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An Acute Oral Toxicity Study of *Gnidia glauca (Fresen.) Gilg*. in Albino Rats as per OECD Guideline 425

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Abstract: Toxicology may be defined as the study of harmful / poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings. The present study was aimed to determine LD_{50} and to establish the safety of methanolic extract of *Gnidia glauca (Thymelaeceae)* barks and roots by acute oral toxicity study in female rats as per OECD guideline 425. Rats were sequentially administered both the extracts in single dosages of 175, 550, and 2000 mg/kg of body weight. All the animals were individually studied for mortality, wellness parameters and body weight for 14 days. No mortality and no significant changes were observed in body weight and wellness parameters at 175, 550 and 2000 mg/kg body wt. doses, which reveal the safety of these extracts in the doses up to 2000 mg/kg body weight. Conclusively, LD_{50} value of *Gnidia glauca* bark and root extracts was found to be more than 2000 mg/kg body weight.

Keywords: Acute Oral Toxicity, *Gnidia glauca,* OECD Guideline 425.

Introduction

'Toxicology' traditionally known as the 'science of poisons' began with early cave dwellers who recognized poisonous plants and animals and used their extracts for hunting or warfare. Later, with time, it included the practice of determining the safety of a particular compound. Comprehensively, in its present form, toxicology may be defined as the study of harmful / poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings[1]. After gaining relevant information on the harmful effects of a compound, the levels for its safe usage or the degree of its safety is established, this is known as its (compound) Biosafety level.

Gnidia glauca (Fresen.) Gilg. is a large shrub growing about 3 meters in height belonging to the family *Thymelaeceae*. The bark is brownish, irregularly scaly when mature. It is widely distributed in India, Srilanka and Africa. The genus *Gnidia* has been found to possess a wide variety of traditional phytomedicinal and agrochemical applications. It is used as traditional African medicine for cancers, sore throat, abdominal pain, wounds, burns and snake bites. It is also used as molluscicidal, insecticidal, piscicidal and as arrow poisons[2, 3, 4]. Also, several *Gnidia* species possess remarkable antineoplastic activity [5].

The present study was aimed to determine LD_{50} and to establish the safety of methanolic extract of *Gnidia glauca* barks and roots by acute oral toxicity study in female rats as per Organization for Economic Cooperation and Development (OECD) guideline 425.

The test procedure described in this guideline uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity. Also, this method is of value in minimizing the number of animals

required to estimate the acute oral toxicity of a chemical. In addition to the estimation of LD_{50} and confidence intervals, the test allows the observation of signs of toxicity.

Material and Method

Test material

Gnidia glauca specimen was identified and authenticated (Voucher No. GNGKHMA1) by Botanical Survey of India, Pune. The plant material was collected from Sinhgad fort, Pune. The bark and roots of the plant were air dried in shadow for 10 days.

Animals

Healthy young adult nulliparous and non-pregnant female rats, weighing 120-140 g (10-12 weeks old) at the start of the experiment, were procured from National Institute of Biosciences, Pune (Reg. No. 1091 /ABC/ 01 / CPCSEA). The present study was approved by Institutional Animal Ethics Committee (Reg. No. 1249/AC/09/CPCSEA) of Rajarshi Shahu College of Pharmacy and Research, Pune.

Female rats were selected because literature surveys of conventional LD_{50} tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive[6].

The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 25 ^{0}C (± 3 ^{0}C) and 45-55% respectively with a light-dark cycle of 12 h (light from 06:00 AM to 06:00 PM). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow (Amrut Laboratory) and unlimited supply of filtered drinking water.

Preparation of the extracts

Dried roots and barks of *Gnidia glauca* were powdered and passed through sieve #10. 150 grams of the sieved powder was weighed accurately and subjected to methanolic extraction using soxhlet extractor for 4 hours at 40^{0}C to 42^{0}C. The extract obtained was filtered, concentrated and dried in a hot air oven. Percentage yield of root and bark extracts of *Gnidia glauca* was found to be 5.08 and 8.23 respectively(Table 1).

Methodology

Paragraph 22 of OECD Guideline 425 suggests two types of acute oral toxicity tests i.e. limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test should be performed. Because the literature survey of this herb indicates about its potential toxicity, therefore, the main test was performed.

Procedure for main test

Prior to dosing, animals were fasted overnight before being weighed, and the extracts were orally administered in a single dose (Table 2). The volume given was not more than 2 ml/100 gm body weight (body wt.). Following the period of fasting, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. After the extract was administered, food was withheld for a further 3-4 hours. Control animals were administered with calculated amount of water for injection.

Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550, and 2000 (or 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000 for specific regulatory needs). Because no estimate of the substance's lethality was available, dosing was initiated at 175 mg/kg till 2000 mg/kg as recommended in OECD Guidelines 425[7].

Table 1: Percentage yield of root and bark extracts of *Gnidia glauca*

Sr. No.	Type of Extract	Percentage yield
1	Root	5.08
2	Bark	8.23

Table 2: Dose and frequency of administration of extracts for main test

Agent	Diluent	Route of Administration	Frequency of Administration
Bark extract	Water for injection	Oral route	Single dose
Root extract	Water for injection	Oral route	Single dose

Table 3: Observations for the main test at 2,000 mg/kg body wt.

Observations	30 min			4 hrs			24 hrs			48 hrs			1 wk			2 wks		
	C	R	B	C	R	B	C	R	B	C	R	B	C	R	B	C	R	B
Skin & Fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convulsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

C – Control, **R** – Root extract, **B** – Bark extract, **N** – Normal

Observations

Wellness parameters Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Changes in wellness parameters were compared with that of control animals (Table 3).

Body weight Individual weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Changes in the weight of individual animals were calculated and compared with that of the control animals.

Statistical Analysis

Changes in body weights were expressed as Mean (M) ± Standard Deviation (SD) and their statistical significance was calculated using t-test. LD_{50} was determined by using Acute Oral Toxicity (Guideline 425) Statistical Program (Version: 1.0).

Results

Mortality No mortality was observed at 175, 550 and 2000 mg/kg body wt. doses.

Wellness parameters and Body weight analysis

No significant changes were observed in body weight and wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated as well as the control animals were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animal.

Although, the body weights of all the rats were decreased after the oral administration of extracts, but, the changes of the body weights were found to be statistically insignificant (Table 4). Insignificant decrease in body weight of test animals indicates that the administration of the extracts does not affect the growth of the animals.

Table 4: Effect of extracts on the body weight of rats at 2,000 mg/kg dose after 14 days

Group	Treatment	Body weight (g)		Calculated 't' value	Remarks
		Before treatment M1±SD1	After treatment M2±SD2		
Control	Water for injection	125.33 ± 3.88	120.66 ± 2.54	t=1.919	NS
Treated	2,000 mg/kg Bark extract	131.66 ± 5.95	124.66 ± 5.74	t=2.394	NS
	2,000 mg/kg Root extract	129.33 ± 2.54	122.66 ± 2.64	t=2.139	NS

N = 3; M1, SD1 and M2, SD2 are mean weights and standard deviations before and after treatment respectively; NS = Not significant

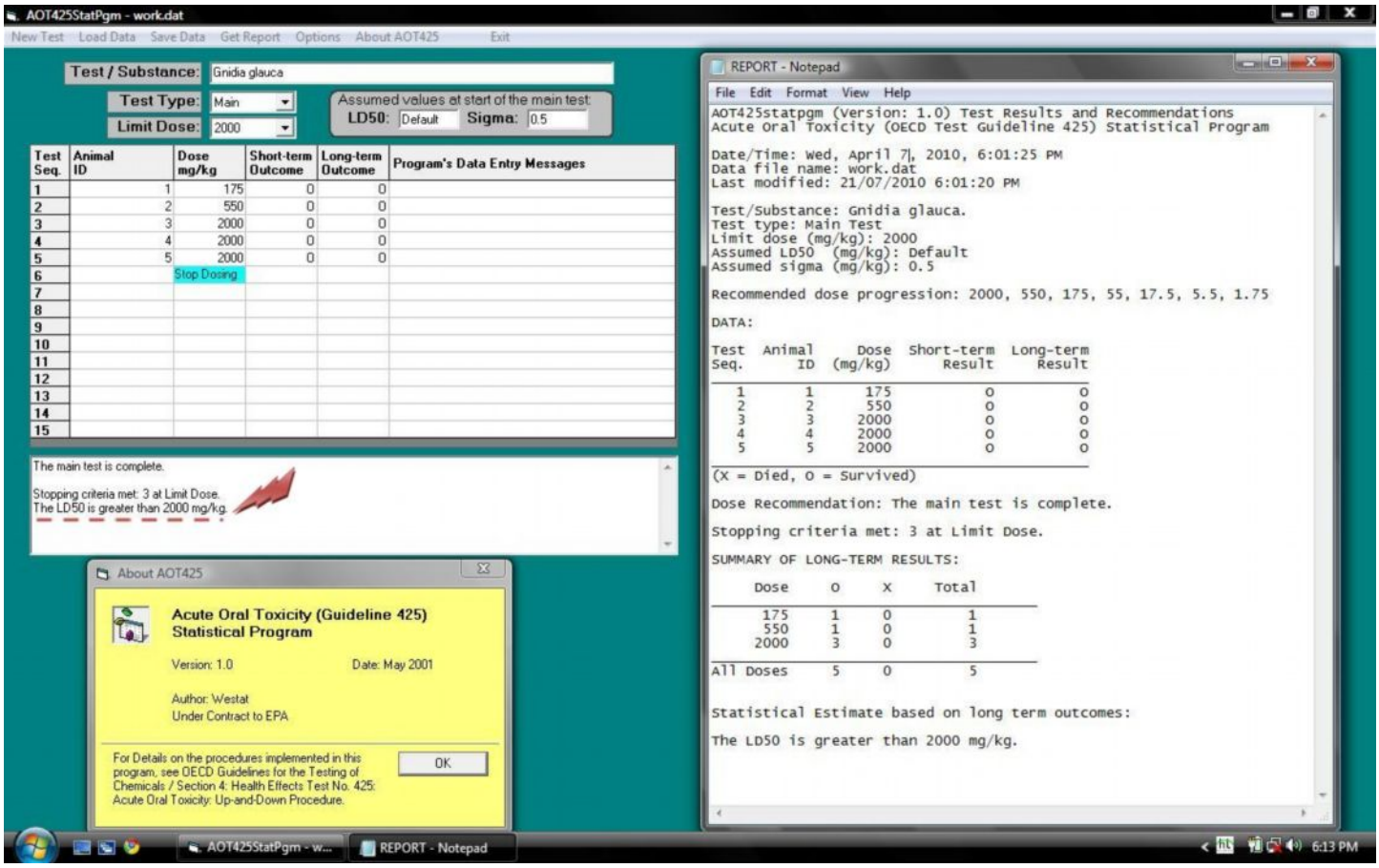

Fig.1. Acute Oral Toxicity (Guideline 425) Statistical Program (Version: 1.0

LD_{50} Value

As per calculations from Acute Oral Toxicity (Guideline 425) Statistical Program (Version: 1.0)[8], the LD_{50} value of *Gnidia glauca* bark and root extracts was found to be more than 2000 mg/kg body weight (Figure 1).

Conclusion

Methanolic extract of *Gnidia glauca* barks and roots did not exhibit acute toxicity when given orally at concentration of 2,000 mg/kg body weight. Also, the normalcy of or insignificant changes in wellness parameters and body weights reveal the safety of these extracts in the doses up to 2000 mg/kg body weight.

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