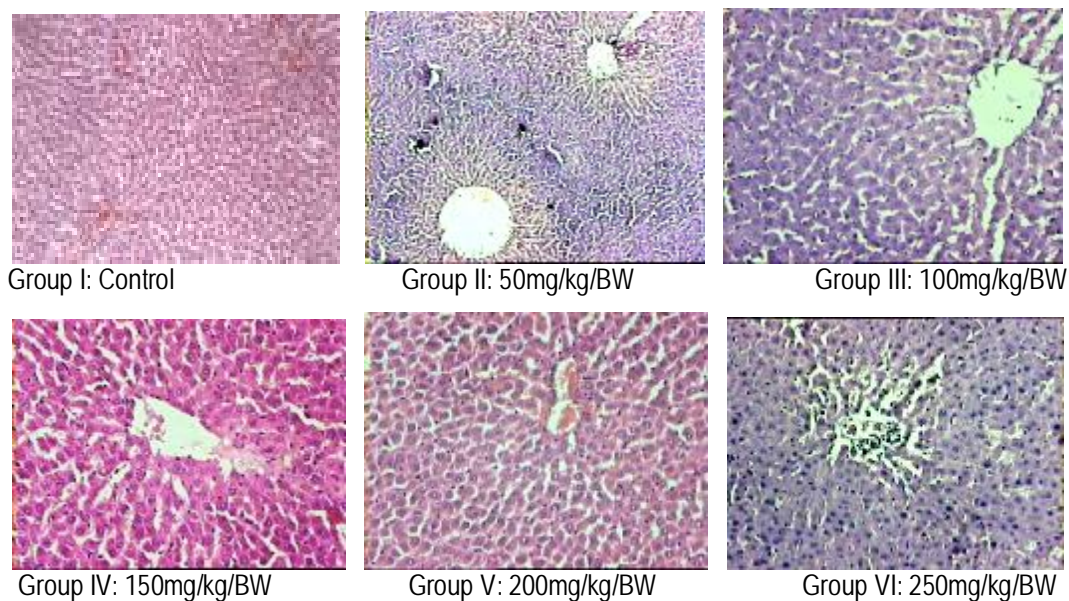


Figure 2: Histoarchitecture of liver of experimental Swiss albino mice exposed to methanolic extracts of the medicinal herb *Cyathula prostrata*. Doses were applied as indicated below each image.

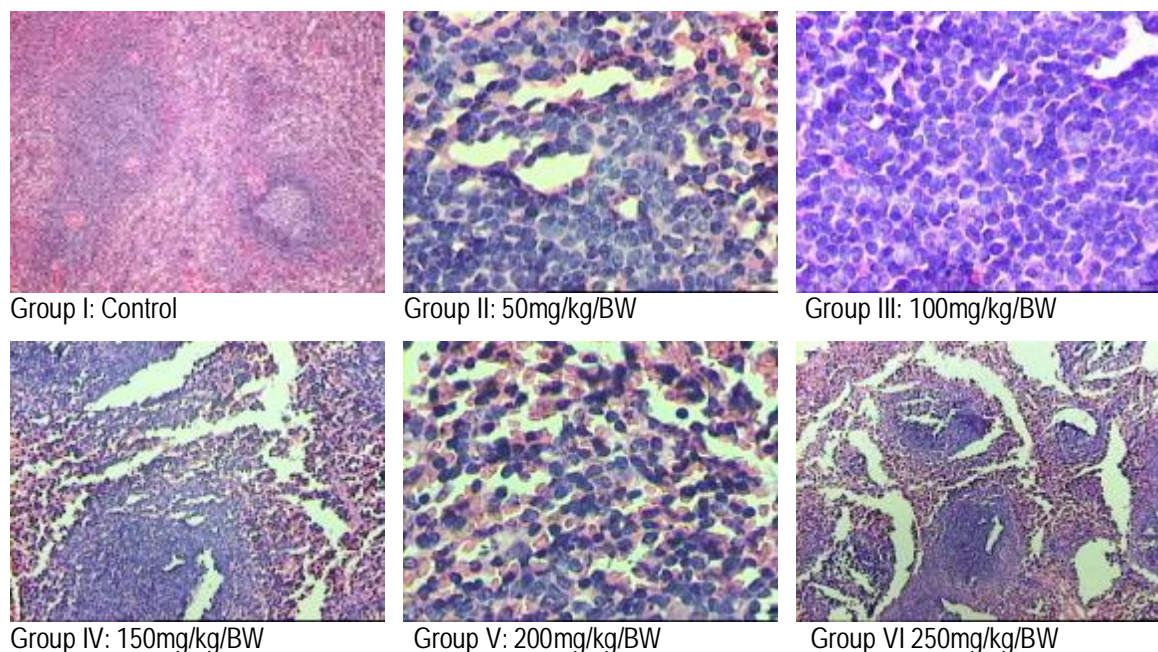


Figure 3: Histoarchitecture of spleen of experimental Swiss albino mice exposed to methanolic extracts of the medicinal herb *Cyathula prostrata*.

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