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ACUTE TOXICITY STUDY OF PROSOPIS CINERARIA PODS

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Abstract:

The medicinal plants need to be evaluated for their toxicity in animals and humans. The aim of this study was to test the acute toxicity of *Prosopis Cineraria Linn.*pods . The acute toxicity study was studied on Albino Swiss mice with a dose of (0.7g/kg) body weight orally. The single administration exposure of Prosopis Cineraria pods powder in the form of boiled aqueous slurry on Albino Swiss mice was carried out. The exposure route was oral with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. No mortality and no significant changes were observed in body weight and wellness parameters.Based on the result of the study *Prosopis Cineraria Linn.*pods can be used for any future *in vivo* and clinical studies.

Keywords: Prosopis cineraria pods, acute toxicity study, Albino Swiss mice ,boiled aqueous extract

Introduction

Toxicology is the study of the adverse effects of chemicals on living organisms. The toxicologist is specially trained to examine the nature of these adverse effects and to assess the probability of their occurrence. The variety of potential adverse effects and the diversity of chemicals present in environment combine to make toxicology a very broad science[1]. A poison derived from a biological source is identified as a toxin.

A chemical agent does not produce toxic effects in biological system unless that agent or its metabolic breakdown products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce a toxic manifestation. The major factors that influence toxicity are the route of administration, the duration and the frequency of exposure to the chemical agents[2].

Animal species commonly utilized in regulatory toxicology studies[3]

Study type	**Primary species**	**Common alternative species**
Acute toxicity	Rat, Mouse	

Multidose toxicity	Rodent(rat), Non rodent(dog)	Mouse, Monkey
Carcinogenicity	Rat, Mouse	
Invivo mutageniciy	Mouse	
Development and Reproduction	Rat, Rabbit	Mouse, Hamster, Monkey
Neurotoxicology	Rat	Mouse
Immunotoxicology or sensitization	Mouse,Guinea pig	Rat

The “Queen of the Desert”, khejri (Prosopis cineraria (L) Druce) belongs to the subfamily

Mimosoidae of Leguminosae family, and has an important place in the economy of the Indian desert. Of the 131 known species of genus Prosopis, only P. cineraria is endemic to the Indian sub-continent. P. cineraria attain a height of 10-15 metres and a bole of 20-30 cm in diameter in 30-40 years.The foliage constitutes a 5-8 m wide umbrella-shaped crown with a cool shade. Owing to the deep root system, a monolayered canopy and the ability to fix atmospheric nitrogen, P. cineraria is compatible with agri horticultural crops. The trees not only boost the growth and productivity of companion plants, but also provide fuel, fodder, food, small timber, medicines, gum and tannin.Its foliage is a nutritive fodder for animals and the wood is of good quality for domestic fuel purpose[4].

Prosopis cineraria being localized in Indian sub-continent, not much research work has been done on the pods .This represents clear under-utilization of this valuable resource.The nutritional value of the pods in terms of per 100g/dry weight has been given as Protein:18.2g, Fat:3.5 g, Minerals:6.2 g, Carbohydrates:58.5 g, Energy:338.3 kcal.

Pod contents of 8 provenaces (15.5°to 29.1°N) were collected, which showed significant

variation such as 8 - 13% protein, 40 - 55% carbohydrates, 8 -15% sugar and 9 - 12% crudefibre and 17-31% sucrose .[5]

Three phytoconstituents methyl nonacosanoate, methyl octadec-9-enoate and β -sitosterol were isolated and characterized from the stems of Prosopis cineraria. According to survey done on food habits of diabetic people in Rajasthan it was found that hypoglycemic foods such as green leafy vegetables and Prosopis cineraria pods, aloe and mint combination are consumed by most of the local people at Ratangarh to normalize blood glucose levels [6].

Objective of the study :In the present work acute toxicity study of ***Prosopis Cineraria Linn.*** pods was conducted by using Revised Draft Guideline 420, Acute Oral Toxicity-Fixed Dose Procedure as per the recommendations of the OECD Guidelines for the Testing of Chemicals.The main objective of this study was to find out whether the aqueous solution of the Prosopis Cineraria pods show any toxic effects when administered as a test material.

Materials and methods:Prosopis cineraria pods were collected from,Jodhpur,Rajasthan and authenticated at B.S.I.,Pune(BSI/WC/Tech./76).Air dried pods were finely powered and stored appropriately.

Justification for selection of species and strain **:** Albino swiss mice is a suitable model for acute toxicity study and is recommended in the test method. The Albino swiss because of its availability and of the availability and of the existing historical database for comparative evaluation, which is believed to be of value in predicting the likely toxicity of the test material in humans[7].

The acute toxicity studies of ***Prosopis Cineraria Linn.*** pods were conducted at Animal Testing Unit (CPCSEA/315) Ramnarain Ruia College Matunga, Mumbai-400019

Route of administration: Intragastic by gavage No.16 using a metal cannula attached to

a graduated syringe.

Justification for route: Oral route is the intended therapeutic route of test material for

oral administration in humans.

Vehicle for administration*:* Water

Volume of dose:1cm^3 per animal

Environmental conditions*:* The animal room was maintained at a temperature of 28-30°C and relative humidity of 65-70%.The rate of air exchange was continuous through a system of inlet fresh air exhaust and outlet bad air exhaust.The lighting was controlled by a timer to give a cycle of 12 hours continuous light and 12 hours continuous darkness.

Accommodation: Animals were housed in solid floor polypropylene cages with rice husk bedding and facilities for food and water.

Diet: Mice feed pellets supplied by Amrut Laboratory Animal Feed, Maharashtra Chakan Oil Mills Ltd.

Water: Potable water was provided in glass bottles with stainless steel caps.

Observation period*:* 14 days (including holidays)

Clinical signs*:* Animals were observed daily for signs of toxicity throughout the study period

Body weights: Individual animal body weights were recorded daily throughout the study period.

Food and water intake*:* Animals were supplied with known amounts of food and water and their daily food and water intake was recorded.

Cage side observations*:* Daily cage side observations for condition of fur and skin, subcutaneous swelling, abdominal distension, eye dullness/pupil diameter,ptosis(drooping of eyelids),colour and consistency of faeces,wetness or soiling of the perineum, condition of teeth, breathing abnormalities and gait were recorded through out the study period.

Test System:Two Female Albino Swiss mice were acquired through Haffikne Bio-pharma. Corp. Ltd. Mumbai.Their age at the start of the study was 8-10 weeks and their body weight was in the range of 30-35 g.The identification was done by cage tag and marking on the inner surface of the ear.The study period was of two weeks.

Preparation of sample dose: 60 mg of pod powder was weighed .Pod slurry was made in $10cm^3$ water and the slurry was boiled for 10 minutes and the volume was brought down to $2cm^3$. One cm^3 (20 mg of boiled extract) of the supernatant liquid was administered to the animal (0.7 g/kg).

***Dose regimen*:**

Number of animals	Sex	Concentration of dose in g/kg per animal	Total volume administered in cm^3 per animal
2	Female	0.7g/kg	$1cm^3$

A control group was also maintained comprising of 2 females. The animals of control group were administered distilled water as sham treatment. Animals were subjected to fasting, food but not water was withheld 3-4 hours prior to dosing[9].Following the period of fasting, the animals were weighed and the test substance administered. After the substance had been administered, food was withheld for a further period of 1-2 hours. Only oral route was used for administration of the test material.Administration of the test material was done only once on the day of dosing after physical restriction of the animals.

The following parameters were observed .

Table A

Sr. No.	Parameters	Observations
1	Condition of fur	Normal

2	Skin	Normal
3	Subcutaneous swelling	Nil
4	Abdominal distension	Nil
5	Eyes-dullness	Nil
6	Opacity of eyes	Nil
7	Pupil diameter	Normal
8	Colour and consistency of faeces	Normal
9	Wetness or soiling of the perineum	Nil
10	Condition of teeth	Normal
11	Breathing abnormalities	Nil
12	Ptosis	Nil
13	Gait	Normal

Cage side observations

Body weight changes*:* A change in body weight is an important factor to monitor the health of the animal. Loss of body weight is frequently the first indication of the onset of the adverse effect. The dose at which the body weight loss is 10% or more is considered to be toxic dose irrespective of whether or not; it is accompanied by any other changes.

Animal from the dose group did not show decrease in body weight, greater than 10%.Table B shows variation in the weight changes of the animals.

Food and Water consumption*:* Food consumption can indicate an adverse effect of a drug at an early stage. Measurement of water consumption is carried out in studies of diuretic compounds that are known or expected to affect the kidneys. Animals from the dose group did not show significant changes in consumption of food and water. Tables C and D show variation in food and water consumption respectively.

Mortality*:* It is a main criterion in accessing the acute toxicity (LD50) of any drug. There was no mortality recorded even at the given dose of 0.7 g/kg body weight as shown in table E

OBSERVATIONS AND RESULTS

TABLE B

Daily body weight record (in grams)

Dose Group (g/kg)	SEX	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
2.00	F	33.65	33.75	33.35	33.25	33.30	33.60	33.54	33.70	33.40	33.55	33.79	33.88	33.70	33.75

TABLE C

Daily food intake record (in grams)

Dose Group (g/kg)	SEX	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
2.00	F	9	8.5	8.5	8	8	7.5	7.5	8	8	8	8	8.5	8.5	9

TABLE D

Daily water intake record (in cm^3)

Dose Group	SEX	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14

(g/kg)															
2.00	F	12	12.5	12.5	12.5	12	11	11	11	11	10	10	11.5	11.5	11.5

TABLE E

Mortality record

Animal No.	SEX	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
1	F	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	F	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Conclusion

From the results of the study, it was observed that there were no significant changes in body weight, food and water consumption by the animal from the dose group. There was no mortality recorded at the given dose of 0.7g/kg body weight, which indicates that the boiled aqueous extract of pods of Prosopis Cineraria has no significant toxic effect in mice.This study provides very important data on the acute toxicity profile of the boiled aqueous extract of pods of Prosopis Cineraria that should be very useful for any future *in vivo* and clinical studies.

Conflict of interest statement: The authors report no conflict of interest.

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