

Acute Toxicity Study for *Argemone mexicana* L. Root Bark Powder

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Summary

The aim of the present work is to carry out acute toxicity study of root bark powder of *Argemone mexicana* L as it is one of the potent liver tonic. The exposure of this powder in the form of aqueous slurry on Swiss mice was carried out and the exposure route was oral single administration with water. The observations of changes in body weight, food and water intake as well as cage side observations were reported. The root bark powder was found to be nontoxic.

Keywords: Hepatocurative, acute toxicity, root bark.

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Introduction

The use of natural medicines is increasing and is a persistent aspect of present day health care. More and more people are using herbal medicines as OTC products. There is a belief of many consumers that naturalness is a guarantee of harmlessness, but this is not true. Many traditionally used medicines can produce dangerous and sometimes even lethal poisoning. The world health organization (WHO) is fully aware of the importance of herbal medicines to the health of many people throughout the world. Thus herbal medicines have been recognized as a valuable and readily available resource of primary health care and WHO have endorsed their safe and effective use. A few herbal medicines have withstood scientific testing but others are simply used for traditional reasons to protect, restore and improve health. The WHO has set guidelines for toxicity studies of herbal medicines. It supports appropriate usage of herbal medicines and encourages the remedies, which are proved to be safe and effective. The route for administration for subacute, subchronic and chronic toxicity can be any one of the above stated routes, but most often it is by oral route.

Toxic Dose

One could define a '**poison**' as any agent capable of producing a deleterious response in a biological system, seriously injuring function or producing death. Among chemicals there is a wide spectrum of doses needed to produce deleterious effects, serious injury or death. Some chemicals, which produce death in microgram doses, are extremely poisonous, while others may be relatively harmless after doses in excess of several grams.

A chemical agent does not produce toxic effects in biological system unless that agent or its metabolic breakdown (biotransformation) 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 which influence toxicity are the route of administration, the duration and the frequency of exposure to the chemical agent. Toxicologists usually divide the exposure of animals into Acute toxicity, Subacute toxicity, Subchronic toxicity, Chronic toxicity.

Limit Test

All chemicals can produce toxicity under some experimental conditions, for instance, if a sufficiently large dose is administered. It is therefore, misleading to conduct acute toxicity studies at unreasonably high dose levels for the sake of demonstrating lethality and / or toxicity, which may be irrelevant to the use of compound itself. An extremely high dose of a practically nontoxic compound for example, can cause gastrointestinal blockage, which in turn can result in gastrointestinal tract dysfunction. Toxicity in such a case is not related to the intrinsic characteristic of the test substance, since effect manifested is a direct result of the physical blockage caused by the biologically inert substance. There must be a point, however, at which an investigator may conclude that a test substance is practically nontoxic or nonlethal after an acute exposure. This test limit for oral toxicity generally is considered to be 5.0 g / Kg body weight. If no mortality is observed at this dose level, a higher dose level generally is not necessary.

The safety of all medicinal products is of the utmost importance. All applications for new medicines undergo extensive evaluation of their risk to-benefit ratio and, once granted, products are closely monitored for the occurrence of adverse effects. Rabbits fed with edible linguda (*Diplozium esculentum*) a low level fern, exhibited systemic toxicity and died following 11 days post feeding. The safety of herbal remedies is of particular importance as most of these products are self-prescribed, available as OTC (over the counter) products and are used to treat minor and often chronic conditions. The trend in the usage of plants as medicines traditionally has enabled one to record the acute and obvious signs of toxicity of the plants, which can be well recognized, and hence their use is avoided.

Acute Toxicity Study of *A. mexicana* Linn. Root Bark Powder

An acute toxicity study was carried out on *Argemone mexicana* Linn. root bark powder using mice as the experimental model. The study was carried out to assess the acute toxicity of the plant root bark slurry on oral administration. Study protocol is given below in table 1.

Table 1
Study Protocol

Name of the study	Acute toxicity study
Test material	<i>A. mexicana</i> L. root bark powder (Slurry)
Animal model	Albino Swiss Mice
Animals procured from	Raj Biotech (INDIA) Ltd., Pune
Sex	Male and Female
Weight range of animals	Between 35 to 55 g
No. of dose groups	Three groups
Animals per group	3 males and 3 females
Route of administration	Intragastric administration with the help of gavage No. 16
Dose volume	2.0 ml per animal
Vehicle for administration	Distilled water
No. of administrations	Single
Concentration of dose	3.0, 5.0, and 7.0 gm/Kg body weight
Study duration	Acclimatization for 14 days, one day drug administration and 14 days observation period including holidays
Parameters observed	Cage side observations, daily food and water intake, daily body weight and daily mortality record etc

Animal Maintenance

The animals were housed in polyurethane cages. The cages were provided with rice husk bedding and were cleaned daily. The animals were provided with drinking water ad libitum and were fed on commercially available Mice feed supplied by AMRUT FEED. The specifications of the feed are listed below in table 2.

Table 2

Name	Percentage
Crude Protein	20 - 21 % minimum
Ether Extractive	04 - 05 % minimum
Crude Fiber	04 % maximum
Ash	08 % maximum
Calcium	1.2%
Phosphorus	0.6 % minimum
NFE	54 %
ME Kcal/Kg	3600
Pallet Size	12 mm

The feed was enriched with stabilized vitamins such as Vit. A and D₃, Vit. B₁₂, Thiamine, Riboflavin, Folic acid and supplemented with all minerals and microelements. Measured quantities of water and feed were supplied daily in each cage. The consumption of water and food was estimated from the amount of water remaining in feeding bottles and from the amount of feed remaining in the feed hopper.

Cage Side Observations

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from the stage of dosing to the end of the study. Cage-side observations included daily recording of condition of the fur; damaged areas of skin; subcutaneous swellings or lumps (the size, shape and consistency), areas of tenderness, abdominal distension, eyes - for dullness, discharges, opacities, pupil diameter, ptosis (drooping of upper eyelid), the colour and consistency of the faeces, wetness or soiling of the perimenum, condition of teeth, breathing abnormalities, gait, etc. Any changes or abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behavior before and after the administration of an oral dose of *Argemone mexicana* Linn. root bark powder as slurry following table 3 shows the dosage regime. Table 4 shows the observations for the parameters studied. Table 5 shows the mortality record.

Table 3
Doses Regime

Sr. No.	Sex	Dose gm/Kg Body Wt.	No. of animals used	Total Vol. administered in cm3
1	Male	3.00	03	2.00
2	Female	3.00	03	2.00
3	Male	5.00	03	2.00
4	Female	5.00	03	2.00
5	Male	7.00	03	2.00
6	Female	7.00	03	2.00

Table 4
Cage Side Observations for all animals

Sr. No.	Parameters	Cage Side Observations
1	Condition of the fur	Normal
2	Skin	Normal
3	Subcutaneous swellings	Nil
4	Abdominal distension	Nil
5	Eyes -dullness	Nil
6	Eyes - opacities	Nil
7	Pupil diameter	Normal
8	Ptosis	Nil
9	Colour & consistency of the faeces	Normal
10	Wetness or soiling of the perimenum	Nil
11	Condition of teeth	Normal
12	Breathing abnormalities	Nil
13	Gait	Normal

Table 5
Mortality Record

Group Gm/Kg	3	3	5	5	7	7
Sex	Male	Female	Male	Female	Male	Female
Hr. 1	-	-	-	-	-	-
Hr. 2	-	-	-	-	-	-
Hr. 3	-	-	-	-	-	-
Hr. 4	-	-	-	-	-	-
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	-	-	-	-	-	-
Mortality	0/3	0/3	0/3	0/3	0/3	0/3

Body Weight Changes

Body weight is an important factor to monitor the health of an animal. Loss in body weight is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10 % or more reduction in the body weight, is considered to be a toxic dose. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes. All the animals from treated groups did not show any significant decrease in body weights for all the 14 days as compared with the 0 day values, indicating no signs of toxicity against root bark slurry of *Argemone mexicana*. The variation in body weight changes of males and females and the data is given in Table 6.

Food and Water Consumption

There was no significant change in food and water intake of the test animals at all dose levels. The data for food and water consumption is given in Tables 7 and 8 respectively.

Table 6
Daily Body Weight Record in Grams.

Group gm/Kg	3	3	5	5	7	7
Sex	Male	Female	Male	Female	Male	Female
Day 0	51	34	43	34	54	47
Day 1	50	33	43	34	54	46
Day 2	52	33	42	35	55	45
Day 3	52	34	43	36	54	45
Day 4	51	35	44	36	55	46
Day 5	54	35	45	37	55	46
Day 6	54	34	45	36	56	46
Day 7	55	35	45	37	56	47
Day 8	55	36	46	37	56	47
Day 9	54	34	45	38	57	47
Day 10	54	36	46	38	56	46
Day 11	55	35	46	38	57	47
Day 12	55	36	46	37	57	47
Day 13	56	36	45	38	58	48
Day 14	55	36	46	38	58	48

(All the values expressed as mean of three animals in each group)

Table 7
Daily Food Intake Record in Grams.

(All the values expressed as mean of three animals in each group)

Group Gm/Kg	3	3	5	5	7	7
Sex	Male	Female	Male	Female	Male	Female
Day 0	14	11	15	14	20	14
Day 1	14	12	15	14	20	15
Day 2	15	11	14	15	19	14
Day 3	13	11	15	15	20	15
Day 4	15	10	15	14	20	15
Day 5	15	11	16	14	21	14
Day 6	16	12	16	14	21	14
Day 7	15	12	16	14	21	14
Day 8	16	11	16	14	22	14
Day 9	17	11	16	14	21	15
Day 10	17	12	17	15	22	14
Day 11	17	12	17	14	22	14
Day 12	18	12	18	14	22	15
Day 13	18	11	18	14	22	15
Day 14	19	11	18	14	23	13

Table 8
Daily Water Intake Record in ml.
 (All the values expressed as mean of three animals in each group)

Group gm/Kg	3	3	5	5	7	7
Sex	Male	Female	Male	Female	Male	Female
Day 0	15	11	15	14	20	13
Day 1	15	11	15	14	20	13
Day 2	14	12	14	13	19	14
Day 3	14	12	14	13	21	14
Day 4	15	12	14	15	21	14
Day 5	16	13	13	15	21	14
Day 6	16	12	15	14	20	13
Day 7	16	12	15	14	22	11
Day 8	17	12	16	14	22	12
Day 9	17	11	15	14	22	12
Day 10	17	12	16	13	23	12
Day 11	18	11	16	13	21	12
Day 12	18	12	17	14	23	13
Day 13	19	11	17	13	22	13
Day 14	19	11	18	14	23	12

Mortality

Mortality is the main criteria in assessing the acute toxicity (LD₅₀) of any drug. There was no mortality recorded even at the highest dose level i.e. 7.0 gm / Kg. body weight.

Conclusion

From the results of this study, it is observed that there is no change in body weight, food and water consumption by the animals from all dose groups (3.00 gm/Kg body weight to 7.0 gm/Kg body weight), There was no mortality recorded even at the highest dose level i.e. 7.0 gm / Kg body weight, which proves that the root bark powder of this plant has no significant toxic effect in mice.

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