

EVALUATION OF TOXICITY STUDY OF *LEUCAS ASPERA*

Shirish S. Pingale

P.G. Department of Chemistry, Gramonnati Mandal's Arts, Com. & Sci. College, Narayangaon, Pune 410504 (Affiliated to S. P. Pune University), India.

ABSTRACT The aim of the present research work is to carry out acute toxicity study of powder of *Leucas aspera* leaves. The exposure of powder of *Leucas aspera* leaves on Swiss mice was carried out and the exposure route was oral single administration with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. All these observations indicate that powder of *Leucas aspera* leaves found to be nontoxic.

KEYWORDS : Acute Toxicity, Powder Of *Leucas Aspera* Leaves

INTRODUCTION

The use of natural medicines is increasing and is a persistent aspect of present health care system. There is a belief of many consumers that naturalness is a guarantee of harmlessness, but this is not true. Some traditionally used medicines can produce dangerous and sometimes even lethal poisoning. The world health organization 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 sub-acute, sub-chronic and chronic toxicity can be any one of the above stated routes, but most often it is by oral route [1, 2, 3].

Toxic Dose

Poison is 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, Sub-chronic toxicity, Chronic toxicity [4].

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 5ml/Kg body weight. If no mortality is observed at this dose level, a higher dose level generally is not necessary [5]. 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. 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.

MATERIALS AND METHODS**Acute Toxicity Study fresh juice of *Leucas Aspera* leaves**

An acute toxicity study of powder of *Leucas aspera* leaves was carried out by using mice as the experimental model. The fully grown yellow fresh leaves of *Leucas Aspera* were collected from Awsari Forest Park, Ambegaon, Pune. These leaves were dried in shade on filter paper and kept in oven at 40 degree Celsius for 12 hours for complete drying. The dried leaves were crushed by electric and sieved to mesh size 80. The leaves powder is preserved in plastic airtight container with proper labeling. This powder of *Leucas aspera* leaves was used for toxicity study. The aim of this work was to assess the acute toxicity of powder of *Leucas aspera* leaves on oral administration. Study protocol is given below in table 1.

Table 1: Study Protocol

Name of the study	Acute toxicity study
Test material	powder of <i>Leucas aspera</i> leaves
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	2 males and 2 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	2.0, 4.0, 6.0, 8.0 and 10gm/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 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.

RESULTS AND DISCUSSION**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 color and consistency of the faeces, wetness or soiling of the perimenium, 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 powder of *Leucas aspera* leaves as distilled water slurry. Following table 2 shows the dosage regime. Table 3 shows the observations for the parameters studied. Table 4 shows the mortality record.

Table 2: Doses Regime

Sr. No.	Sex	Dose gm/Kg Body Wt.	No. of animals used	Total Vol. administered in cc
1	Male	2	2	2
2	Female	2	2	2
3	Male	4	2	2
4	Female	4	2	2
5	Male	6	2	2
6	Female	6	2	2
7	Male	8	2	2
8	Female	8	2	2
9	Male	10	2	2
10	Female	10	2	2

Table 3: 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 perimenium	Nil
11	Condition of teeth	Normal
12	Breathing abnormalities	Nil
13	Gait	Normal

Table 4: Mortality Record

Group gm/Kg	2	2	4	4	6	6	8	8	10	10
Sex	Male	Female	Male	Female	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	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

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 zero-day values, indicating no signs of toxicity against powder of *Leucas aspera* leaves. The variation in body weight changes of males and females.

Food and Water Consumption

There was no significant change in food and water intake of the test animals at all dose levels.

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. 10 gm/Kg. body weight.

CONCLUSION

From the results of this study, it is observed that there is no considerable change in the body weight, food and water consumption by the animals from all dose groups (2.00 gm/Kg body weight to 10.0 gm/Kg body weight). There was no mortality recorded even at the highest dose level i.e. 10.0 gm/Kg body weight, which proves that powder of *Leucas aspera* leaves has no significant toxic effect in mice.

REFERENCES

- Research guidelines for evaluating the safety and efficacy of herbal medicine, World Health Organisation Regional Office for the Western Pacific Manila, (1993), 1-9.
- John H. Duffus, Fundamental Toxicology for Chemists, Ed. John H. Duffus and Howard G. J. Worth, Royal Society of Chemistry, (1996), 1-5.
- Michael A. Gallo, Casarett and Doull's Toxicology - The Basic Science of Poison, Ed. Curtis D. Klassen, International edition, McGrath-Hill Health Professions Division, 5th edition, (1996), Ch. 1, 3-5.
- Trevar J.W., The error of determination of toxicity, Proc. R. Soc. Lond., (1927), Vol.101B, 483-514.
- David L. Eaton and Curtis D. Klassen, Casarett and Doull's Toxicology, The Basic Science of Poison, Ed. Curtis D. Klassen, International edition, McGrath-Hill Health Professions Division, 5th edition, (1996), Ch. 2, 13.
- EPA: EPA fact sheet: Background on acute toxicity testing for chemical safety, August (1984).
- FDA: "Final report on acute studies workshop" Sponsored by the U.S. Food and Drug Administration on November 9, 1983.
- Kennedy G.L.etal, "J. Appl. Toxicol.", (1986), 24, 457-463.
- Pingale Shirish S. *, Raghunath D. Pokharkar and Mugdha S. Pingale, Standardisation of Herbal Drug as a Potent Liver Tonic, Pharmacologyonline Newsletter, 1:13-19 (2008).
- Pingale Shirish S. *, Pokharkar Raghunath D. and Pingale Mugdha S., Acute toxicity Study for Argemone mexicana L. Root Bark Powder, Pingale et al., Pharmacologyonline Newsletter, 1: 24-31 (2008).
- Pingale Shirish S. * and Pokharkar Raghunath D., Acute toxicity study for Cissus quadrangularis whole plant powder, Pharmacologyonline Newsletter, 2: 256-262 (2008)
- Pingale Shirish S., Acute toxicity study for Centella asiatica whole plant powder, Pharmacologyonline Newsletter, 3: 80-84 (2008)
- Pingale S.S, Acute Toxicity Study of Ocimum Sanctum, IRJP (1) Dec 2010, 409-413
- Pingale Shirish Sadashiv1, Markandeya Anil Ganpat2 and Gawali Sunita3, Toxicity Study for Celosia argentea Leaves IRJP, 2 (1), Jan 2011 263-266
- Pingale Shirish S., Hepatoprotective Action of Terminalia belerica on CCl4 Induced Hepatic Disorders, Scholars Research Library, Der Pharma Chemica, 2011, 3(1): 42-48,
- Pingale Shirish S.1 and Virkar Popat S2., Evaluation of Acute Toxicity For Abutilon Indicum, Scholars Research Library, Der Pharmacia Lettre, 2011, 3(3):37-42.
- Pingale Shirish S. and Shewale Shrikant S, Acute Toxicity Study of Phyllanthus Amarus, International Journal of Pharmaceutical Sciences Review and Research, Pg 81-84, Volume 9, Issue 1, July - August 2011;
- Pingale Shirish Sadashiv and Avvaru Radha Krishna, Acute Toxicity Study for Achyranthes aspera Leaves, Journal of Pharmacy Research, Vol. 4. Issue 7. July 2011, 2221-2222.
- Pingale Shirish Sadashiv, Acute Toxicity Study For Tinospora Cordifolia, International Journal of Research in Ayurveda and Pharmacy, IJRAP 2011, 2(5), 1571-1573,
- Pingale Shirish Sadashiv, Acute Toxicity Study for Leucas Aspera, Der Pharmacia Lettre, 2011; 3 (5)132-137.
- Pingale Shirish S. and More Bharat P., Toxicity Study of Terminalia chebula, Pg. No. 2127-2134, World Journal of Pharmaceutical Research, Vol 3, Issue 2, 2014.