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Acute toxicity studies of aqueous leaf extract of *Capparis grandiflora* Wall Ex Hook.f & Thomson

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ABSTRACT

The acute toxicity of the aqueous leaf extract of C.grandiflora, a climbing shrub found mainly in the adjacent regions of Coimbatore, Nilgiry and Tiruchirappalli, South India was investigated. For the acute toxicity study, 1000-3000 mg/kg of the aqueous leaf extract were administered to rats and obvious toxic symptoms and mortality 24 hours postadminstration of the extract were determined. However, the initial reactions included excitement, tremors, and restlessness, loss of appetite and general weakness .No muscular numbness of the hind and fore legs, salivation or diarrhoea was observed .The symptoms were dose dependent with signs noticeable with increasing dosage. Post mortem, haematological and histopathological examination did not show any significant ($P<0.05$) damage as a result of the extract administration. No significant changes in body weights of treated rats were observed when compared with untreated control groups Phytochemical screening of the aqueous stem bark extract revealed the presence of cardiac glycosides, flavonoids, saponins, and tannin. The results suggest that the aqueous extract is not acutely toxic to the mice.

Key-words: *Capparis grandiflora*, acute toxicity, phytochemicals, histopathology.

INTRODUCTION

Recent years have witnessed a renewed interest in plants as pharmaceuticals in the Western world. This interest is channelled into the discovery of new biologically-active molecules by the pharmaceutical industry and into the adoption of crude extracts of plants for self-medication by the general public. [1]Natural products are the cornerstone of health care delivery especially in resource poor settings. Present estimates indicate that about eighty percent of the world's population relies on traditional medicine for health care delivery. [2-4] A number of studies have reported the toxic effects of herbal medicines .[5-8] Studies of medicinal plants using scientific

approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments. *Capparis Grandiflora* Wall ex Hook.f and Thomson is a climbing shrub with spreading branches found mainly in the adjacent regions of Coimbatore, Nilgiry andTiruchirappalli.[9,10] Communication with the traditional practitioners have revealed the use of the leaf juice as a stomachic, diuretic , anti rheumatic, shortness of breath and anti tumour. *Capparis* species has been reported to have anthelmintic, antimicrobial and anti inflammatory activities. [11, 12] The plant is used in traditional medicine for treatment of headache, fever, ulcers and skin infections of mixed origin. Phytochemical screening of the leaves revealed the presence of saponins, cyanogenic glycosides, flavonoids, and tannins. [13] So much has been done in screening medicinal plants for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far out number those of toxicity, probably as a result of the greater demands for resources and time such exercise warrant. Pharmacological and toxicological evaluations of medicinal plants are essential for drug development.[14,15] In the present study, the acute toxicity and histopathology of the aqueous extract of the leaves was evaluated in mice to assess its safety or otherwise, since the findings are important considering the usage of the plants by human beings.

EXPERIMENTAL SECTION

Plant

The fresh leaves of *Capparis grandiflora* wall. ex Hook.f & Thomson (Capparidaceae), collected at the flowering stage in the month of March 2010 from the tribal areas of Attapady, Palakkad district, Kerala state, South India were authenticated by the Botanical survey of India,Coimbatore,Tamilnadu (BSI). A voucher specimen (no. no.BSI/SRC/5/23/10-11/Tech-565) was deposited in the departmental herbarium. Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction.

Extraction

Air-dried, powdered leaves were Soxhlet extracted with water and evaporated in vacuo. (yield13.5 % w/w). Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

Animals

Swiss albino mice (20-32 g) aged 8- 12 weeks, bred in the KMCH College of Pharmacy, Kovai Estate, and Coimbatore, India were used for the study. They were kept in clean plastic cages in a 12 h light/dark cycle with litter changed every week. They were fed with mice cubes and water *ad libitum*. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998).

Phytochemical screening

The aqueous leaf extract was screened as described by standard methods. [16-18]

Acute toxicity studies

Thirty male Swiss albino mice of average weight 25.3 g were acclimatized for a week in cleaned cages and randomly divided into 3 groups of 3 animals each. Groups 1, 2 and 3were intraperitoneally/ orally administered 10, 100 and 1000 mg/kg body weight aqueous leaf extract reconstituted in water following the method of Lorke (1983).[19] The control group was administered water orally/intraperitoneally. The animals were observed frequently from the day of treatment. The nature and time of any adverse effect was noted, Observation was carried out

for 14 days and the experiment terminated. All animals were weighed and euthanized in a chloroform chamber and gross pathologic examination conducted. Sections of tissues such as lung, kidney, spleen, liver, and heart were obtained for histopathological studies. Determination of median lethal dose (LD50): Based on the result of the acute toxicity test, white Swiss albino mice of average weight 25.3 g divided into 3 groups of one animal per group were intraperitoneally/orally administered 1500, 2000 and 3000 mg/kg body weight, aqueous leaf extract in water. Death was monitored over a period of 24 h. The acute toxicity LD50 was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no mortality at all. The animals were observed and the studies terminated after two weeks. Recovery and body weight gain after each investigation was taken as a sign of surviving the test.

Haematology

The packed cell volume (PCV), haemoglobin and white blood cell were measured using the standard microhematocrit method, [20]and plasma protein was determined by method of Coles .[21]

Statistics

The mean and standard deviation and the level of significance for the differences between means were computed by student's t test.

RESULTS

The results revealed that the aqueous extract of leaves of *C. grandiflora* had high contents of Triterpenoids, tannins, steroids and carbohydrates. Alkaloids, cardiac glycosides, flavonoids and saponins were moderately present (Table 1).

Table 1: phytochemical screening of *Capparis grandiflora*

Extract	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Saponins	Tannins	Terpenoids	Steroids
Aqueous extract	++	--	--	--	++	++	++	++

++ = Present, -- = Absent

Table 2: Effect of administering different doses of *Capparis grandiflora* aqueous extract on body weight of mice over a period of two weeks

Dose(mg/kg b.wt)	Day 0	Day 7	Day 14
Control	27.20±0.20	27.90±0.20	28.50±1.20
10	19.20±0.14	22.20±0.34	24.50±0.22
100	29.50±0.45	29.70±0.14	29.85±1.22
1000	30.00±0.20	28.20±0.56	27.10±0.36
1500	20.10±0.22	20.40±0.14	18.80±0.12*
2000	25.60	24.80	23.40*
2500	24.70	24.90	22.00*
3000	28.90	28.40	26.80*

Clinical signs of toxicity observed in all the cases include initial excitement, tremors, and restlessness, loss of appetite and general weakness which was seen as the doses increased. No muscular numbness of the hind and fore legs, salivation or diarrhoea was observed. No death

was observed in the first 24 h and throughout the period of experiment. Table No. 2shows the average body weights of the rats before and after the treatment.

No significant changes in body weights of treated rats were observed when compared with untreated control groups. The relative weights of the organs (heart, liver, spleen, kidney and lungs) were not significantly ($p<0.05$) different from the control (Table 3).

Table 3: Effect of administering different doses of *Capparis grandiflora* aqueous extract on the organ weight of mice (g/100g)

Dose(mg/kg b.wt)	Heart	Liver	Lungs	Kidney	Spleen
Control	0.20	1.20	0.20	0.60	0.40
10	0.60	1.80	0.30	0.80	0.40
100	0.20	1.80	0.20	0.80	0.40
1000	0.45	2.60	0.60	0.90	0.30
1500	0.60	1.60	0.80	0.65	0.40
2000	0.50	1.60	0.70	0.80	1.00
2500	0.40	1.60	0.60	0.80	0.80
3000	0.40	1.30	0.50	0.70	0.60

Histopathological examination of the organs did not reveal any abnormalities (data not shown). The packed cell volume, haemoglobin, white blood cell and total plasma protein were not significantly ($p<0.05$) different from the control (Table 4).

Table 4: Effect of administering different doses of *Capparis grandiflora* aqueous extract on the haematological profile of the mice

Dose(mg/kg b.wt)	PCV (%)	Hb(g/dl)	WBC(x 10^9/L	Total plasma protein(g/dl)
Control	56.75	17.32	7.15	5.40
10	54.7	16.25	7.10	5.30
100	50.7	16.07	7.00	5.10
1000	50.00	16.00	6.90	5.90
1500	49.00	16.00	6.75	5.20
2000	49.00	15.75	6.35	5.00
2500	48.00	15.50	6.20	4.90
3000	48.00	15.00	6.00	4.80

However, the white blood cell and total plasma protein of group treated with 3000mg/kg body weight showed decreased values.

DISCUSSION

Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD50/ED50) of drugs and Xenobiotics. [22] Phytochemicals are thought to have a positive or negative effect on an animal. Tannins and Flavonoids are thought to have both proxidant and antioxidant effects on the body. While the antioxidant protects the tissues and organs, the proxidant damages the tissues and organs. The weight changes of the animals during the period of observation which was more visible at higher doses, suggest the presence of tannins and other phenolics which are thought to interfere with absorption of nutrients making them unavailable and thereby reducing feed intake .[23] Even though the animals were fed with adequate diet, the aqueous extract at higher doses could have caused the interference since phytochemical studies showed the presence of tannins and other compounds which interferes with absorption of nutrient such as proteins and minerals resulting

in weight loss. The organ weights were not significantly different form the control. This suggests that the aqueous extract did not interfere with the organs. The congestion seen in the lungs could be as a result of the inhalation of the chloroform used to euthanize the animals. It appears that the different dose of the aqueous extracts did not affect the haematological parameters of the animals. This is very surprising because the extracts contained the presence of saponnins which has been reported to have deleterious haemolysing effect on circulating erythrocytes. [24] The absence of gross and histopathological lesions in the organs could suggest the level of safety of the aqueous extract on the animals. In conclusion, to our knowledge, this is the first investigation on the toxicity studies of *C.grandiflora*.This study has shown that acute administration of the aqueous leaf extract of *C.grandiflora* may be safe as the LD50 could not be determined at the doses given. The results of the present study suggest that the aqueous fraction is not acutely toxic to the rats thereby providing a support to the use of *C.grandiflora* leaves in indigenous system of medicine. However, further long-term toxicological studies (chronic toxicity), are needed in order to establish it as medicine. Though the phytochemical screening revealed many chemical constituents, which could affect the animal positively or negatively as a result of prolong usage, it is recommended that a long-term study be conducted. The effect on haemoglobin concentration (Hb), white blood cell (WBC) count and packed cell volume (PCV) indicated the unlikelihood of the extract to induce anaemia even after long use. [25]

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