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Evaluation of Acute oral toxicity of aerial parts of *Artemisia parviflora* Roxb. in Swiss albino mice

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ABSTRACT

Artemisia parviflora Roxb. (syn. A. japonica; Asteraceae) is a member of Indian tarragon family and is commonly found in many areas of north western Himalayas, central Himalayas, the Nilgiri hills, Arunachal Pradesh and Uttarakhand in India. A. parviflora is a medicinal plant used in traditional system of Indian medicine for its wound healing, febrifuge, digestive, anthelmintic properties. Here, we have performed the acute oral toxicity of ethanolic extract of aerial parts of A. parviflora. In acute toxicity study total four group of mice (n=5 per group) were orally treated with doses of 0.10, 0.50, 1.0 g/kg body weight. And parameters like behavioral change, body weight change, and other abnormalities were observed. Our study showed that A. parviflora did not produce any hazardous symptoms or death in acute toxicity study showing a LD50 higher than 1 g/kg body weight. The administration of this plant material did not showed any significant changes in the body weight or any type of adverse effect on the body of animal. Hence, taken together the study proves that oral administration of ethanolic extract of aerial parts of A. parviflora produced no significant toxic effect in male and female Swiss albino mice, which could stand as an assurance for the medicinal use of this plant in folk medicine.

Keywords: Acute toxicity, *Artemisia parviflora*, Asteraceae.

INTRODUCTION

Artemisia parviflora Roxb. (syn. *Artemisia japonica*), family Asteraceae, is a member of Indian tarragon family and is commonly found in many areas of north western Himalayas, central Himalayas, the Nilgiri hills, Arunachal Pradesh and Uttarakhand in India. It is also found in Booni valley, Ponch, Rawalpindi, Sawat and Northern areas of Pakistan. In traditional medicine, various parts of this plant (leaves, stem, seeds and fruits) have been widely used by tribal people for its wound healing properties, treatment of skin diseases, febrifuge, depurative properties, digestive and in ethnoveterinary medicine. The volatile oil is reported to have potent antifungal activity. Decoction of leaves is used as vermifuge and leaves juice is used externally on cuts, wounds and skin conditions[1]. The leaves are also considered as digestive, anthelmintic, back pain reliever and in treatment of round worms [2,3]. The shoots are used as incense, sometimes the leaves and twigs are also burned as incense [4]. Dried and grounded seeds, leaves and fruits used for stomachache, high blood pressure and diabetes [5]. The expressed juice of the plant is used in the treatment of vaginitis [6]. In United States, Dunkel et al holds patent for "insecticidal or insect behaviorally active preparations from aromatic plants" claims to provide insect control preparation, which uses the supercritical CO_2 extract of two or more aromatic plants, namely *Artemisia japonica, Geranium viscosissimum* and *Balsamorhiza sagittata*; and their vapor composition [7].

As the clinical and pharmacological interest of the efficacy and safety of herbal remedies has grown during the past ten years because of the realization that many people are self medicated using these agents there is renewed interest in the gallery of scientists in evaluating the same [8,9]. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Unfortunately, many people underestimate the toxicity of natural products and do not realize seriousness of the matter that these natural products could be toxic or than the synthetic drugs [10]. A typical example of toxic herbal product are the leaves of *Atropa belladonna* [11] and *Digitalis purpurea* [12], which shows severe systemic toxicity if taken internally.

The ethanolic extract of *A. parviflora* showed a LD_{50}- Lethal dose, 50 percent kill for rodent mouse (*Mus musculus*), of 750 mg/ kg [13]. No toxic effects were reported other than lethal dose value. In another report extract of *A. parviflora* was tested on rodent rat (*Rattus rattus*) showed LD_{50}- Lethal dose value to be greater than 1 g/ kg [14]. There are no reports of toxicity in ethnobotanical data however, a decoction of the leaves is said to promote a plump figure, but too much is said to be deleterious and can cause hypertension [6]. One method for evaluation of herbal toxicity is the acute oral toxicity test in which the herbal preparation is given orally as a single and very high dose to laboratory animals like Swiss albino mice. The tested animals are then observed for a period of 14 days for activity, behavior, and indications of toxicity or illness [10]. Therefore, we investigated the possible toxic effect of aerial parts of *A. parviflora* in order to ensure its safe use.

MATERIALS AND METHODS

Plant material

Fresh samples of *Artemisia pariviflora* (Fig,1) were collected from forests of Nilgiri hills, Ooty, Tamil Nadu, India in the month of June, 2011 and authenticated by Botanical Survey of Medicinal Plants and collection unit, Ootacamund. The voucher specimens were preserved in the Department of Pharmacognosy, J.S.S. College of Pharmacy, Mysore, for further reference. The plant was dried under shade to a constant weight and coarsely powdered in a electronic mixer, sieved through mesh no. 40 and stored in air tight, well closed container till further use. The plant material was used for study as per the study protocol given in table 1.

Table 1. Study protocol

Name of study	Acute oral toxicity study
Test material	Ethanolic extract of *Artemisia parviflora* Roxb.
Animals	Swiss albino mice
Animals procured from	JSS Medical College, Mysore
Sex	Both male and female
Weight range	25 to 30 g
Number of animals per group	n= 5/group
Route of administration	*po* (oral gavage)
Vehicle	0.5% Carboxy methyl cellulose
No. of administration	One per day
Dose concentration	0.10 g/kg, 0.50 g/kg and 1.0 g/kg body weight
Study duration	Acclimatization for 7 days and one day drug administration for 14 days
Parameters	Cage side observation, daily food and water intake, body weight and mortality record.

Extraction and preliminary phytochemical screening

The coarsely powdered aerial parts of *A. parviflora* (AP) were extracted with 95% ethanol The herb to solvent ratio was kept 1:10 to ensure complete extraction. The plant material was extracted by cold maceration for 72 hours with various solvents with intermittent agitation. After incubation, the extracts were filtered through Whatman filter paper and the extracts were collected and stored at 4°C in refrigerator till further use. The extracts obtained were subjected to following chemical tests for identification of various phytoconstituents as per the methods given by Harborne.

Animals

Adult male and female Swiss albino mice were, aged 2 to 3 months, weighing 25g to 30g were obtained from JSS Medical College, JSS University, Mysore. The animals were maintained in standard conditions (22-24°C; 12:12 h dark/ light cycle). Water and industrialized dry pellet were provided *ad libitum*. The specifications of feed are mentioned in table 2. Measured quantities of water and pellets were supplied daily in each cage. The experimental protocol were approved by the Institutional Animal Ethical Committee, JSS College of Pharmacy, Mysore (Process no. 096/2011) and were kept in accordance with the OECD and institutional guidelines.

All animals were housed in the polyurethane cages. The cages were provided with wheat husk bedding and were cleaned daily. The animals were acclimatized to laboratory conditions for 7 days and animals showing any sign of abnormality or pathological change were not included in the study.

Table 2. Composition of feed

Name	Percentage (%)
Protein	24
Fat (ether extract)	5.0
Fat (acid hydrolysis)	5.7
Crude fiber	5.1
Nitrogen free extract	48.7
Total digestible nutrients	76.0
Pellet diameter	10mm

Experimental design

Healthy Swiss albino mice were fasted for 4 h, but with free access to water. Animals were randomly divided in four groups having five animals each (n = 5/ group). The group 1 (control group) received orally distilled water. Group 2, 3, 4 were orally treated with A. parviflora extract in doses of 0.10 g/kg, 0.50 g/kg and 1 g/kg respectively. The animal were observed for general behavioral and body weight changes, hazardous symptoms and mortality for a period of first four hour and then 14 days after treatment.

RESULTS AND DISCUSSION

Cage side observation

The examinations were carried out on daily basis after the administration of doses for any abnormality or toxic effect. On day 1, after the administration of doses the animals were kept under observation for first four hours and then on daily basis the other parameters like condition of fur, skin, breathing abnormalities etc were observed. Any changes or abnormalities were recorded could be an indication of toxicity. The test animals at all dose levels of ethanolic extract showed no significant changes in behavior before and after administration. Table 3 presents the dosage regime for ethanolic extract of *A. parviflora*, table 4 shows the general cage side observations for all parameters studied. Table 5 shows the mortality record of the animals in different groups.

Table 3. Dosage regime

Sr. No.	Group	No. of animals (n)	Dose (in g/kg body weight/day)
1	Group 1(Control)	5	0.5% CMC
2	Group 2	5	0.10
3	Group 3	5	0.50
4	Group 4	5	1.0

Table 4. Cage side observation for animal

Sr. No.	Parameters	Cage side observations
1	Condition of fur	Normal
2	Skin	Normal
3	Eyes-dullness	Nil
4	Pupil diameter	Normal
5	Color and consistency of faeces	Normal
6	Wetness or soiling of the perineum	Nil
7	Condition of teeth	Normal
8	Breathing abnormalities	Nil
9	Gait	Normal

Table 5. Mortality record of animal group

Sr. No.	Group	Mortality at different dose		
		0.10 g/kg/day	0.50 g/kg/day	1.0 g/kg/day
1	Group 1(Control)	Nil	Nil	Nil
2	Group 2	Nil	Nil	Nil
3	Group 3	Nil	Nil	Nil
4	Group 4	Nil	Nil	Nil

Preliminary phytochemical study

Phytochemical screening shows the presence of alkaloids, sterols/ triterpenoids, tannins, flavonoids, phenols and carbohydrates.

Body weight changes and Mortality

Body weight change is an important factor to monitor the animal health. Any loss in body weight is frequently the first indicator of onset of an adverse effect. A dose, which causes 10% or more reduction in the body weight, is considered a toxic dose. All the animals treated showed normal body weight at the end of 14^{th} day as compared to day zero observations.

There was no mortality recorded at the end of the study, even at the highest dose of 1 g/kg body weight of plant extract.

CONCLUSION

Medicinal plants and their derivatives have been used as an alternate to allopathic medicines in many countries. Despite of wide spread use, few studies have been undertaken to ascertain the safety and efficacy of traditional medicine [15,16]. *A. parviflora* is a medicinal plant used in traditional system of Indian medicine for its wound healing, febrifuge, digestive, anthelmintic properties. This study was done according to the international guidelines drawn under OECD. The study concludes that there was no mortality up to the dose of 1 g/kg body weight which is in concurrent to previous reports on the LD_{50} value of *A. parviflora* in rats [13]. The administration of this plant material did not showed any significant changes in the body weight or any type of adverse effect on the body of animal. All the groups were observed for any abnormalities like diarrhea, general behavioral changes during the first four hour of administration of dose and then daily for a period of 14 days. Hence, taken together the study proves that oral administration of ethanolic extract of aerial parts of *A. parviflora* produced no significant toxic effect in male and female Swiss albino mice, which could stand as an assurance for the medicinal use of this plant in folk medicine.

Fig. 1 Two shoots of *Artemisia parviflora* showing leaf morphology

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