



Acute Oral Toxicity Study of Hydro-Methanolic Extract of *Abrus precatorius* L. Seeds in Wistar Rats.

Shazia Tabasum*¹, Swati Khare², Kirti Jain¹

¹Department of Botany, Govt. Science and Commerce College Benazir, Bhopal, M.P., India.

²Department of Botany, Govt. MLB Girls PG autonomous College, Bhopal, M.P., India.

*Corresponding author's E-mail: shaziatabasum49@gmail.com

Accepted on: 23-04-2016; Finalized on: 31-05-2016.

ABSTRACT

The present study was designed to ascertain the acute toxicity of crude Hydro-methanolic seed extract of *Abrus precatorius* L. *Abrus precatorius* L. commonly known as Ratti belongs to family Fabaceae. It is native to India but found in other tropical subtropical countries. Various parts of the plant are used for the treatment of a range of diseases and are used in Ayurvedic formulations. Acute toxicity study was carried out as per OECD (423) guidelines in Wistar albino rats weighing 185-238 g. The extract was dissolved in 0.1% carboxymethylcellulose (CMC) solution and suspension was administered orally at four different doses of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. The animals were observed almost constantly for behavioural changes and mortality during firstly for 4 hours and then every day for a period of two weeks. Besides other cage side observations, alterations regarding body weight, food and water intake were also kept under observation. There was no abnormality observed in any of the four experimental groups. Hence, acute toxicity test provides evidence that Hydro-methanolic extract of *Abrus precatorius* L. seeds is safe up to dose level of 2000mg/kg body weight.

Keywords: *Abrus precatorius*, Hydro-methanolic extract, acute oral toxicity, OECD (423) guidelines.

INTRODUCTION

Plants provide a wide variety of biochemical components useful to mankind. These substances can be extracted and used in the preparation of drug, or the plant itself can be used directly as a medication. Medicinal plant preparations are administered with the hope of promoting health and treating various diseases. A large section of people particularly in developing countries believe that all medicinal plants being natural are safe and free from harmful effects. However, the belief that traditional use of plants from ancient times ascertains their safety does not necessarily hold true. Medicinal plants which are being used, on large scale for the treatment of particular disease are reported to be having serious side effects. Scientific studies on efficacy and safety of some medicinal plants indicated that there are many phytochemicals that have toxic effects. All medicinal herbs contain many xenobiotic agents and the biotransformation products of these substances can be potentially harmful. The effects may arise instantly with obvious signs after the intake or in their prolonged use without any sign or symptom that can be severe and sometimes fatal¹.

Abrus precatorius L. of family Fabaceae is locally known as Ratti. It is a wild plant, native to India and is also found in other tropical and subtropical regions^{2,3}.

The plant is a slender, perennial, woody, twinning climber with herbaceous branches and pinnate leaves⁴. Flowers are borne in clusters and are purple pink or reddish in colour and also white in rare occasions⁵. Fruits are pods bearing characteristics red seeds with dark spot at the

base. Mature fruits split and turn backwards revealing 3-7 hard seeds. Plant is famous for its beautiful but toxic seeds^{6,7}. Seeds are rich in various phytochemical components like alkaloids, flavonoids, carbohydrates, proteins, steroids, triterpenoids etc^{8,9}. Toxicity of the seed is due to the presence of a toxic protein, Abrin⁴. The plant is used in traditional and folk medicines from centuries. Seeds are said to be emetic, antiphlogistic, tonic, purgative and aphrodisiac¹⁰. *Abrus precatorius* seeds are also reported to possess different pharmacological activities like antimicrobial¹¹, anticancer¹², nephroprotective¹³, antifertility^{4,14}, antibacterial¹⁵, antidiabetic¹⁶, antioxidant¹⁷, immunomodulatory⁸ etc. Taking into account the both ethnobotanical and pharmacological uses, the present study was carried out to record clinical observations and to detect the LD₅₀ of the crude Hydro-methanolic extract of the *Abrus precatorius* seeds by oral route in the case of acute toxicity in Wistar rats.

Toxicity is the basic science of poisons and a poison is any substance that produces adverse effects in a biological system. The objective of toxicity studies is to make available sufficient information for the assessment of toxicology properties of chemicals and commercial products and to find out whether a substance is safe or not. Toxicity is generally divided into four types which are Acute, Sub-acute, Chronic and Sub-chronic. Organization for Economic Co-operation and Development (OECD) pointed out Acute-toxicity as the adverse effect occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours^{18,19,20}. From regulatory point of view, the most



important aim of acute toxicity testing is to sort out the substances in accordance to their intrinsic toxicity as mainly required for labelling, packaging and classification of hazardous substances. This requirement saves the health of people from harm by regulating the exposure to potentially dangerous substances. Classification of chemical substances is done on the basis of the medium lethal dose LD₅₀ value which is the statistically derived single dose of a test substance expected to cause death in 50 percent of the animals in an experimental group.

MATERIALS AND METHODS

Collection and authentication of plant material

The seeds used in the study were purchased from the local market Bhopal Madhya Pradesh, India and were authenticated by Dr. Zia-Ul-Hassan, Professor and HOD, Department of Botany, Safia Science College, Bhopal. A voucher specimen (sample No. 520/Bot/Saifia/2015) was deposited in the Department for future reference.

Extraction

The seeds collected were cleaned, shade dried and reduced to coarse powder with the help of an electrical grinder. The coarsely powdered seeds were extracted with petroleum ether and the marc remaining was extracted successively by cold maceration process using 70% methanol for 7 days respectively. The extracts were then filtered through Muslin cloth and finally with Wattman No.1 filter paper, concentrated on rotary evaporator at low temperature and reduced pressure. Both were preserved in air tight container, labelled and kept at 2-4 °C in refrigerator for further use. Present study was done on crude hydro-methanolic extract. Test sample was prepared as a suspension of weighed amount of extract in 0.1% CMC (carboxymethylcellulose) solution.

Ethical Approval

The use of animals for the present study was reviewed and approved with approval reference no. PBRI/IAEC/PN-412 by the Institutional Animal Ethical Committee of Pinnacle Biomedical Research Institute Bhopal, Madhya Pradesh (Reg. no. 1283/PO/c/09/CPCSEA) and maintained as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India.

Animals

Adult male Wistar albino rats weighing 185-238 g were selected as model for the present study. Animals were procured from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal. These were housed in polypropylene cages maintained under standard laboratory conditions (24±2 °C; 50±5% humidity; 12 hours

light/12 hours dark cycle) with standard diet and water *ad libitum* and were acclimatized to the laboratory conditions for a week before starting the experiment. Paddy husk was used as bedding and was changed twice in a week.

Acute toxicity studies

The acute toxicity study was performed for methanol (70%) extract of *Abrus precatorius* L. seeds according to OECD (423) guidelines. A total of 30 rats (6 rats per group), were randomly selected and marked for individual identification. The test groups included a control group (normal saline) and four other treatment groups with dosages at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. Individual doses were calculated on the basis of animal weight. The treatment groups were fasted overnight prior to drug administration. The animals were observed almost constantly for behavioural changes, mortality and appearance during firstly for first 4 hours, periodically during the 24 hours and then every day for a period of two weeks. Measured amount of food and water were given to each cage daily. Alterations regarding body weight, food and water intake were also kept under observation besides the cage side observations.

RESULTS AND DISCUSSION

Acute toxicity studies are designed to determine the dose that will cause mortality or severe toxicological effects when given once or over a few administrations in 24 hours²¹. Mortality, behavioural signs, body weight and food consumption are very sensitive indicators to assess the acute toxicity (LD₅₀ of any test substance). The body weight is also an important factor to monitor the health of animals¹⁹. The results of the acute oral toxicity studies showed that oral administration of the hydro-methanolic seed extract of *Abrus precatorius* L. to rats up to 2000 mg/kg body weight resulted in no death of any test animal during the observation period of 14 days. All animals survived and gained bodyweight over the period. There was no significant loss of fur and skin lesions.

Animals did not show any sign of aggression or abnormal behaviour during handling. Eyes appeared clear and normal, and there was no lethargy, convulsions, tremors, salivation, or coma in experimental animals which are signs associated with oral toxicity.

All other side cage observations were also found to be normal. Results of acute toxicity study are presented in Table 1.



Table 1: General appearance and behavioural observations of acute oral toxicity study for control and treated groups

Observations	Control Group	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Food intake	Normal	Normal	Normal	Normal	Normal
Body weight	Normal	No change	No change	No change	No change
Digestion	Normal	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Normal	Normal
Changes in skin and fur	No effect	No effect	No effect	No effect	No effect
Sub-cutaneous swellings	Not observed	Not observed	Not observed	Not observed	Not observed
Eyes and mucous membranes changes	Not observed	Not observed	Not observed	Not observed	Not observed
Respiratory abnormalities	Not present	Not present	Not present	Not present	Not present
Colour and consistency of the faeces	Normal	Normal	Normal	Normal	Normal
Urination	Normal	No effect	No effect	No effect	No effect
Abdominal distension	Not present	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present	Not present
Tremors	Not observed	Not observed	Not observed	Not observed	Not observed
Convulsions	Not observed	Not observed	Not observed	Not observed	Not observed
Lethargy	Not present	Not present	Not present	Not present	Not present
Diarrhoea	Not present	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive	Alive

CONCLUSION

Acute toxicity study is an important test in the toxicological investigation of unknown materials. LD₅₀ although not regarded as a biological, is the index of the acute toxicity. Such study also serves to provide information regarding the doses that should be selected for subsequent studies. As no mortality and adverse effects were observed in the experimental animals during the period of acute oral toxicity study, it can be concluded that LD₅₀ of the crude Hyrdo-methanolic seed extract of *Abrus precatorius* L. is more than 2000 mg/kg body weight. Hence the study provides evidence that extract is safe up to dose level of 2000 mg/kg body weight.

Acknowledgement: The authors would like to acknowledge for the Financial Support from University Grants Commission (UGC), New Delhi under MANF to carry out this research work. The facilities provided by the Pinnacle Biomedical Research Institute Bhopal, Madhya Pradesh also need special appreciation for carrying out this work.

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Source of Support: Nil, Conflict of Interest: None.