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Original Research Article

Acute toxicity study of seeds of *Achyranthes aspera*, bark of *Berberis aristata* and roots of *Coleus forskohlii* in Wistar rats

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ABSTRACT

Background: *Achyranthes aspera* is a species of plant in the family *Amaranthaceae*. *Berberis aristata* is a shrub belonging to the family *Berberidaceae* and the genus Berberis. *Plectranthus barbatus* is a tropical perennial plant related to the typical coleus species. It produces forskolin, an extract useful for pharmaceutical preparations and research in cell biology. It is belonging to Lamiaceae. The present study has been undertaken to study the toxic effects of hydro alcoholic extracts of *A. aspera*, *B. aristata*, *C. forskohlii* in albino Wistar rats and to establish the hazardous safety category of hydro alcoholic extracts of these plants as per organization for economic cooperation and development (OECD-423) guidelines and GHS classification system respectively.
Methods: In acute toxicity study, the hydro-alcoholic extracts of all the above three plants were given orally at the dose of 2000 mg/kg b. w. to three rats in each group respectively in step I. Then, all the animals were observed for initial 4 hours and followed by fourteen days for their clinical signs and mortality in step II.
Results: In step I, all the animals were normal and there was no mortality after 48 hours. In step II with the same dose, all the animals showed no adverse effects and no mortality when followed up to 14 days observation period.
Conclusions: The result indicates that the hydro alcoholic extracts of *A. aspera*, *B. aristata*, *C. forskohlii* plants can be utilized safely for therapeutic use in pharmaceutical formulations and it falls under category '5' or 'unclassified' of GHS system.

Keywords: *Achyranthes aspera*, *Berberis aristata*, *Coleus forskohlii*, OECD and acute toxicity

INTRODUCTION

Medicinal plants have long been used for the treatment of certain diseases. According to literature, the use of plants as a source of medicine dates back more than 5000 years.[1] The World Health Organization, estimates populations using medicinal plants around 65-80%.[2] The medicinal plants contain active molecules that are at the origin of the therapy. Researchers have shown that medicinal plants have various effects on body and can be used for infertility, hypertension, diabetes mellitus, asthma, infections and even certain cancers.[3-5] However, although medicinal plants have several therapeutic virtues, they are not free from any danger of intoxication. Several researchers have pointed out the potential toxicity, as well as the risks associated with the use of certain species of plants and vegetables.[6]

During the past few decades, traditional system of medicine has received marvelous attention for in vivo studies.[7] Toxicology is the important part of pharmacology which deals with the undesirable effect of phytocompounds on living organisms previous to the use

as drug or chemical in clinical use.[8] Several studies are concentrated on toxicity analysis so as to determine the safety of medicinal plants and their products. Toxicity analysis is essential, as some herbs consumed might have some toxic effects and many reports have been published for toxicity caused due to long term consumption of herbs. The occurrence of toxicity mechanism could differ depending on the cell membrane and chemical properties of the toxicants in human beings. It might happen within the cell membrane or on the cell surface or tissue underneath as well as at the extracellular matrix. According to OECD guidelines, in order to ascertain the protection and effectiveness of a new drug, toxicological studies are extremely significant in animals like mice, rat, guinea pig, dog, rabbit, monkey etc.

Toxicological studies aid to extend decision whether a new drug must be adopted for clinical use or not. OECD guidelines such as 401, 423 and 425 do not permit the use of drug clinically without its clinical trial as well as toxicity studies.[9] The toxicity of *Achyranthes aspera* was studied only upto 500 mg and that prompted us to carry out toxicity studies at higher doses.[10] The objectives of the present study was to study the acute toxicitis of seeds of *Achyranthes aspera*, bark of *Berberis aristata* and roots of *Coleus forskohlii* in Wistar rats, to establish the LD 50 values and to categorize the herbal extract according to the GHS classification system.

METHODS

Collection and authentication of plant materials

The seeds of *Achyranthes aspera*, bark of *Berberis aristata* and roots of *Coleus forskohlii*, were collected freshly. The taxonomic identity of the plant was confirmed by botanist. The plant material was rinsed thoroughly under running tap water and then with distilled water to eradicate the surface pollutants cleaned, shade dried, powdered to mesh size 60. The powdered material was stored in air tight container.

Preparation of extract

Hydroalcoholic extract of each fraction of plants powder was prepared by maceration method. Powdered plant materials were soaked in mixture of ethanol and water (60:40) at room temperature for 7 days in a separate labeled beaker and then filtered using Whatman no. 1 filter paper. Then the filtrate was evaporated and dried and then used for acute toxicity test.

Experimental animals, housing and feeding conditions

Healthy young adult Wistar rats were used for the study. Female rats were used because they are slightly more sensitive than male. Healthy young adult animals of commonly used laboratory strains were employed. Female rats were 8 to12 weeks old, nulliparous and non-pregnant and weighing around 150-180g. The temperature in the experimental animal room was 22ºC (+3ºC), humidity of 30-70%. Artificial lighting with 12 hours light and dark cycle was maintained. Pelleted rodent feed and RO water was provided *ad libitum*. Animals were housed in poly propylene rat cages (approximate internal dimensions of 370 mm × 210 mm × 150 mm) with Corn cob bedding (3 animals per cage). The Institutional Ethical Committee of Osmania Medical College, Koti, Hyderabad, India approved the protocol for these experiments under number IAEC/Pharma/OMC/28/2015.

Administration of doses

All the animals were individually marked for identification kept 5 days for acclimatization and were fasted overnight before the dosing. Carboxy methyl cellulose (CMC) was used as vehicle based on preliminary solubility test. The herbal extracts of *A. aspera, B.* aristata, *C.* forskohlii at a single dose of 2000 mg/kg body weight were administered orally using oral gavage tube to three animals in each group respectively. Then all the animals were fasted further for a period of 3 to 4 hours after dosing. This is the step I in acute toxicity testing. Then we observe the animals for 48 hours. Our further procedure depends on the observation of mortality. If, there is 50% mortality, we will step down the dose to 300 mg/kg body weight and if there is no mortality, we will proceed to step II, where we give same dose to confirm the step I and observe the animals up to 14 days.

Mortality

All the animals were observed for mortality twice daily throughout the study period especially for the first 48 hours. As per principles of acute oral toxicity testing-acute toxic class method of OECD-423, we need to follow step wise procedure.[11] That is, to facilitate the use of a minimum number of animals per step. The test extract/compound is given orally to a group of experimental animals at one of the defined doses. Each step using three animals of female sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; as per (Figure 1).

No further testing is needed, dosing of three additional animals, with the same dose and dosing of three additional animals at the next higher or the next lower dose level.

Statistical analysis

The results were expressed as mean±SEM. Statistical analysis was carried out by using SPSS ver21.

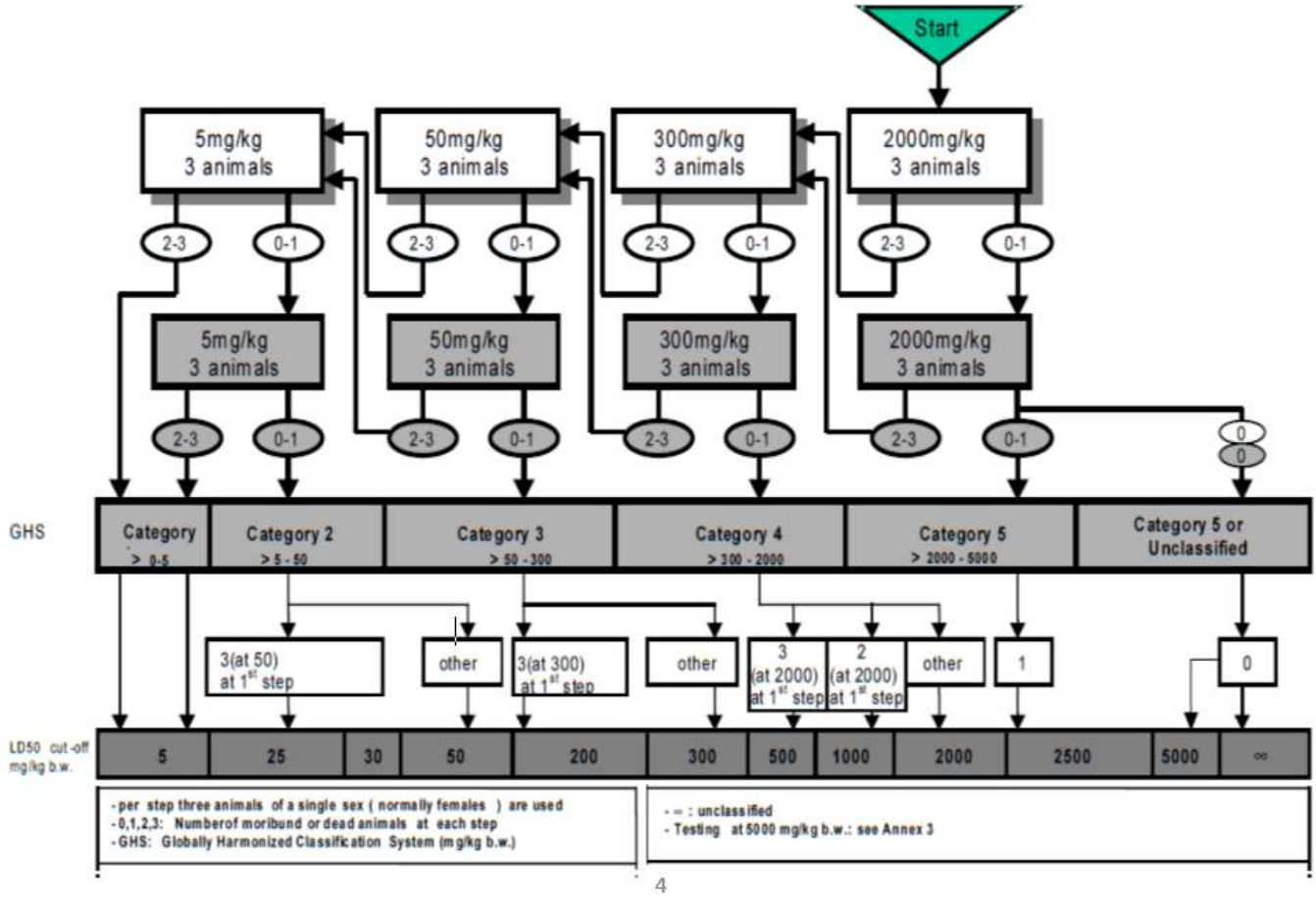

Figure 1: Test procedure with a starting dose of 2000 mg/kg body weight.[11]

RESULTS

Clinical signs

All the animals were closely observed for their clinical signs with following frequency. Daily once during the acclimatization period, just before dosing, during the first 30 minutes after the dosing and at approximately 1, 2, 3 and 4 hours after the dosing on day 0. Further every day for the period of 14 days. Following parameters were observed, condition of skin and fur, eyes and mucus membrane, respiratory, circulatory and autonomic and central nervous system, somato-motor activity and behavioral pattern. Specific observations made for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The results are shown in Table 1 and 2.

Mortality was observed in the study as per OECD 423 guidelines. No mortality was observed in the first 48 hours, hence, proceeded to step II as shown in Figure 1 and followed the similar observation for the entire study period. The results are shown in Table 3.

Body weight

Body weight of all the animals was measured on test day 0 (prior to dosing), day 7, and day 14. All surviving animals had gained body weight by day 7 and day 14 as compared to day 0 and results are shown in Table 4.

Euthanasia

On termination (day-14), the surviving animals were humanely euthanized by CO_2 asphyxiation.

Necropsy

Gross necropsy was performed on all surviving animals on day 14. Macroscopic lesions for individual animal were recorded.

Macroscopic findings

Macroscopic/gross pathological examination was conducted for all the animals after 14 days observation period. No gross pathological lesions were recorded and the results are shown in (Table 5).

Table 1: Signs and symptoms observed during acute toxicity testing of hydro alcoholic extracts of *Achyranthes aspera*, *Berberis aristata* and *Coleus forskohlii*.

Observation	*Achyranthes aspera* step-I	*Achyranthes aspera* step-II	*Berberis aristata* step-I	*Berberis aristata* step-II	*Coleus forskohlii* step-I	*Coleus forskohlii* step-II
Temperature	Normal	Normal	Normal	Normal	Normal	Normal
Change in skin	No effect	No effect	No effect	No effect	No effect	No effect
Eye color change	No effect	No effect	No effect	No effect	No effect	No effect
Food intake	Normal	Normal	Normal	Normal	Normal	Normal
General physique	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Not present	Not present	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present	Not present	Not present
Drowsiness	Not present	Not present	Not present	Not present	Not present	Not present
Breathing difficulty	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Sedation	No effect	No effect	No effect	No effect	No effect	Observed
Tremor	Not present	Not present	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive	Alive	Alive

Table 2: Observation of all the animals for toxicity from the time of administration of extract till 14 days.

Group and dose mg/kg b. w.	Days of observation																		
	0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	30 min	1 h	2 h	3 h	4 h														
***Achyranthes aspera* /step-I, step-II And 2000 mg/kg**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
***Berberis aristata*/ step-I, step-II and 2000 mg/kg**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
***Coleus forskohlii*/ step-I, step-II and 2000 mg/kg**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Three female rats in each group, N= normal. *Examinations were performed within the first 30 minutes and at approximately 1, 2, 3 and 4 hours after treatment on test day 0.

Table 3: Mortality among the animals after the administration of extracts.

Group/step	Dose mg/kg b.w.	No. of animals treated	No. of animals died	Percent mortality (up to 14 days)
***Achyranthes aspera*/I**	2000	3	0	0.00
***Achyranthes aspera*/II**	2000	3	0	0.00
***Berberis aristata*/I**	2000	3	0	0.00
***Berberis aristata*/II**	2000	3	0	0.00
***Coleus forskohlii*/I**	2000	3	0	0.00
***Coleus forskohlii*/II**	2000	3	0	0.00

Key: mg/kg= milligram/kilogram, b.w.= body weight, No.= number.

Table 4: Changes in the body weights of the animals throughout the study.

Group/step	Test day 0 (g)	Test day 7 (g)	Test day 14 (g)	% b. w. change (day 0-day 7)	% b. w. change (day 0-day 14)
***Achyranthes aspera*/step-I**	162.67±1.82	174.61±1.78	190.29±2.08	7.34±0.21	16.99±2.15
***Achyranthes aspera*/step-II**	164.93±4.33	178.02±6.88	191.41±8.65	7.92±1.97	16.02±2.41
***Berberis aristata*/step-I**	160.65±5.41	171.35±6.14	185.68±6.47	6.65±0.23	15.58±0.29
***Berberis aristata*/step-II**	163.02±2.37	176.52±1.90	194.43±4.75	8.29±0.46	19.26±1.98
***Coleus forskohlii*/step-I**	163.86±5.98	175.67±5.62	188.07±6.81	7.22±0.74	14.78±0.07
***Coleus forskohlii*/step-II**	158.55±1.06	169.88±1.19	181.99±2.40	7.15±0.31	14.78±1.22

Dose is 2000 mg/kg body weight and n=3 female rats per group.

Table 5: Macroscopic findings of all the animals during the study.

Group/step	Dose mg/kg b.w.	Sex	Mode of death	Macroscopic/gross pathological observations findings	
				External	Internal
***Achyranthes aspera*/step-I, II**	2000	F	TS	NAD	NAD
		F	TS	NAD	NAD
		F	TS	NAD	NAD
***Berberis aristata* /step-I, II**	2000	F	TS	NAD	NAD
		F	TS	NAD	NAD
		F	TS	NAD	NAD
***Coleus forskohlii*/step-I, II**	2000	F	TS	NAD	NAD
		F	TS	NAD	NAD
		F	TS	NAD	NAD

Key: mg/kg= milligram/kilogram, b.w.= body weight, F= female, NAD= no abnormalities detected, TS= terminal sacrifice.

DISCUSSION

After the administration of hydro alcoholic extracts of *Achyranthes aspera*, *Berberis aristata* and *Coleus forskohlii* there was no abnormal signs and symptoms observed, also there was not a single mortality infect there was significant increase in body weight. After performing autopsy, there was no abnormality detected in gross/macroscopic examination of the animal tissues. So, the result indicates that the hydro alcoholic extracts of *A. aspera*, *B. aristata*, *C. forskohlii* plants can be utilized safely for therapeutic use in pharmaceutical formulations. *Achyranthes aspera* methanol extract was also found to be safe by a study conducted by Pingale et al.[12]

A study by Chandana et al also showed that upto 1000 mg/kg methanol extract of *A. aspera* was safe.[13] A study by Anil et al showed no toxicity to ethanolic extract of *Berberis aristata* root at 1000 mg/kg b. w. dose.[14] On the contrary a study conducted by Padmaja et al, the LD_{50} of aqueous extract of bark of *Berberis aristata* was >5000 mg/kg body weight.[15] The third plant, that is *Coleus forskohlii* was also found to be safe only upto 1000 mg/kg by a study conducted by Majeed et al.[16]

CONCLUSION

The hydro alcoholic extracts of *A. aspera*, *B. aristata*, *C. forskohlii* plants can be utilized safely for therapeutic use in pharmaceutical formulations and the acute oral LD_{50} is determined as 2000<ATE≤5000 mg/kg b. w. LD_{50} (cut off value): the herbal extracts (*Achyranthes aspera*, *Berberis aristata* and *Coleus forskohlii*) falls under category '5' or unclassified with LD_{50} cut-off value of 5000 mg/kg body weight according to the GHS classification system.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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