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TOXICOLOGICAL STUDIES ON *MALACHRA CAPITATA (L.)* EXTRACT IN EXPERIMENTAL ANIMALS

V. Rajalakshimi*, P.V. Sireesha Rani, CH. Sireesha, E. Rajini, N. Naresh

*Department of Pharmacology, Jagan's college of pharmacy, Nellore, Andhra Pradesh, India.

ABSTRACT

The roots of the *Malachra capitata* (L.) (Family: Malvaceae) is traditional remedies for the many disease condition such as pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds. The present investigation was carried out to evaluate the safety of aqueous extract of *Malachra capitata (L.)* (AMC) roots by determining its potential toxicity after acute and chronic administration in rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. General behavior adverse effects and mortality were determined for up to 14 days. In the chronic toxicity study, the AMC was administered orally at doses of 100, 200 and 400 mg/kg once in a week for 6 weeks to rats. Biochemical and hematological parameters were determined after 6 weeks. In the acute study in rats, there was no toxicity/ death was observed at the dose of 2000mg/kg b.w. The onset of toxicity and signs of toxicity also not there. In the chronic toxicity study, no significant treatment-related changes in the levels of haematological, hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study. It suggests that the aqueous extract of *Malachra capitata (L.)* does not appear to have significant toxicity. In view of the dose of *Malachra capitata (L.)* consumed in traditional medicine, there is a wide margin of safety for the therapeutic use of the aqueous extract of *Malachra capitata (L.)* roots.

Key words: *Malachra capitata (L.)*, Traditional Medicine, Acute and Chronic Toxicity, Heamatological Parameters, Biochemical Parameters.

INTRODUCTION

Malachra capitata (L.) is a herb belongs to family: Malvaceae. Description: Mostly erect, coarse, annual or perennial herb 1-2 m tall, throughout densely whitish- or yellowish-tomentose with stellate hairs and usually also moderately to copiously hispid with simple or stellate hairs to 2 mm long; roots long-petioled; stipules lanceolate, 5-15 mm long; blades orbicular to ovate, 2-10 cm long, palmately sinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteate heads, bracts 1-2 cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, suborbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucral bracts wanting; calyx tubular-campanulate, 4-8 mm long, 5-lobed to below middle, lobes ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15 mm long, slightly exceeding staminal column; mericarps 3-3.5 mm long, muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5 mm long, black, whitish-pubescent about hilum. The roots of the *Malachra capitata* (L.) is traditional remedies for the many disease condition such as pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds [1-5]. And also roots are used for the treatment of ulcers and sores In spite of the use of *Malachra capitata (L.)* in traditional medicine and its potential for toxicity, systematic evaluation of its toxic effects is lacking. From the source of literature documentation and relevant traditional approaches on plant drugs, the present

Corresponding Author

V. Rajalakshimi
Email id: rajii.vasudevan86@gmail.com

investigation was carried out to investigate the acute and chronic toxic effects of aqueous extract of *Malachra capitata (L.)* in rodents.

MATERIALS AND METHODS

Collection and authentication of plant material

The Plant material of *Malachra capitata (L.)* roots was collected from Tirunelveli District, in the Month of August 2011. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The roots of the *Malachra capitata* (L.) are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at 35^{o}C for 4 days. The dried roots of *Malachra capitata* was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of aqueous extract of *Malachra capitata* was found to be 10.5 % w/w. The aqueous extract was administered to the animals by suspending each time in 1% CMC.

Experimental animals

Adult Wistar rats of either sex weighing 180-250 gms were used in pharmacological and toxicological studies. The inbred animals were taken from the animal house and maintained in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at 22±1˚C with humidity at 55±5%. They were fed balanced rodent pellet diet from Poultry Research station, Nandanam, Chennai-35 and tap water ad *libitum* throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).

Acute toxicity study of *Malachra capitata (L.)* extract in rats

The procedure was followed by using OECD 423 (Acute Toxic Class Method) [6]. The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of AMC was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*. Food was withheld for a further 3-4 hours after administration of AMC and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, 1/20th (100mg/kg), 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study.

Study of Chronic Toxicity of *Malachra capitata (L.)* extract in rats

Design of Treatment

Animals were divided into 5 groups of six rats each.

Group I - Normal saline (0.9%, NaCl, 5ml/kg, p.o) once in a week for 6 weeks.

Group II- Vehicle 1% SCMC (5ml/kg, p.o) once in a week for 6 weeks.

Group III-V- Aqueous extract of *Malachra capitata (L.)* roots at the dose of 100, 200 and 400 mg/kg, p.o respectively.

Animals from each group were sacrificed at the 6th week, after the last dose. Different haematological and serum biochemical tests were then performed.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinised bottles for haematological studies and clean non-heparinised bottles and allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Methods for estimation of haematological parameters

Estimation of Hemoglobin [8], RBC count [8], WBC count [8], different leucocytic count [7], Elongation time [7] and ESR [9]were determined according to the standard procedures.

Determination of serum biochemical parameters

Blood Glucose, [10] Serum Bilirubin [11], Serum Gluconate – Oxaloacetate Transaminase (SGOT) [11], Serum Glutamate – Pyruvate Transaminase (SGPT) [11], Serum Alkaline Phosphatase (ALP) [11], Blood Cholesterol [10], Blood Urea [10] , Serum Uric Acid [10], Blood Creatinine [10] and Serum protein[10] were estimated by standard procedures.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the

groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

The body weight of the rats before and after administrations were noted that there is slightly increased the body weight. But there are no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses (Table 1).

Chronic toxicity study

The chronic oral administration of aqueous extract of *Malachra capitata (L.)* roots caused no noticeable change in the general behaviour of the rats and, compared to the control group (saline and vehicle), no significant changes in body weight, food intake and utilization of food in the AMC treated rats. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of study.

Effect of aqueous extract of *Malachra capitata (L.)* roots on the haematological and biochemical parameters of rats

In the chronic toxicity study, the haematological parameters, hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells in the treated rats did not differ significantly ($P > 0.01$) from that of the control group (Table 2) and all the values remained within normal limits throughout the experimental period. As shown in Table 3 & 4, no significant treatment-related changes in the levels of hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study.

Table 1. Acute toxicity study of aqueous extract of *Malachra capitata (L.)* (AMC) in rats

S.No	Groups	Dose/kg b.w, p.o	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of study
			Before Test	After Test			
1	AMC	2000 mg	165 g	170 g	No signs of Toxicity	Nil	14days
2	AMC	2000 mg	180 g	185 g	No signs of Toxicity	Nil	14days
3	AMC	2000 mg	160g	165 g	No signs of Toxicity	Nil	14days
4	AMC	2000 mg	180 g	185 g	No signs of Toxicity	Nil	14days
5	AMC	2000 mg	210 g	215 g	No signs of Toxicity	Nil	14days
6	AMC	2000 mg	205 g	210 g	No signs of Toxicity	Nil	14days

Table 2. .Effect of aqueous extract of *Malachra capitata (L.)* (AMC) on heamotological profiles in rats

Design of treatment	Group I Saline(0.9 % W/V)	Group II Vehicle (1%SCMC)	Group III AMC	Group IV AMC	Group V AMC
Dose mg/kg	5 ml/kg,p.o	5 ml/kg,p.o	100mg/kg,p.o	200mg/kg,p.o	400mg/kg,p.o
Neutrophil (%)	24.4± 0.12	24.2 ± 0.14	37.7 ± 0.41[a]	36.5 ± 0.52[a]	38.1 ± 0.17[a]
Eosinophil (%)	1.2 ± 0.01	0.9 ± 0.21	1.7 ± 0.02[a]	0.9 ± 0.04[a]	0.8 ± 0.02[a]
Lymphocyte (%)	74.1 ± 0.17	74.5 ± 0.13	67.3 ± 1.12[a]	58.4 ± 1.12[a]	52.6 ± 1.17[a]
Monocyte (%)	3.4 ± 0.61	2.6 ± 0.41	2.7 ± 0.22[a]	2.5 ± 0.24[a]	1.8 ± 0.17[a]
Clotting time (seconds)	76.2 ± 1.17	83.2 ± 1.14	95.5 ± 1.82[a]	95.1 ± 1.29[a]	104.3 ± 1.11[a]
Haemoglobin(gm%)	14.2 ± 0.17	13.2 ± 0.51	15.7 ± 0.22[a]	14.6 ± 0.21[a]	13.3 ± 0.11[a]
RBC cells(cu.mm)×10^9(%)	8.2 ± 0.14	7.4 ± 0.15	7.5 ± 0.4[a]	6.7 ± 0.12[a]	7.8 ± 0.12[a]
WBC cells (cu.mm)×10^9 (%)	7.7 ± 0.16	7.7 ± 0.11	7.8± 1.12[a]	8.6± 1.22[a]	11.2± 1.27[a]

a- Group I & II Vs group III, IV &V. P < 0.01 when compared to control group
Each value represents the mean ± S.E.M six rats in each group

Table 3. Effect of aqueous extract of Malachra capitata (L.) (AMC) on hepatic parameters in rats

Groups	Design of treatment	Dose Mg/kg	Glucose Mg/dl	Bilirubin Mg/dl	SGOT 1 Unit/L	SGPT 1 Unit/L	ALP 1 Unit/L	Cholestrol mg/100ml
I	Saline (0.9 % W/V)	5 ml /kg,p.o	84 ± 2.5	0.5 ± 0.01	50.2 ±0.4	31.5 ±0.2	8.2 ±0.17	61.2 ±1.1
II	Vehicle (1% SCMC)	5ml/kg,p.o	91 ± 2.3	0.6 ±0.01	54.4 ±0.2	34.2 ±1.5	8.5 ±0.13	64.1 ±1.5
III	AMC	100mg/kg, p.o	94 ± 1.5[a]	0.6 ± 0.01[a]	51.2 ±0.2[a]	35.6 ±0.4[a]	10.2 ±0.31[a]	52.4 ±1.2[a]
IV	AMC	200mg/kg, p.o	105 ±1.4[a]	0.6 ± 0.01[a]	53.3 ±0.2[a]	36.5 ±0.4[a]	11.4 ±0.34[a]	55.2 ±1.4[a]
V	AMC	400mg/kg, p.o	102± 1.2[a]	0.6 ± 0.01[a]	56.4 ± 0.4[a]	37.5±0.1[a]	12.4±0.21[a]	71.2±1.2[a]

a- Group I, II Vs group III, IV & V.
b- P < 0.01 when compared to control groupEach value represents the mean ± S.E.M six rats in each group

Table 4. Effect of aqueous extract of *Malachra capitata (L.)* (AMC) on renal parameters in rats

Groups	Design of treatment	Dose mg/kg	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Protein gm/dl
I	Saline(0.9 % W/V)	5 ml/kg,p.o	20 ± 0.25	4.4 ± 0.2	0.9 ± 0.02	6.8 ±0.12
II	Vehicle (1%SCMC)	5 ml/kg,p.o	21 ± 0.12	4.5 ± 0.4	1.3 ± 0.04	6.9 ±0.22
III	AMC	100mg/kg,p.o	24 ± 0.13[a]	3.7 ± 0.5[a]	1.2 ±0.02[a]	6.7 ± 0.32[a]
IV	AMC	200mg/kg,p.o	27 ± 0.22[a]	3.8 ±0.4[a]	1.4 ±0.02[a]	7.1 ± 0.21[a]
V	AMC	400mg/kg,p.o	29± 0.12[a]	3.7±0.5[a]	1.6±0.02[a]	7.6± 0.22[a]

a- Group I & II Vs group III, IV &V. P < 0.01 when compared to control group
Each value represents the mean ± S.E.M six rats in each group

DISCUSSION AND CONCLUSION

In this study, the aqueous extract of *Malachra capitata (L.)* was found to be non-toxic in rats when administered orally in doses up to 2000 mg mg/kg, p.o. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. Based on this animal study, may be described as being practically non-toxic.

In the six weeks chronic toxicity study, the AMC at the doses of 100, 200 & 400mg/kg did not appear to affect the bodyweight or the behaviour of the rats and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered AMC did not retard the growth of rats. After six weeks treatment, there were also no treatment related changes in the haematological parameters (i.e. hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells) between control and treated groups indicating that the AMC was not toxic to the circulating red cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds [12] and an important index of physiological and pathological status in man and animals [13].

In addition, most of the hepatological and renal parameters (i.e. Glucose, creatinine, Bilirubin, SGOT, SGPT, ALT, urea, uric acid, protein and cholesterol,) were also unchanged by the doses of AMC 100, 200 & 400mg/kg. The lack of significant alterations in the levels of ALP, creatinine, Bilirubin, SGOT, SGPT and cholesterol, good indicators of liver and kidney functions, respectively [14]. The transaminases (SGOT and SGPT) are well known enzymes used as biomarkers predicting possible toxicity [15]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases [16]. The transaminases were not significantly increased at the doses of AMC 100, 200 & 400mg/kg. It suggests that chronic ingestion of AMC did not alter the hepatocytes and kidneys of the rats, and, furthermore the normal metabolism of the animals. The relevance of this result may be associated with the biological value of the plant *Malachra capitata (L.)*.

In conclusion, the present investigation demonstrates that at doses consumed in the traditional medicine, the aqueous extract of *Malachra capitata (L.)* may be considered as relatively safe, as it did not cause either any lethality or changes of in the general behavior in both the acute and chronic toxicity studies in rats. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

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