



ACUTE TOXICITY STUDY OF *PHYLLANTHUS AMARUS*

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ABSTRACT

The study was designed to examine the acute toxicity of *Phyllanthus amarus*. Aerial parts of *Phyllanthus amarus* were collected from Avsari Forest Park, the fresh juice of aerial parts were prepared and used for study. The acute toxicity study is carried out as per OECD guideline in Swiss mice weighing 35 to 50 gm. The dose of 2, 4, 6 and 8gm/kg body weight plant material were administered orally in the form of aqueous slurry. The groups were almost continuously observed for mortality and behavioral changes during firstly for 24 hrs and then daily for 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 results provide evidence that *Phyllanthus amarus* plant material was found to be nontoxic.

Keywords: *Phyllanthus amarus*, acute toxicity study, fresh juice.

INTRODUCTION

Phyllanthus amarus is used for support in treatment of in Hepatitis, Jaundice, Gonorrhoea, Frequent menstruation, and Diabetes. It has also been used on the skin for sores, swelling, and itchiness. Entire plant is having its medicinal value and the whole plant is used in Ayurvedic formulation. Research shows that this herb acts primarily on the liver. This action in the liver confirms its use as a remedy for jaundice. *Phyllanthus amarus* has been traditionally used to promote liver functions¹⁻²².

It also shows Pharmacological Activities like Analgesic, Antibacterial, Antifungal, Antidiarrheal, Antihepatotoxic, Antihypercholesterolemic, Antihyperglycemic, Antihyperlipemic, Antimutagenic, Antipyretic, Antispasmodic, Antitumor, Antiviral, Cardiotoxic, Cytotoxic, Hepatitis B Surface antigen inactivation, etc properties. *Phyllanthus amarus* is bitter, astringent, cooling, diuretic, stomachic, febrifuge and antiseptic. It is useful in dropsy, jaundice, diarrhoea, dysentery, intermittent fevers, diseases of urino-genital system, cabbies ulcers and wounds²³⁻²⁸.

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. Phytochemical interactions of poisons lead to injury or death of living tissues. Toxicology is like science and an art like medicine. It includes observational data gathering and data utilization to predict outcome of exposure in human and animals. The ancient humans categorized some plants as harmful and some as safe²⁹.

All organisms are exposed constantly and unavoidably to foreign chemicals or xenobiotics, which include both manmade 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 chronic. The aim of the present work is to study the toxic effect of *Tridax procumbens* L leaves powder as well as fresh juice of leaves of the same plant³⁰⁻³¹.

MATERIALS AND METHODS

Phyllanthus amarus plants were collected from Avsari Forest Park (Pune), Maharashtra, India. Herbaria of the plant were prepared and authenticated from BSI (Botanical Survey of India), Pune, India. After collection of the required quantity of plant material, it was carefully segregated, cleaned and grinded by mortar and pestle to a very fine paste. The plant material was then used for study as per the study protocol given in table No. 1.

Animal Maintenance

All animals were housed in polyurethane cages. The cages were provided with wheat 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. 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 recorded from the amount of water left in the feeding bottles and from the amount of feed left in the feed hopper.



Table 1: Study Protocol

| | |
|--------------------------------|--|
| Name of the study | Acute toxicity study |
| Test material | <i>Phyllanthus amarus</i> plant material. |
| Animal model | Albino Swiss Mice |
| Animals procured from | Raj Biotech (INDIA) Ltd., Pune |
| Sex | Male and Female |
| Weight range of animals | Between 30 to 50 g |
| No. of dose groups | Four 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 | 2, 4, 6 and 8g/Kg body weight plant material |
| 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 2: 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. |

RESULTS AND DISCUSSION

Cage Side Observations

The examination of the behavior of animals was reported by recording 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 of leaves powder and methanol extract showed no significant changes in behavior before and after the administration. Table 3 shows the dosage regime for *Phyllanthus amarus* Table 4 shows the general cage side observations for all parameters studied. Table 5 shows the mortality record for whole plant powder of *Phyllanthus amarus*.

Table 3: Doses Regime

| Sex | Dose g/Kg Body weight | No. of animals used | Total Vol. administered in cm ³ |
|--------|--------------------------|------------------------|--|
| Male | 2 | 1 | 2 |
| Female | 2 | 1 | 2 |
| Male | 4 | 1 | 2 |
| Female | 5 | 1 | 2 |
| Male | 6 | 1 | 2 |
| Female | 6 | 1 | 2 |
| Male | 8 | 1 | 2 |
| Female | 8 | 1 | 2 |

Table 4: Cage Side Observations for All Animals

| Parameters | Cage Side Observations |
|------------------------------------|---------------------------|
| Condition of the fur | Normal |
| Skin | Normal |
| Subcutaneous swellings | Nil |
| Abdominal distension | Nil |
| Eyes –dullness | Nil |
| Eyes – opacities | Nil |
| Pupil diameter | Normal |
| Ptosis | Nil |
| Colour & consistency of the faeces | Normal |
| Wetness or soiling of the perineum | Nil |
| Condition of teeth | Normal |
| Breathing abnormalities | Nil |
| Gait | Normal |

Table 5: Mortality Record for plant material as aqueous slurry

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



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. There was no significant change in food and water intake of the test animals at all dose levels of the plant material and extract 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. 8g/Kg body weight plant material of *Phyllanthus amarus*.

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 (2 g/Kg body weight to 8mg/Kg body weight). There was no mortality recorded even at the highest dose level i.e. 8g/ Kg body weight fresh plant material, which proves that *Phyllanthus amarus* plant material have no any significant toxic effect in Swiss mice.

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