

ACUTE TOXICITY STUDY OF *OCIMUM SANCTUM*

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ABSTRACT

Ocimum sanctum is an annual plant, 30-60 cm high, much branched; stem and branches usually purplish, sub-quadrangular; leaves 2.5-5 by 1.6-3.2cm, elliptic oblong-obtuse, pubescent on both side and minutely gland-dotted. Flowers are in racemes. It is commonly found in India and used in many traditional medicines. Acute toxicity studies of leaves powder of this plant material were carried out as per OECD guideline in Swiss mice weighing 25 to 35 gm by administering a dose 3, 5 and 7gm/kg body weight orally in the form of aqueous slurry. The groups were almost continuously observed for mortality and behavioral changes during first 24 hr and then daily for a fortnight. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There was no abnormality observed in any of these three groups. The whole plant powder was found to be nontoxic.

KEYWORDS: *Ocimum sanctum*, vehicle, acute toxicity

INTRODUCTION

Toxicity is the fundamental science of poisons. The organization for Economic and Development (OECD) mentioned acute toxicity as the advance effect occurring within a short time of oral administration of a simple dose of a substance or a multiple doses given within 24 hours (OECD Guideline 1999) Phychochemical interactions of poisons lead to injury or death of living tissues¹. Toxicology is like science and an art like medicine. It includes observational data gathering & data utilization to predict outcome of exposure in human and animals. The ancient humans categorized some plants as harmful and some as safe².

All organisms are exposed constantly and unavoidably to foreign chemicals or xenobiotics, which include both man-made chemicals such as drugs industrial chemicals pesticides, pollutants pyrolysis products in cooked foods, alkaloids secondary plant metabolites, and toxins produced by moulds, plants and animals. Poisons are any agent capable of producing a deleterious response in a biological system, seriously injuring function or producing death. Toxicologists usually divide that exposure of animals into four categories which are acute, sub acute, sub chronic and chronic³.

Literature pertaining to *Ocimum sanctum* suggests that Tulsi has been used for thousands of years as a prime herb in Ayurvedic treatment, for its diverse healing properties. Tulsi is considered to be an adaptogen, balancing different processes in the body, and helpful for adapting to stress⁴. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity⁵.

Tulsi's extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, and malaria⁶. Traditionally, Tulsi is taken in many forms: as an herbal tea, dried powder, fresh leaf, or mixed with ghee. Essential oil extracted from Karpoora Tulsi is mostly used for medicinal purposes and in herbal cosmetics. Widely used in skin

preparations for its anti-bacterial activity. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects⁷.

Recent studies suggest that Tulsi may be a COX-2 inhibitor, like many modern painkillers, due to its significant amount of eugenol (1-hydroxy-2-methoxy-4-allylbenzene). Studies have also shown Tulsi to be effective for diabetes, by reducing blood glucose levels⁸. The same study showed significant reduction in total cholesterol levels with Tulsi. Another study showed that Tulsi's beneficial effect on blood glucose levels is due to its antioxidant properties. Tulsi also shows some promise for protection from radiation poisoning and cataracts⁹.

MATERIALS AND METHODS

Ocimum sanctum leaves were collected from Rajgurunagar (Pune), Maharashtra, India. Herbaria of the plant were authenticated by BSI (Botanical Survey of India), Pune, India. After collection of the required quantity of plant material, it was carefully segregated, cleaned and dried in shade to constant weight. The plant material was kept in preset oven for eight days at 45°C. The dried plant material free of moisture was powdered and sieved through a BSS Mesh No. 85 sieve and then stored in an airtight container¹⁰.

The study protocol used for the study is given in table No. I

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 II.

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. 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 whole plant powder as slurry following table III shows the dosage regime. Table IV shows the general cage side observations for the parameters studied. Table V shows the mortality record.

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

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 g / 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 g/Kg body weight to 7 g/Kg body weight), There was no mortality recorded even at the highest dose level i.e. 7 g/ Kg body weight, which proves that *Ocimum sanctum* leaves powder have no any significant toxic effect in mice. The same study is also carried out for

fresh juice of whole plant material. 2ml of fresh juice is given to test animals, which has also no any toxic effect.

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Table I: Study Protocol

Name of the study	Acute toxicity study
Test material	<i>Ocimum sanctum</i> Whole plant powder slurry
Animal model	Albino Swiss Mice
Animals procured from	Raj Biotech (INDIA) Ltd., Pune
Sex	Male and Female
Weight range of animals	Between 25 to 35 g
No. of dose groups	Three groups
Animals per group	1 male and 1 female
Route of administration	Intragastric administration with the help of gavage No. 16
Dose volume	2.0 ml per animal
Vehicle	Distilled water
No. of administrations	Single
Concentration of dose	3, 5 and 7 g/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

Table II: Composition Of Feed

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.

Table III: Doses Regime

Sr. No.	Sex	Dose g/Kg Body Wt.	No. of animals used	Total Vol. administered in cm ³
1	Male	3	1	2
2	Female	3	1	2
3	Male	5	1	2
4	Female	5	1	2
5	Male	7	1	2
6	Female	7	1	2

Table IV: 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 perineum	Nil
11	Condition of teeth	Normal
12	Breathing abnormalities	Nil
13	Gait	Normal

Table V: Mortality Record

Group	3	3	5	5	7	7
Sex	Male	Female	Male	Female	Male	Female
Hr. 1	Nil	Nil	Nil	Nil	Nil	Nil
Hr. 2	Nil	Nil	Nil	Nil	Nil	Nil
Hr. 3	Nil	Nil	Nil	Nil	Nil	Nil
Hr. 4	Nil	Nil	Nil	Nil	Nil	Nil
Day 1	Nil	Nil	Nil	Nil	Nil	Nil
Day 2	Nil	Nil	Nil	Nil	Nil	Nil
Day 3	Nil	Nil	Nil	Nil	Nil	Nil
Day 4	Nil	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil	Nil
Day 7	Nil	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	0/1	0/1	0/1	0/1	0/1	0/1

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