

## **ACUTE TOXICITY STUDY FOR *CENTELLA ASIATICA* WHOLE PLANT POWDER**

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### **Summary**

Centella asiatica is a perennial, slender, herbaceous creeper with kidney shaped leaves of 2-5 cm diameter found in India, China and S. Africa. It is used for wound healing, bronchitis, dysentery, fever, inflammation, leucoderma and as a nerve tonic and jaundice. The acute toxicity study of Centella asiatica was studied on Swiss mice with a dose of 3, 5 and 7 g/Kg body weight orally. The single administration exposure of the whole plant powder in the form of aqueous slurry on Swiss mice was carried out and 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. The whole plant powder was found to be nontoxic

**Keywords:** *Centella asiatica*, creeper, 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 (1-6). Phychochemical interactions of poisons lead to injury or death of living tissues (1-6). 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 (6). The ancient humans categorized some plants as harmful and some as safe (6).

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 [3]. Toxicologists usually divide that exposure of animals into four categories which are acute, subacute, subchronic and chronic (7).

### Experimental

*Centella asiatica* plants were collected from various places in Pune and Ahmednagar district; washed thoroughly and dried at room temp in shade. They were powdered, sieved through sieve of mesh to 85 (BSS) and stored in airtight containers. Three dose groups are considered for the toxicity study of *Centella asiatica* whole plant powder. The study protocol used for the study is given in following table AS1 [8-13].

**Table AS1: Study Protocol**

<b>Name of the study</b>	Acute toxicity study
<b>Test material</b>	<i>Centella asiatica</i> plant powder slurry
<b>Animal model</b>	Albino Swiss Mice
<b>Animals procured from</b>	Raj Biotech (INDIA) Ltd., Pune
<b>Sex</b>	Male and Female
<b>Weight range of animals</b>	Between 35 to 55 g
<b>No. of dose groups</b>	Three groups
<b>Animals per group</b>	1 male and 1 female
<b>Route of administration</b>	Intragastric administration with the help of gavage No. 16
<b>Dose volume</b>	2.0 ml per animal
<b>Vehicle</b>	Distilled water
<b>No. of administrations</b>	Single
<b>Concentration of dose</b>	3, 5 and 7 g/Kg body weight
<b>Study duration</b>	Acclimatization for 14 days, one day drug administration and 14 days observation period including holidays
<b>Parameters observed</b>	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 AS2.

**Table AS2**

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

The feed was enriched with stabilized vitamins such as Vit. A and D<sub>3</sub>, Vit. B<sub>12</sub>, 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 AS3 shows the dosage regime. Table AS4 shows the general cage side observations for the parameters studied. Table AS5 shows the mortality record.

**Table AS3: Doses Regime**

<b>Sr. No.</b>	<b>Sex</b>	<b>Dose g/Kg Body Wt.</b>	<b>No. of animals used</b>	<b>Total Vol. administered in cm<sup>3</sup></b>
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 AS4: 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 AS5: Mortality Record**

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

### 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 (9). 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 (LD<sub>50</sub>) 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 *Centella asiatica* plant powder have no 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 no any toxic effect.

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