

## ACUTE AND SUB ACUTE TOXICITY STUDY OF A HERBOMINERAL SIDDHA FORMULATION THUTHUVALAYATHY CHOORANAM

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### ABSTRACT

The present study investigated the acute and sub acute toxicity study of Thuthuvalayathy Chooranam, a herbomineral Siddha formulation indicated for the management of Swasa kasam (Bronchial asthma) in experimental animal models. Thuthuvalayathy Chooranam contains Thuthuvalai (*Solanum trilobatum, Linn*) Echchura muli (*Aristolochia indica.Linn*) Chittarattai (*Alpinia officinarum.Linn*) karunchirakam (*Nigella sativa .Linn*) Iluppai (*Madhuca lonifolia*) Chukku (*Zingiber officinale, Rose.*) Milagu (*Piper nigrum, Linn*) Thippili (*Piper longum, Linn.*) Kadukkai (*Terminalia chebula. Retz*) Perungayam (*Ferula asafoetida.*) thippili-ver (*Piper longum, Linn.*) Indhuppu (*Sodium chloride impure*) Vengaaram (*Sodii biberas;sodii boras.*) The aim of

the study is to evaluate the safety of the Thuthuvalayathy Chooranam through acute and sub acute toxicity study. In an acute toxicity study the drug was administered orally at a dose 2700mg/kg p.o and the animals were observed for any toxic symptoms upto 72hrs. The results indicated there were no toxic symptoms up to the dose level of 2700mg/kg p.o. In a Sub acute toxicity study Thuthuvalayathy Chooranam was tested at a dose ranging from 270mg/kg, 1,350 mg/kg and 2700mg/kg p.o once daily for 30 days. The animals were sacrificed on 31<sup>st</sup> day .The liver, heart, lung, stomach and kidney were processed for histo pathological study. The result of the sub acute toxicity study did not show evidence of any changes in body weight, food and water intake when compared with the control animals. The vital organs of animals treated with Thuthuvalayathy Chooranam for 30 days did not show any histopathological evidence of pathological lesions.

**KEY WORDS:** Thuthuvalayathy Chooranam, Acute and sub acute toxicity study, Siddha medicine.

## INTRODUCTION

Thuthuvalayathy Chooranam contains Thuthuvalai (*Solanum trilobatum*, Linn) used in the treatment of respiratory diseases like cough, asthma, chronic febrile infections, tuberculosis, cardiac and liver diseases. The ethanolic extracts of *S. trilobatum* showed mast cell degranulation inhibition property. The extract exhibit antimicrobial activity against Gram(+) and Gram (-) bacteria. Echchura muli(*Aristolochia indica*.Linn) cures vatha, pitha diseases, cough, asthma, fever, body pain and all poison.. Chittarattai (*Alpinia officinarum*.Linn) The phytochemical diary heptanoid phenylhept (HMP) in lesser Galangal exhibit the anti-inflammatory properties on mouse macrophage cell line and human peripheral blood mononuclear cells (PBMCs) in vitro. It significantly inhibited lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production. It also inhibited the release of LPS-induced proinflammatory cytokines interleukin-1 beta (IL-1 beta). karunchirakam (*Nigella sativa* .Linn) Nigellone has proved to be an excellent prophylactic agent for both bronchial asthma and asthmatic bronchitis. Iluppai (*Madhuca lonifolia*) Chukku (*Zingiber officinale*, Rose.) Nigellone has proved to be an excellent prophylactic agent for both bronchial asthma and asthmatic bronchitis. Milagu (*Piper nigrum*, Linn) It is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, Fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhoea. Thippili (*Piper longum*, Linn.) Piperine relieves inflammation, pain and asthma improves and digestion. It has analgesic, ant-pyretic, anti-inflammatory activity. Kadukkai (*Terminalia chebula*. Retz) Fruits are used in fever, cough, asthma, urinary disease, piles etc.Perungayam (*Ferula asafoetida*.) The smoke of powered asafoetida with vigna mungo is inhaled for asthma. Thippili-ver (*Piper longum*, Linn.) It contain piperine, piper lonumine and dihydrostigmasterol which reduced inflammation of respiratory diseases. Indhuppu (*Sodium chloride impure*) It is indicated for dyspepsia and other abdominal disorders, cough and asthma. Vengaaram (*Sodii biboras;sodii boras*.) The ingredient in this drug said to possess expectorant, stimulant, carminative, antispasmodic action, immunomodulatory, bronchodailator, anti-oxidant, anti-inflammatory, anti-histamine actions and also cost effective. In the present study the acute and sub acute toxocity study of the Thuthuvalayathy Chooranam was investigated to assess its safety and tolerability profile in long term treatment.

## MATERIALS AND METHODS

**Collection of plants:** The ingredients of this formulation Thuthuvalayathy Chooranam contains raw drugs Thuthuvalai root bark (*Solanum trilobatum*, Linn) Echchura muli root (*Aristolochia indica*, Linn) Chittarattai (*Alpinia officinarum*, Linn) Karunchirakam (*Nigella sativa*, Linn) Iluppai oil cake (*Madhuca lonifolia*) Chukku (*Zingiber officinale*, Rose.) Milagu (*Piper nigrum*, Linn) Thippili (*Piper longum*, Linn.) Kadukkai (*Terminalia chebula*, Retz) Perungayam (*Ferula asafoetida*.) thippili-ver (*Piper longum*, Linn.) Indhuppu (*Sodium chloride impure*) Vengaaram (*Sodii biboras*; *sodii boras*) were purchased from a well reputed country shop in Chennai.

### Preparation of Test Substance

The Thuthuvalayathychooranam is light green in colour. The test substances are insoluble in water, in order to obtain and ensure the uniformity in drugs distribution, the drugs is dissolved by aqueous Tween 80 solution (10%).

### Experimental Animals

Acute and sub-acute toxicity studies were carried out in Swiss albino mice and Wistar rats, respectively. Animals were obtained from animal house, Kings Institute, Chennai and stocked at animal house, National Institute of Siddha, Chennai. All the animals were kept under standard environmental condition ( $27 \pm 2$  degree C). Adult mice (6 weeks old) of either sex weighing 20-25 gm were housed in polypropylene cages, 5 animals per cage with free access to water and standard pellet diet (Sai Meera food Pvt. Ltd, Bangalore). 6-8 weeks old Wistar albino rats of either sex weighing 150-200 gm were housed, 5 per cage in polypropylene cages with free access to food and water. The principles of laboratory animal care were followed. (1248/ac/09/CPCSEA/4-01/2011) dated 20/12/2011.

### Administration of Doses

Thuthuvalayathychooranam was suspended in aqueous Tween 80 solution (10%), with uniform mixing and it was administered to the groups in a single oral dose. The control groups were received equal volume of the vehicle. The animals were weighed before giving the drug. The dose level was calculated according to body weight, and surface area. Since the clinical dose for Thuthuvalayathy chooranam was 1.5gm it was converted to animal dose 0.27gm/ kg and then administered. The principle of laboratory animal care was followed.

### **Acute Toxicity Study**

For acute toxicity studies, 20 mice were used for the study. The mice were divided into 2 groups containing 10 animals. The animals were fasted overnight and the drug was administered orally. Group I received distilled water (vehicle for formulation) and served as the control. Group II received 0.27gm /kg b.w of Thuthuvalayathy chooranam aqueous extract single dose orally. The animals were observed continuously for the first 4hrs then occasionally up to 24hrs and then daily up to 14 days, post treatment to observe for any toxic symptoms and mortality.

### **Sub-Acute Toxicity Study**

For sub-acute toxicity studies, rats were divided into 4 groups of 10 animals each (5 males and 5 females). The animals were fasted overnight and the drug was administered orally. Group I received distilled water for 28 days and other 3 groups were received the test drug Thuthuvalayathy chooranam at the dose of 0.027gm , 0.135gm, 0.27gm/kg b.w., once daily for 28 days. All the rats were observed for any physiological and behavioral changes and mortality. Food and water consumption was checked daily. Body weight was recorded at the beginning and weekly intervals throughout the study

### **Observations**

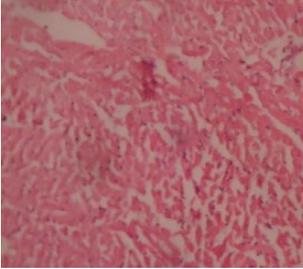
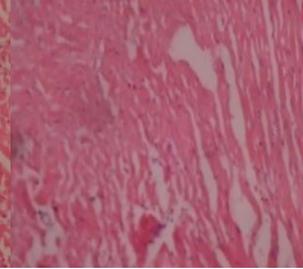
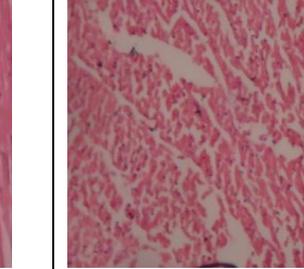
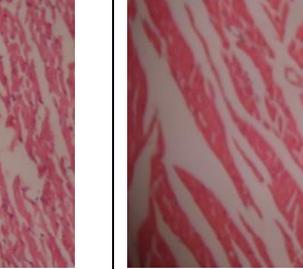
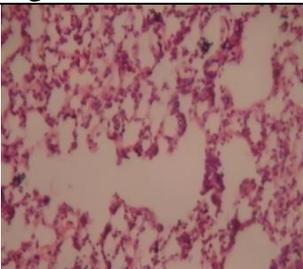
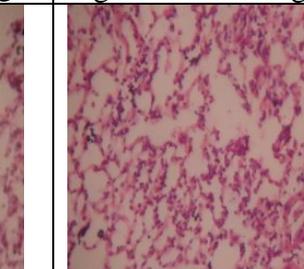
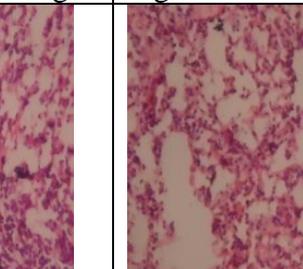
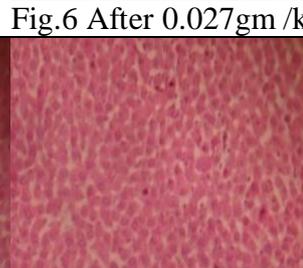
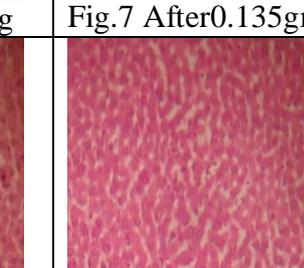
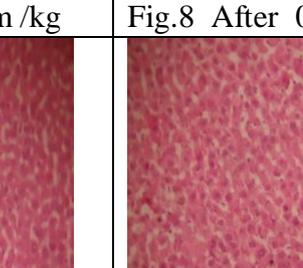
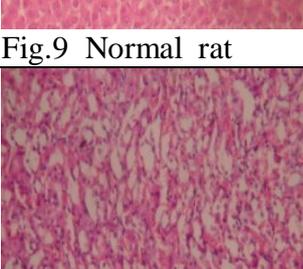
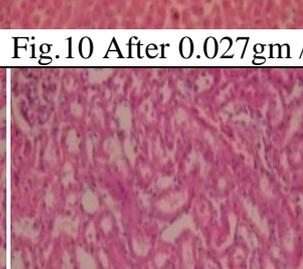
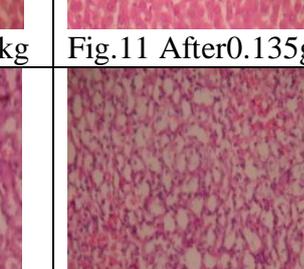
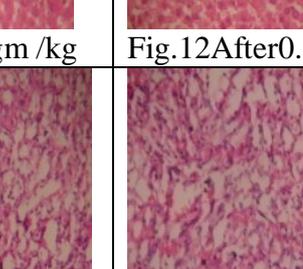
Observations were made and recorded systematically and continuously observed as per the guideline [*WHO guidelines, 1993*] after test drug administration. Animals were observed individually (visual observations included skin changes, alertness, grooming, aggressiveness, sensitivity to sound, touch and pain, restlessness, tremors, convulsion, righting reflex, gripping reflex, pinna reflex, corneal reflex, writhing reflex, papillary reflex, urination, salivation, lacrimation for first 4 hrs, then periodically during the first 24 hrs. Animals were observed for body weight and mortality for 14 days. If animals dying during the period of study, the animals were sacrificed. At the end of the 14<sup>th</sup> day all animals were sacrificed and necroscopy was done.

### **Histopathology**

Tissue samples of organs from control and treated animals were preserved in 10% formalin for preparation of sections using microtome. The organs included brain, heart, lungs, stomach, liver, kidneys, spleen, intestine, pancreas and sex organs of the animals were preserved and they were subjected to histopathological examination. The organ pieces (3-5 micron) were fixed in 10% formalin for 24 hours and washed in running water for 24 hours.

Samples were dehydrated in tissue processor and then cleaned in benzene to remove absolute alcohol. Embedding was done by passing the cleared sample through three cups containing molten paraffin at 50 degree c and then a cubical block of paraffin made by the L moulds it was followed by microtome and the slides were stained with haematoxylin–eosin stain. Stained sections of each organ were examined under light microscope at high (40X) power magnification. All the his to pathological slides were prepared at Vels University, pallavaram, Chennai.

**Image-1. Histopathology slides of the Organs in both Male & Female rats.**

| Slides | Control Group  | 1x Group   | 5x Group  | 10x Group  |
|--------|--|--|---|--|
| Heart  | <br>Fig.1 Normal rat    | <br>Fig.2 After 0.027gm/kg    | <br>Fig.3 After 0.135gm/kg    | <br>Fig.4 After 0.270gm/kg    |
| Lung   | <br>Fig.5 Normal rat  | <br>Fig.6 After 0.027gm/kg  | <br>Fig.7 After 0.135gm/kg  | <br>Fig.8 After 0.270gm/kg  |
| Liver  | <br>Fig.9 Normal rat  | <br>Fig.10 After 0.027gm/kg | <br>Fig.11 After 0.135gm/kg | <br>Fig.12 After 0.270gm/kg |
| Kidney | <br>Fig.13 Normal rat | <br>Fig.14 After 0.027gm/kg | <br>Fig.15 After 0.135gm/kg | <br>Fig.16 After 0.270gm/kg |

Stomach

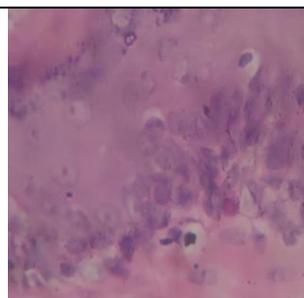


Fig.17 Normal rat

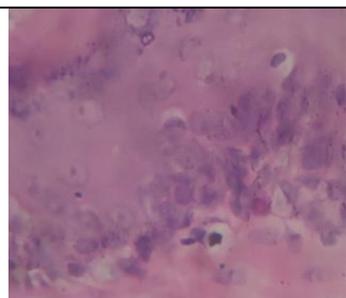


Fig.18 After 0.027gm /kg

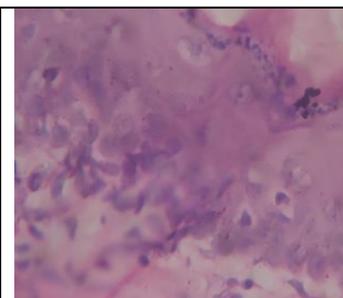


Fig.19 After 0.135gm /kg

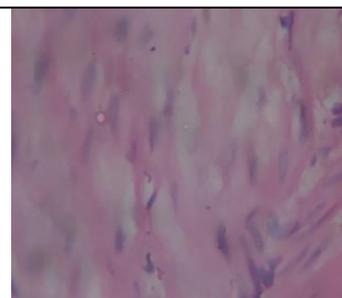


Fig.16 After 0.270gm /kg

## RESULT AND DISCUSSION

No weight loss, abnormal animal behaviors, metabolic functions [urination, lacrymation, defaecation etc.] and mortality were noted on oral administration at the dose of 0.27gm /kg b.w of Thuthuvalayathy chooranam formulation and all the animals were found to be normal during and at the end of the observation period (14 days). In the sub-acute toxicity study, there was no death in the treatment period either in control or in the treated groups. There was no significant change observed in food and water consumption. There was no change in the general behavior and other physiological activities of the animals. In necropsy of the animal organs showed normal appearance and weight. In Histopathological studies, No abnormal findings were observed in the organs such as Heart, Liver, Lungs, Kidney and Stomach in X, 5X and 10X compared with control group. These observations were similar in the male and female rats (Fig. 1-20).

## CONCLUSION

The study revealed that Thuthuvalayathy chooranam formulation at different doses of 270, 1350, 2700 mg/kg did not provoke toxic effects in the animal's tissues and it was safe when administered to Bronchial Asthma patients.

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