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Research Article

Acute and Sub Acute Toxicity Studies of Some Indigenous Medicinal Plants

Haja Sherief Sheik[1*], Niraimathi Vedhaiyan[2] and Sengottuvelu Singaravel[1]
[1]Department of Pharmacology, Nandha College of Pharmacy, Koorapalayam Pirivu, Perundurai Main Road, Erode, Tamil Nadu, India.
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai, Tamilnadu, India.

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Abstract

The present investigation was studied to evaluate the toxicity of the medicinal plants extract. The three medicinal plants *Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds were evaluated for its toxicological studies and also for its consequence on biochemical parameters and histological variations. Acute and sub acute toxicity studies with these plants were done on Swiss albino mice and wistar albino rats respectively. During acute toxicity studies (72hr), there were no any clinical signs found in general behavior and mortality at any dose level used (50mg-2000mg/kg b.w.). In sub acute toxicity study (28days), these medicinal plants (250 and 500mg/kg, b.w.) did not cause any changes in hematological and biochemical parameters like hemoglobin, RBC, Hb, WBC, differential count and produced no significant change in organ weight. The record of biochemical parameters like SGOT, SGPT, ALP and total protein in above extracts treated rats were did not show any toxicity signs. From the above results it was concluded that above plants were safe and non-toxic in experimental animals.

1. INTRODUCTION

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern medicines in a number of studies. In developing countries, all over the world, 80% of population continues to use traditional medicine for primary medical problems[1]. Herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased recently. Their utilization is often based on long-term clinical experience. Despite the usage of plants in folk medicine over ages, only lately has pharmacology and toxicity of these plants begun to receive attention from scientists. With the upsurge in the use of herbal remedies in the last two decades, there is need for a thorough scientific evaluation of these medicinal plants[2].

Citrus maxima Burm. syn. *Citrus decumana* Watt., *Citrus grandis* Osbeck. (Family – Rutacaea) is also known as (English) Chinese grape fruit, Pomelo, Jabong, Pummelo, (Hindi) Sadaphal and (Sanskrit) adhukarkatika. Its leaves are traditionally used to produce sedative effect in cases of epilepsy, cholera and convulsive coughing. The essential oil from fresh leaves exhibits dermatophytic and fungistatic activity. The hot leaf decoction is applied on swellings and ulcers. Its leaves have anti-tumor activity[3,4,5].

The genus *Cyperus* includes common weeds found in upland and paddy fields in temperate to tropical regions. In Asian countries, the rhizomes of *Cyperus rotundus*, which are used as traditional folk medicines for the treatment of stomach and bowel disorders, and inflammatory diseases, have been widely, investigated[6-9].

Cyperus rotundus (*Cyperaceae*) is a traditional herbal medicine used widely as analgesic, sedative, antispasmodic, antimalarial, stomach disorders and to relieve diarrhea[10]. The tuber part of *C. rotundus* is one of the oldest known medicinal plants used for the treatment of dysmenorrheal and menstrual irregularities[11]. Infusion of this herb has been used in pain, fever, diarrhea, dysentery, an emmenagogue and other intestinal problems[12-14].

Abelmoschus moschatus Medik. leaves and seeds are considered as valuable traditional medicine. The aromatic seeds of this plant are aphrodisiac, ophthalmic, cardio tonic, antispasmodic and used in the treatment of intestinal complaints and check queasiness. To give a scientific basis for traditional usage of this medicinal plant, the seed and leaf extracts were evaluated for their antioxidant, free radical scavenging, antimicrobial and antiproliferative activities[15].

In the present study, the acute and sub acute toxicity study of *Citrus maxima*, *Cyperus rotendus* and *Abelmoschus moschatus* were investigated to accesses its safety and tolerable profile in long-term treatment.

2. MATERIALS AND METHODS

2.1 Plant Material

Leaves of *Citrus maxima* were collected from Ooty, tubers of *Cyperus rotundus* was collected from outskirts of Erode, and seeds of *Abelmoschus moschatus* were collected from Thirunelveli district. Authentication has been done by taxonomist, Agriculture University, Coimbatore (Tamilnadu). The voucher specimen (No.: BSI/ SRC/ 5/ 23/ 2012- 13/ Tech. 562, 775) has been deposited in the herbarium for future references.

2.2 Preparation of Extract

The above plant materials were washed with fresh water to remove adhering dirt and foreign particles. The plant materials were shade dried, crushed and grinded to get coarse powder. The coarse powder was then placed with 90% ethanolic solution in a round bottomed flask. 500g of the coarse powder of the plant materials in 1.0 liter of 90% ethanolic solution were macerated for 7 days. The mensturm was collected, concentrated by vacuum distillation and then air dried in an evaporating dish till constant weight was obtained.

2.3 Animals

Swiss albino mice of either sex weighing 25-30g and Wistar albino rats of either sex weighing 180 – 220 gm, were used for this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative

Corresponding Author:
S.Haja Sherief., Assistant Profesor,
Department of Pharmacology, Nandha College of pharmacy,
Perundurai main road, Erode-638052,
Tamil Nadu, India.
Email: sherief_1974@rediffmail.com

humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chaw (M/s.Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (NCP/IAEC/No.16/2012-13) of Nandha College of Pharmacy and Research Institute, Erode, Tamilnadu and were in accordance with the guidelines of the IAEC.

2.4 Acute Toxicity Study

Healthy Swiss albino mice of either sex weighing 25-30g were divided in to six groups of three animals each. The animals were housed under standard conditions and room temperature (25 ^{0}C ±2 ^{0}C) was maintained. All animals were fed with standard pellet diet and had free accesses two water *ad libitum*. The extracts were administered orally at doses of 50, 300, 500, 1000 and 2000mg/kg b.w. and control group received normal saline. The animals were observed for 72 hrs for any signs of behavioural changes, toxicity and mortality[16].

2.5 Sub-Acute Toxicity Study

Wistar albino rats of either sex weighing 150-200g were assigned to each group. Group I served as control received 0.1% carboxy methyl cellulose (CMC) 1ml/kg. Group II –III received the *Citrus maxima* extract at the dose of 250 and 500 mg/kg respectively. Group IV –V received the *Cyperus rotundus* extract at the dose of 250 and 500 mg/kg respectively. Group VI –VII received the *Abelmoschus moschatus* extract at the dose of 250 and 500 mg/kg respectively. All the test drugs were administered orally, once daily for 28 days. Body weight, food intake and water intake were monitored at regular intervals. The animals were sacrificed on 29th day for biochemical and histopathological studies. Prior to the sacrifice, animals were isolated in individual cages and fasted for 12 h, with water provided *ad libitum*.

Then, they were anaesthetized with ether and the blood was collected by sino-orbital puncture. Blood samples for the determinations of hematological parameters were collected in heparinized tubes and used for the following determinations, hemoglobin(Hb), red blood cell (RBC) count, white blood cell (WBC) count and differential count (DC) [neutrophils(N), lymphocytes (L), eosinophils (E), basophils (B) and monocytes (M)][17].

Non-heparinized tubes were used for serum biochemistry determinations. To obtain the serum, blood samples were placed at room temperature for approximately 30 min. Then, the tubes were centrifuged at 3000 x *g* for 10 min and the supernatants were taken to perform the following determinations: Serum glutamate oxaloactate (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP)[18] blood urea nitrogen[19] and serum creatinine[20].

After blood collection, the animals were sacrificed by cervical decapitation and the organs such as brain, heart, liver, spleen, kidney and testis were removed and weighed. The organs were preserved in 10% buffered formaldehyde for histopathological observations.

2.6 Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's *t* test. *P* values <0.05 were considered significant.

3. RESULT AND DISCUSSION

Table 1: The effect of plant extracts *(Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds) on organ weight changes in control and treated rats of sub acute toxicity studies.

Group	Treatment and Dose	Organ Weights (gms)					Mortality (%)	Body Weight (gm)
		Liver	Kidney	Lung	Heart	Spleen		
CONTROL	0.1% CMC 10ml/kg, p.o	5.01±0.42	1.15±0.09	0.96±0.18	0.62±0.06	0.59±0.04	NIL	226.56±19.87
AEAM	250mg/kg, p.o	4.98±0.51	1.26±0.11	1.06±0.94	0.59±0.09	0.63±0.07	NIL	217.62±22.41
AEAM	500mg/kg, p.o	5.16±0.39	1.36±0.16	1.02±0.14	0.63±0.07	0.67±0.06	NIL	214.78±19.06
AECR	250mg/kg, p.o	5.08±0.52	1.28±0.09	1.14±0.09	0.57±0.06	0.67±0.07	NIL	218.96±23.12
AECR	500mg/kg, p.o	4.89±0.50	1.29±0.09	0.94±0.12	0.70±0.06	0.59±0.04	NIL	214.72±20.62
AECM	250mg/kg, p.o	5.06±0.54	1.41±0.12	0.98±0.16	0.69±0.05	0.63±0.05	NIL	208.76±19.62
AECM	500mg/kg, p.o	4.92±0.41	1.27±0.13	1.20±0.18	0.65±0.06	0.59±0.06	NIL	206.84±18.64

Table 2: The effect of plant extracts *(Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds) on hematological parameters (RBC, WBC and Hb) in rats after 28 days treatment

Group	Treatment and Dose	RBC million cells/mm^3	WBC cells/mm^3	Haemoglobin (gm %)
CONTROL	0.1% CMC 10ml/kg, p.o	4.19 ± 0.40	8669.8 ± 142.41	14.48± 0.61
AEAM	250mg/kg, p.o	4.74 ± 0.32	8526.7 ± 102.33	14.70 ± 0.45
AEAM	500mg/kg, p.o	4.88 ± 0.28	8789.2 ± 138.14	15.65 ± 0.67
AECR	250mg/kg, p.o	4.91 ± 0.33	8807.0 ± 165.28	16.75 ± 1.12
AECR	500mg/kg, p.o	4.14 ± 0.34	8436.8 ± 208.54	15.98 ± 0.40
AECM	250mg/kg, p.o	4.78 ± 0.27	8699.3 ± 188.04	16.61 ± 0.89
AECM	500mg/kg, p.o	4.77 ± 0.19	8714.0 ± 205.33	15.75 ± 0.52

Table 3: The effect of plant extracts *(Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds) on differential leucocyte count in rats after 28 days treatment

Group	Treatment and Dose	Differential Count %			
		Neutophils	*Eosinophils*	*Monocyte*	*Lymphocyte*
CONTROL	0.1% CMC 10ml/kg, p.o	63.12 ± 3.76	1.9 ± 0.15	3.87 ± 0.19	21.60± 1.60
AEAM	250mg/kg, p.o	59.43 ± 2.98	2.02 ± 0.11	4.02 ± 0.31	23.10 ± 1.31
AEAM	500mg/kg, p.o	61.33 ± 4.53	1.90 ± 0.13	3.98 ± 0.28	22.81 ± 1.40
AECR	250mg/kg, p.o	60.13 ± 1.91	2.10 ± 0.07	3.27 ± 0.20	24.16 ± 1.68
AECR	500mg/kg, p.o	61.22 ± 3.08	1.86 ± 0.11	3.12 ± 0.13	25.16 ± 1.56
AECM	250mg/kg, p.o	61.53 ± 2.63	1.90 ± 0.12	3.88 ± 0.18	23.91 ± 1.25
AECM	500mg/kg, p.o	59.16 ± 3.95	2.10 ±0 .18	4.56 ± 0.30	23.98 ± 1.45

Table 4: The effect of plant extracts *(Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds) on liver functions in rats after 28 days treatment.

Group	Treatment and Dose	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilurubin (mg/dl)	Total Protein (g/dl)
CONTROL	0.1% CMC 10ml/kg, p.o	79.55±3.6	118.2±4.7	187.52±10.27	0.61±0.05	6.74±0.43
AEAM	250mg/kg, p.o	65.98±4.9	109.08±8.1	179.36±13.91	0.59±0.03	6.61±0.21
AEAM	500mg/kg, p.o	66.28±6.5	108.5±4.2	176.81±12.18	0.60±0..02	6.44±0.24
AECR	250mg/kg, p.o	65.93±4.9	113.55±4.4	171.61±13.85	0.59±0.03	6.16±0.24
AECR	500mg/kg, p.o	71.47±4.1	106.00±3.2	173.78±15.96	0.61±0.02	6.87±0.19
AECM	250mg/kg, p.o	66.46±4.6	102.9±4.7	174.94±14.4	0.65±0.03	6.15±0.29
AECM	500mg/kg, p.o	69.38±1.7	105.9±5.1	175.51±12.46*	0.55±0.03	5.92±0.22*

Table 5: The effect of plant extracts *(Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds) on kidney functions in rats after 28 days treatment.

Group	Treatment and Dose	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)
CONTROL	0.1% CMC 10ml/kg, p.o	39.79±3.0	0.94±0.03	3.97±0.05
AEA	250mg/kg, p.o	39.48±1.82	0.81±0.03	3.69±0.14
AEAM	500mg/kg, p.o	36.08±1.71	0.78±0.07	3.26±0.19
AECR	250mg/kg, p.o	33.36±2.17	0.77±0.03	2.96±0.27
AECR	500mg/kg, p.o	37.05±2.03	0.91±0.05	3.48±0.24
AECM	250mg/kg, p.o	31.08±1.24	0.82±0.05	3.18±0.19
AECM	500mg/kg, p.o	30.97±2.82	0.70±0.04	2.72±0.10

All the doses (50, 300, 500, 1000 and 2000 mg/kg) of plant extracts employed for acute oral toxicity studies were found to be non-toxic. Plant extracts did not produce any mortality even at the highest dose (2000 mg/kg) employed. As 2000mg/kg of body weight was well tolerated by the animals without any behavioral changes. Two sub maximal doses (250 and 500 mg/kg) which was found to be safe were employed for further toxicological investigations. In the sub-acute toxicity study, the plant extracts treated groups did not show any significant changes in body weight compared to the control group (Table 1).

The weights of the liver, kidney and heart were remaining unchanged in the experimental groups compared with the control group (Table 1). The hematological (Table 2 and 3) and biochemical parameters (Table 4 and 5) did not show any significant changes in plant extracts treated groups when compared to the control groups. The histopathological section of various organs such as the liver, kidney, lung, heart and spleen revealed normal architecture on comparison with the control group. Moreover, no lethality was recorded up to the maximum of 500 mg/kg body weight of plant extracts during the 28days of treatment.

In the toxicity studies, including the acute and sub-acute toxicities were elucidated in experimental rats. Although poisonous plants are omnipresent[21], herbal medicine is used by up to 80% of the population in developing countries. Despite widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. The present investigation shows that the plant extracts is non-toxic by oral route in rats, at least up to maximum doses of 2000 mg/kg body weight acutely and 500 mg/kg body weight sub-acutely.

The histopathological section of various organs such as the liver, kidney, lung, heart and spleen revealed normal architecture. There was no significant change in any liver function parameters, such as SGPT, SGOT and ALP, compared to the control group. Increase in these parameters would have indicated hepatotoxicity. The normal levels of blood urea and serum creatinine indicate that the test drug did not interfere with renal function and renal integrity was preserved. Also there was no significant changes in various hematological parameters such as Hb, RBC, WBC, platelets and differential count compared to the control group, which indicates that plant extracts may not be toxic and does not affect circulating red cells hematopoiesis or leucopoiesis.

4. CONCLUSION

Based on the results of the acute toxicity studies, it was concluded that a dose of 2000 mg/kg body weight of *Citrus maxima* leaves, *Cyperus rotendus* tubers and *Abelmoschus moschatus* seeds extract given orally appeared to be non-toxic. The results of sub-acute toxicity supports that the above plant extracts were safe since no marked changes in hematological, biochemical and histopathological parameters.

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