

**ACUTE AND LONG TERM TOXICITY STUDIES OF *SIDDHA* FORMULATION  
*SADHAKUPPAI CHOORANAM***

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Article Received on 20/05/2018

Article Revised on 10/06/2018

Article Accepted on 30/06/2018

**ABSTRACT**

The polyherbal *Siddha* formulation *Sadhakuppai Chooranam* (SKC) has been indicated in classical literature for the management of worm infestations in pediatrics. Though these age old traditional formulations have been clinically beneficial, till now no scientific data exists to confirm its safety and efficacy. Hence the present study was conducted to determine the acute and long term safety profile of *Sadhakuppai Chooranam* (SKC). Acute toxicity was carried out in 20 Swiss albino mice of both sexes divided into vehicle control group and therapeutic group and the test drug was administered to the groups in a single oral dose (2592mg/kg bwt). Animals were observed for body weight and mortality for 14 days. Long term toxicity study was conducted in 40 Male and Female wistar albino rats of both sexes and was divided into 4 groups (Group I-Vehicle control, Group II- 259 mg, Group III-1296 mg, Group IV-2592 mg). The vehicle control groups received equal volumes of vehicle (i.e water). Administration was by oral gavage once a day for 30 days and the animals were observed for clinical signs, body weight, food and water intake and mortality. The study results showed that both acute and long term toxicity studies did not produce toxic effects when administered 10 times of therapeutic dose and LD50 > 2000mg/kg hence indicating the safety of *Sadhakuppai chooranam*.

**KEYWORDS:** *Sadhakuppai Chooranam* (SKC), Acute toxicity study, Long term toxicity study, Herbal drug, *Siddha* medicine.

**INTRODUCTION**

The antique *Siddha* system of medicine has hailed and flourished among the people of South India since time immemorial. This system of medicine is believed to be established by the sages of South India known as *Siddhars*. These *Siddhars*, had a vast knowledge about the medicinal uses of flora and fauna of this universe and therefore contributed an enormous wealth of literature indicating the medicinal use of these herbs. As these herbs are in use over centuries, a wealth of literature is available in manuscripts indicating the medicinal use of these herbs. According to World health organization (who) there are nearly 3 billion population that are affected by worm infestation globally.<sup>[1]</sup> Among them the urban and rural areas of India have nearly 450 million Children affected by worm infestation.<sup>[2]</sup>

Though *Sadhakuppai Chooranam* has been indicated by *Siddha* texts for the management of *Kudal Kirumi* (Worm infestations), many issues related to deficient scientific evidence concerning the efficacy and safety of the drug remains unanswered.<sup>[3]</sup> Hence the Pre-clinical toxicity studies seem to be essential for determining a safe dose for human trials and to confirm the traditional

claims of ancient literature.<sup>[4]</sup> Therefore the study was performed with an objective to determine the acute and long term safety profile of *Sadhakuppai chooranam* in experimental animals.

**MATERIALS AND METHODS**

The following animal study was conducted after the approval of Institutional Animal Ethical Committee for the use of animals and the study design. (1248/ac/09/CPCSEA/June/ 2011).

**Acute toxicity studies**

Acute toxicity was carried out in 20 Swiss albino mice of both sexes which were 6 weeks old and weighing 20-25 gms. The animals were acclimatized for a period of 7 days prior to drug administration. Then the animals were administered with a single exposure of 10 times (2592 mg) of the recommended therapeutic dose of test compound the study duration will be 14 days.

**Long term toxicity studies**

Long term toxicity studies was conducted in 40 Male and Female wistar albino rats of 6-8 weeks old weighing 150-200 gms and the laboratory procedures were

followed as described above. The period of administration of the test substance to animals are depending on the expected period of clinical use. Since the clinical dose of the test drug is 20 days and as per WHO guidelines the administration period is reported to be 1 month.

#### Test drug

The ingredients of *Sadhakuppai Chooranam* are *Sadhakuppai* (*Anethum graveolens*), *Seeragam* (*Cuminum cyminum*), *Peruncheeragam* (*Pimpinella anisum*), *Karuncheeragam* (*Nigella sativa*), *Elam* (*Elettaria cardamomum*), *Lavangapattai* (*Cinnamomum verum*) *Athimadhuram* (*Glycyrrhiza glabra*), *Kothumalli* (*Coriandrum sativum*), *Lavangam* (*Syzygium aromaticum*) and *Cheenakarkandu* (*White sugar candy*). All the above ingredients were taken in equal quantity, powdered individually and was placed on the cloth tied on top of a vessel containing milk for purification of Chooranam and was steamed until the milk evaporated. The purified powder was taken and then dried.<sup>[5]</sup>

The *Sadhakuppai choornam* is brown in colour with mild acrid taste and mild odour. The test substance is insoluble in water, in order to obtain and ensure the uniformity in drug distribution, the drug is dissolved by aqueous Tween 80 solution (10%).

#### Test Animals and grouping

Test animals were obtained from the animal laboratory of the King institute, Chennai and stocked at National Institute of Siddha, Chennai. All the animals were kept under standard environmental condition (27+ or - 2°C). The animals had free access to water and standard pellet diet (Sai Durga foods pvt.ltd, Bangalore). The animals were housed in polypropylene cages provided with bedding of husk. Dark and light cycle each of 12 hours.

In acute toxicity study, the 20 Swiss albino mice were divided into two groups of vehicle control (Group-1) containing 10 mice (5 male, 5 female) and Toxic dose 10X therapeutic dose (Group-2) containing 10 (5 male, 5 female). In Long term toxicity studies 40 Male and Female wistar albino rats of both sexes and was divided into 4 groups of 10 rats ((5male,5 female) in each group and were administered with equal volume of vehicle in vehicle control group (Group-1) (i.e water), 259 mg(1X therapeutic dose in Group-2), 1296 mg(5X Therapeutic dose in Group-3), 2592 mg(10XTherapeutic dose in Group-4).

#### OBSERVATIONS

##### Acute toxicity study

In acute toxicity study, the control and 2592mg/kg b.wt treated animals were observed for their behavioural signs and mortality for a period of 14 days. Observations were made and recorded systematically and continuously observed as per the guideline after substance 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. At the end of the 14<sup>th</sup> day all animals were sacrificed and necropsy was done.

##### Long term toxicity study

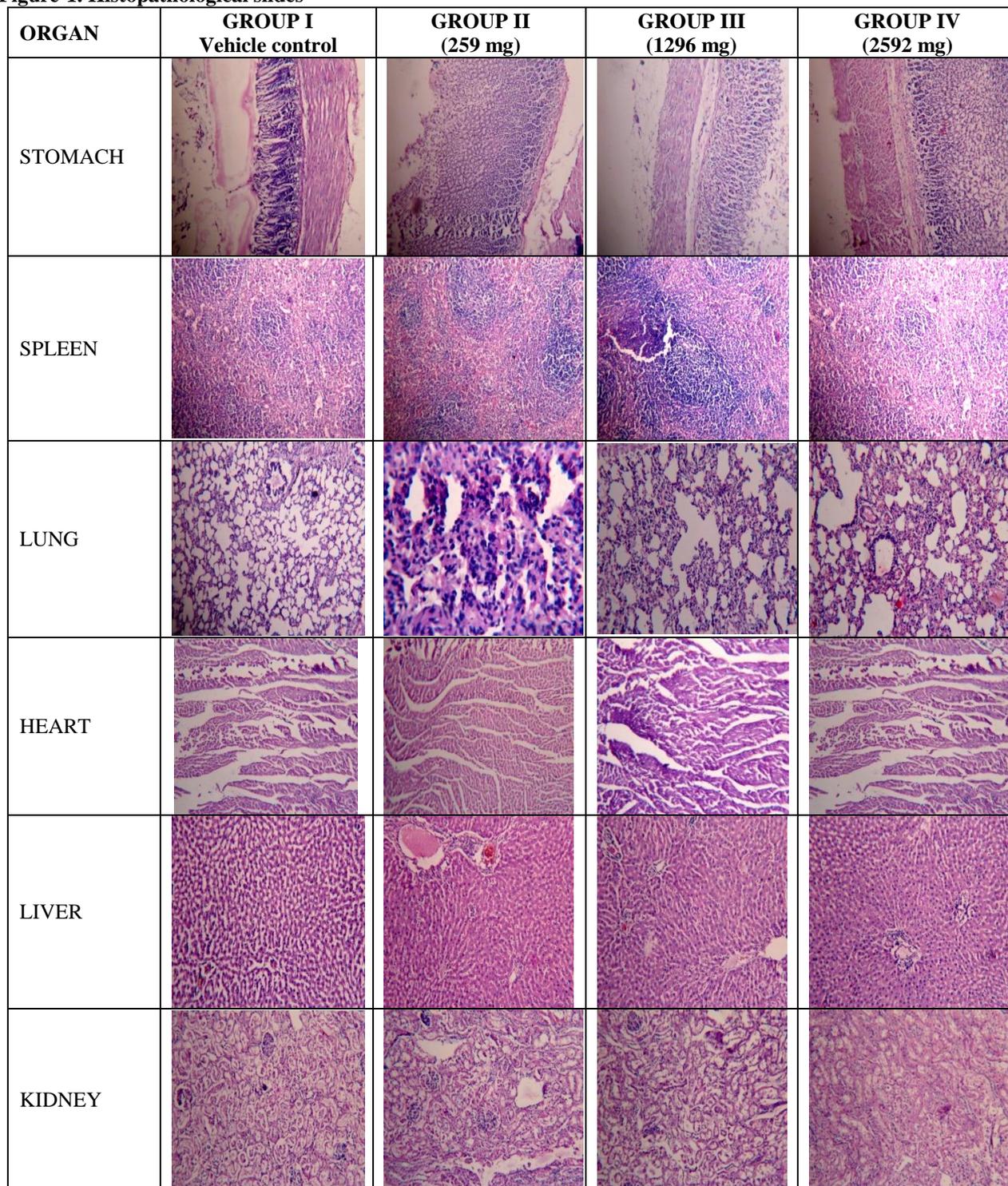
In long term toxicity study, body weight changes, food and water intake and Hematological and biochemical parameters such as Haemoglobin PCV, RBC, Erythrocyte count was estimated by Hemocytometer method of Ghai (1995). Total Leukocyte Count was estimated by Hemocytometer method of John (1972). Total (bilirubin test kid- malloy & evelyn 1937) direct and indirect bilirubin were determined. Alkaline phosphatase, Alanine amino transferase (ALT) and Aspartate amino transferase (AST) were measured by using ALT and AST test kit (kind & king). Total protein TP concentration was determined. Albumin was determined based on its reaction with bromocresol green (binding method). Urea was determined according to urease – berthelot method and plasma creatinine was estimated using jaffe reaction. All the animals were sacrificed on day 31 under ether anesthesia. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, brain, heart, and lungs were recorded.

##### Histopathology

Tissue samples of organs from control and treated animals were preserved in 10% formalin for preparation of sections using microtome. The organs included liver, kidneys, heart, lungs and stomach 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 histo pathological slides were prepared at Dept. of Pathology, Madras Medical College, Chennai.

Figure-1. Histopathological slides



## RESULTS AND DISCUSSION

Though traditional medicines are widely alleged by the community to be safe and free from side effects, the above toxicity study was performed to scientifically claim the safety of the Herbal formulation *Sadhakuppai chooranam*. The results of acute toxicity studies in Swiss albino mice indicated that *Sadhakuppai chooranam* was non toxic and no behavioral changes, mortality was observed. On the basis of these results, the doses were

selected for the study as per WHO guidelines. *Sadhakuppai chooranam* at the dose 2592mg/kg/bwt did not exhibit any mortality in mice. No behavior changes were noted for the first 4 hours and for the next 24 hours and throughout the study period of 14 days. No weight reduction was noted before and after the acute toxicity study. Reflexes were found to be normal before and after the study. All other observations such as hematological and biochemical parameters were found to be normal

before and after the study. In Necropsy, the organs of the animal such as Liver, Heart, Lungs, Pancreas, Spleen, Stomach, Intestine, Kidney, Urinary bladder, Uterus appeared normal.

Histopathological studies of both control and test organs showed normal study. Histopathology of Liver showed central veins with rows of radiating hepatocytes, portal triads and cells appear normal. Histopathology of Kidneys showed glomeruli tubules, interstitial cells of normal histology. Histopathology of Heart, Lung, Stomach showed normal appearing myocardial fibres, bronchioles, alveoli, widened alveolar septa and chronic inflammatory cells and normal gastric mucosal glands lined by columnar cells respectively(Figure-1).

### CONCLUSION

Thus the present study on acute and long term toxicity confirmed the safety of the *Siddha* formulation *Sadhakuppai chooranam* and the lethal dose was found to be LD50 > 2000mg/b.wt.

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