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Acute and Repeated Oral Toxicity Studies on Siddha Drug *Lavanga Choornam*

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ABSTRACT

The polyherbal *Siddha* formulation *Lavanga choornam* (*LC*) has been indicated in classical literature for the management of "*Pitha paandu noi*" (Iron deficiency Anaemia). 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 Sub acute safety profile of Lavanga choornam (*LC*). The Acute toxicity was carried out in 20 colony inbred animals strains of wistar rats of either sex weighing 200-250 g and divided into control group that received distilled water and test group. The test drug *Lavanga choornam* (*LC*) was administered to the groups in a single oral dose (2000mg/kg bwt). In acute toxicity study the animals were observed for body weight and mortality for 14 days. Repeated oral toxicity study was conducted in 20 Male and Female wistar albino rats of both sexes and was divided into 2 groups (Group I- control (1% sodium carboxy methyl cellulose), Group II-500 mg of test drug). Administration was by oral gavage once a day for 28 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 repeated oral toxicity studies did not produce toxic effects when administered 10 times of therapeutic dose and LD50 > 2000mg/kg hence indicating the safety of Lavanga choornam (*LC*)

Keywords: Lavanga choornam, oral toxicity, Iron deficiency Anaemia, Siddha herbal formulation, Pitha paandu

INTRODUCTION

The Siddha system of medicine was established by the saints of South India known as Siddhars. These Siddhars, had a vast knowledge about the natural resources of this universe including plants, minerals and zoological resources and therefore contributed an enormous wealth of literature indicating their medicinal uses. "*Pitha paandu noi*" which is symptomatically identical to "Iron deficiency anemia has been described in the ancient Siddha text of *Yugi vaithiya chinthamani*. [1] The drug lavanga Choornam for the management of Iron deficiency anemia was selected from the Siddha literature *Agasthiyar attavanai vagadam*. [2] Though *Lavanga choornam* (*LC*) has been indicated by Siddha texts for the management of "*Pitha paandu noi*" (Iron deficiency Anaemia) many issues related to deficient scientific evidence concerning the efficacy and safety of the drug remains unanswered. 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. Therefore the study was performed with an objective to determine the acute and long term safety profile of *Lavanga choornam* (*LC*) in experimental animals.

MATERIALS AND METHODS

Test drug

The raw materials *Lavangam* (*Syzygium Aromaticum*), *Elam* (*Elettaria cardamomum*), *Thalisa pathiri* (*Abies*

Webbiana), *Adhimadhuram* (*Glyccirrhiza glabra*) were procured from the local markets of Chennai and were identified and authenticated by the head of the pharmacological department (Gunapadam) of Govt siddha medical college, Chennai, Tamilnadu. The purification process was done according to the procedures mentioned in the Siddha literature. The ingredients of *Lavanga chooranam* were collected, processed and prepared by the methods prescribed in *Agathiyar Attavanai Vaagadam*. [2]

Preparation of drug for dosing:

The acute oral toxicity and repeated oral toxicity test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals. All the drugs used for the study was suspended each time with 1% (w\v) solution of sodium carboxy methyl cellulose before administration. Histamine hydrochloride and fine chemicals used in these experiments were obtained from sigma chemicals company, U.S.A., other analytical grade chemicals were obtained from S.D fine chemicals Ltd, Mumbai.

Experimental animals:

Colony inbred animals strains of 20 Wistar rats of either sex weighing 200- 250 g were used for the toxicological study. The animals were kept under standard conditions 12:12 (day\ night cycles) at 22 ^{O}C room temperature, in polypropylene cages. The animals were fed on standard pellet diet (Hindustan Lever Pvt Ltd, Bangalore) and tap water. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions.

Acute toxicity studies

Colony inbred animals strains of 20 Wistar rats of either sex weighing 200- 250 g were used for the toxicological study. 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 (2000 mg) of the recommended therapeutic dose of test compound the study duration will be 14 days as per OECD guide lines "unclassified". In acute toxicity study, the control and 2000mg/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 hours, then periodically during the first 24 hours. Animals were observed for body weight and mortality for 14 days. At the end of the 14th day all animals were sacrificed and necropsy was done. Group 1 Control animals received distilled water. Group 2 Test group animals received highest dose of 2000 mg \kg \p.o of *LC*. The animals were closely observed for 14 days for behavioural toxicity and for any mortality. On 15th day all the animals were sacrificed for gross pathological changes of the organs. [3]

Repeated oral toxicity study (28 days)

Repeated oral toxicity studies can be used to get additional information regarding the toxicity profile of a chemical. Repeated oral toxicity studies are defined as those studies where the chemical is administered to the animal for a period covering approximately 10 % of the expected life of the animal. Usually, the dose levels are lower than for acute studies and allow chemicals to accumulate in the body before lethality occurs, if the chemicals possess this ability. In Repeated oral toxicity study (28 days) colony inbred animals strains of 20 Wistar rats of either sex weighing 200-250 g were used for the toxicological study. The animals were acclimatized for a period of 7 days prior to drug administration. Group 1 Control animals received 1% sodium carboxy methyl cellulose (LC), 2 ml \ kg \ p.o. for 28 days. Group 2 drugs

suspended in *Lavanga Choornam* was given at dose level of 500mg \ kg\ p.o. for 28 days.

Body weight, food intake and water intake was recorded at two intervals with simultaneous observation for toxic manifestation and mortality, if any. On 29th day all the animals were sacrificed by over dosage of ether anaesthesia. Blood was collected and was used for haematological studies. Sections of liver, kidney, and heart were dissected out and kept in 10% formalin for histopathological studies. [4]

RESULTS AND DISCUSSION

Effect of Siddha formulation (LC) on biochemical markers of liver and kidney after 28 days of repeated oral dosing (500 mg \kg\ po) in rats

Groups	AST (IU\L)	ALT(IU\L)	Cholesterol (Mg\dl)	Urea (mg\dl)	Uric acid (mg\dl)
Control	70.28± 0.972	30.730±0.418	45.271±0.81	24.62±0.648	1.88+0.841
Test Group (LC-500mg\kg\p.o)	77.85±2.582	36.723±2.267	50.813±2.67	23.37±0.34	3.245±0.35

n=6; values are expressed as mean ± S.D followed by students paired 't' test ; Ns= non significant when compared to control groups

Though traditional medicines are widely believed to be safe and free from adverse effects, the above toxicity study was performed to scientifically claim the safety of the Herbal formulation .The results of acute toxicity studies in Swiss albino mice indicated the safety of *Lavanga choornam* (*LC*). There was no weight reduction and reflexes were found to be normal before and after the study. Increment in body weight determines the positive health status of the animals. [5] All other observations such as hematological and biochemical parameters were found to be normal before and after the study. Biochemical parameters have significant roles as a marker in toxicological study because of their response to clinical signs and symptoms produced by toxicants. [6]

ALT is found primarily in the liver and is the most sensitive marker for liver cell damage. AST is found primarily in the red blood cells, cardiac and skeletal muscles, and kidney and is not specific to liver as ALT. The abnormal elevation of the liver enzymes (ALT and AST) is usually associated with liver damage or alteration in bile flow during which, it leaks this enzyme into the blood. [6] Urea clearance falls as the kidney fails and as a result, urea tends to accumulate with diseased kidneys that are unable to excrete these substances at normal rate; this will raise blood urea level. [7-8] In normal adult rat serum urea is measured about 15-45 mg/dl. In the present toxicity study the urea was found to be within the normal range indicating no damage to the kidneys and renal function.

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. The Kidneys showed glomeruli tubules, interstitial cells of normal histology. The other vital organs 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.

The acute toxicity study method uses defined doses (2000 mg \ kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemicals which cause acute toxicity. Since the formulation is relatively non toxic in clinical practice the highest dose of 2000 mg \kg \p.o (as per OECD guide lines "unclassified ") was used

in the acute oral toxicity study. Repeated oral toxicity studies are defined as those studies where the chemical is administered to the animal for a period covering approximately 10 % of the expected life of the animal. Usually, the dose levels are lower than for acute studies and allow chemicals to accumulate in the body before lethality occurs, if the chemicals possess this ability.

CONCLUSION

Siddha medicine formulation mainly made up of herbs, metals, herbs and metal in combination. This study medicine mainly made up of herbs, with proper purification showed no toxicity and hence proved safe for treatment. The study drug *lavanga choornam* is recommended for human usage. The test drug *lavanga choornam* at the dose of 500 mg \kg and at the dose of 2000 mg\kg showed no mortality changes in rats. No behavior changes were noted for the first 4 hours and for the next 24 hours and throughout the study period of 14 days of the acute toxicity study. No reduction in weight, in complete course of the study. Normal Reflexes were found throughout the study. All other observations were found to be normal before and after study. In necropsy, the organs of the animal such as liver, heart, lungs, pancreas, spleen, stomach, intestine, kidney, urinary bladder, uterus all appeared normal.

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

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