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

J. Ravi Kumar1*, V. Prasanna2, T. Chakradhar1, K. C. Haritha1

1Department of Pharmacology, Osmania Medical College, Koti, Hyderabad, Telangana, India
2Department of Pharmacology, R. V. M. Institute of Medical Sciences and Research Center, Mulugu, Siddipet, Telangana, India

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*Correspondence:
Dr. J. Ravi Kumar,
Email: jravipharma@gmail.com

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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 toxicities 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 8 to12 weeks old, nulliparous and non-pregnant and weighing around 150-180g. The temperature in the experimental animal room was 22°C (+3°C), 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.
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 CO₂ 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*                  |
|                           | 30 min              |
|                           | 1 h                 |
|                           | 2 h                 |
|                           | 3 h                 |
|                           | 4 h                 |
|                           | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 |
| Achyranthes aspera I      | N N N N N N N N N N N N N |
| Achyranthes aspera II     | N N N N N N N N N N N N N |
| Berberis aristata I       | N N N N N N N N N N N N N |
| Berberis aristata II      | N N N N N N N N N N N N N |
| Coleus forskohlii I       | N N N N N N N N N N N N N |
| Coleus forskohlii II      | 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.
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.\(^\text{12}\)

A study by Chandana et al also showed that upto 1000 mg/kg methanol extract of *A. aspera* was safe.\(^\text{13}\) A study by Anil et al showed no toxicity to ethanolic extract of *Berberis aristata* root at 1000 mg/kg b. w. dose.\(^\text{14}\) 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.\(^\text{15}\) 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.\(^\text{16}\)

**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\(\leq\)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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**Ethical approval:** The study was approved by the Institutional Ethics Committee

**REFERENCES**

1. Kelly K. History of medicine. New York: Facts on file; 2009: 29-50.
2. WHO (World Health Organization). The World Traditional Medicines Situation, in Traditional medicines: Global Situation, Issues and Challenges. Geneva. 2011:1-3-14.
3. Blahi ANM, Zougrou NE, Gnghoue G, Kouakou K. Mechanism of Action of the Aqueous Leaves Extract of Sarcocephaluslatifolius (Smith) on the Reproductive System of Female Rat. J Physiology Pharmacol Advances. 2016;6(12):950-9.
4. Reinhart KM, Coleman CI, Teevan C, Vachhani P, White CM. Effects of garlic on blood pressure in patients with and without systolic hypertension: a meta-analysis. Ann Pharmacother. 2008;42:1766-71.

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**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 |
|----------------------------|-----------------|-----|---------------|------------------------------------------------------|
| *Achyranthes aspera*/step-I, II | 2000            | F   | TS            | NAD                                                  |
|                            |                 | F   | TS            | NAD                                                  |
| *Berberis aristata*/step-I, II | 2000            | F   | TS            | NAD                                                  |
|                            |                 | F   | TS            | NAD                                                  |
| *Coleus forskohlii*/step-I, II | 2000            | F   | TS            | NAD                                                  |
|                            |                 | F   | TS            | NAD                                                  |

Key: mg/kg = milligram/kilogram, b.w. = body weight, F = female, NAD = no abnormalities detected, TS = terminal sacrifice.
5. Taur DJ, Patil RY. Some medicinal plants with antiasthmatic potential: a current status. Asian Pac J Trop Biomed. 2011;1:413-8.
6. Agbaire PO, Emudainohwo JOT, Clarke PBO. Phytochemical screening and toxicity studies on the leaves of Manniophytontfylvum. Inter J Plant Ani Environ Sci. 2013;3(1):1-6.
7. Mazid M, Khan TA, Mohammad F. Medicinal plants of rural India: a review of use by Indian folks. Indo Global J Pharmaceutical Sci. 2012;2(3):286-304.
8. Aneela S, De S, Kanthal LK, Choudhury NS, Das BL, Sagar KV. Acute oral toxicity studies of Pongamiapinnata and Annonasquamosa on albino wistar rats. Int J Res Pharmacy Chemistry. 2011;1(4):820-4.
9. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother. 2011;2:74.
10. Reddy CV, Kamble A. Toxicity study of Achyranthes aspera. Int Letters Natural Sci. 2014;14:85-96.
11. Mir AH, Sexena M, Malla MY. An acute oral toxicity study of methanolic extract from Tridex procumbens in Sprague Dawley’s Rats as per OECD guidelines 423. Asian J Plant Sci Res. 2013;3(1):16-20.
12. Sadashiv PS, Krishna AR. Acute toxicity study for Achyranthes aspera leaves. J Pharmacy Res. 2011;4(7):2221-2.
13. Barua CC, Talukdar A, Begum SA, Pathak DC, Sarma DK, Borah RS. In vivo wound-healing efficacy and antioxidant activity of Achyranthes aspera in experimental burns. Pharmaceutical Biology. 2012;50(7):892-9.
14. Pareek A, Suthar M. Anti-diabetic activity of extract of Berberis aristata root in streptozotocin induced diabetic rats. Pharmacology online. 2010;2:179-85.
15. Joshi PV, Shirkhedkar AA, Prakash K, Maheshwari VL. Anti-diarrheal activity, chemical and toxicity profile of Berberis aristata. Pharm Biol. 2011;49(1):94-100.
16. Majeed M, Nagabhushanam K, Natarajan S, Sarangbani, Vaidyanathan P, Majeed S, et al. Investigation of Acute, Sub-Acute, Chronic Oral Toxicity and Mutagenicity of Coleus forskohlii Briq. Hydroethanolic Extract, Standardized for 10% Forskolin in Experimental Animals. IJPR. 2015;5(1):219-38.

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