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Research article

Toxicological evaluation of a Siddha Medicine *Vembu Karpam* (*Azadirachta indica*): Acute oral toxicity studies

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Abstract

The test drug *Vembu karpam* composed of of chief ingredient Azadirachta indica which is referenced from *Bogar Vaithiya Saram 700*. It has wide clinical role and majorly used to alleviate vatha related disorders especially *Uthara vatha suronitham*.

The aim of the present study was to evaluate the *in vivo acute oral* toxicity Siddha herbal formulation *Vembu karpam*. Acute oral toxicity is carried out as per the OECD-423 guidelines after the animal ethical clearance from Institutional Animal Ethics Committee.

In the acute toxicity study female albino Mice administered single oral dose (0, 300, 2000 mg/kg) of *Vembu karpam*, and observed the physical symptoms and behavioral changes for the crucial phases of the study. In the acute toxicity study, no such mortality or behavioural changes were observed in the treated animals with a varied single dose of *Vembu karpam* upto 2000 mg/kg, which is considered as highly tolerable doses.

These results exhibit the absence of acute oral toxicity after treatment of *Vembu karpam* in rats. However, further clinical studies humans are needed in order to have sufficient safety evidence for its use in humans.

Keywords

Vembu karpam, Siddha Medicine, Toxicity studies

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Introduction

In this current scenario, the plants from herbal medicines such as Siddha and Ayurveda formulations, provides unlimited opportunities for the discovery of new drugs for different diseases.

Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. However, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to the patients.

In relation to drug discovery and development, there are different weights of concern of all relevant groups such as health authorities, pharmaceutical industry, and patients which need to be taken into consideration.

The general public, patients and consumers are primarily interested in fast access to safe and efficient drugs, as well as in animal welfare. Based on their long-term use by humans one might expect plants used in traditional medicine to have low toxicity.

This raises concern about the potential toxic effects resulting from the short-term and long-term use of such medicinal plants. Therefore, evaluating the toxicological effects of the drug *Vembu karpam* intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects.

Azadirachta indica L. (neem) shows therapeutics role in health management due to rich source of various types of ingredients.

The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin.

Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7- benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17 -hydroxyazadiradione, and nimbiol Preliminary studies revealed that neem oil has acaricidal, antibacterial, antifungal, antimalarial, antiparasitic, anti-inflammatory as well as immunomodulatory properties in different animal species

Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of such compounds. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals (Shetty Akhila, *et al.,* 2007).

Materials and Methods

Ingredients

100 years old Vembu (Azadirachta indica) - powder.

Part used – Bark

100 years old bark of Neem Tree

In this study, we have collected *100* Year old neem bark from the traditional area of Papanasam. In traditional siddha literature, the role of the vembu is quite higher and the life time of plant based preparation are discussed many.

This type of plant has major role in engulfing the clinical conditions of various origins.

Authentication of drug:

The herbal ingredients are authenticated by Botanist of Department of Medicinal Botany, Government Siddha Medical College, Palayamkottai, Tamilnadu.

Purifications of Drugs:

The Bark of Vembu was collected outer dead bark are removed off. And other foreign particles is observed and were also cleaned. The inner bark were cut into small pieces and dried in shade. After fully dried condition the bark was once again cleaned.

Method of Preparation:

The purified dried 100 years old Vembu bark is finely powdered.

Animal Studies

Selection of Animals

Healthy adult Wistar albino rat weighing between 170-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU).

Laboratory standardization protocols

A 12 light / dark cycle were maintained. Room temperature was maintained between 22 + 2o C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*.

Animal Acclimatization Procedure

All the selected animals were acclimatized in a separate cage in the laboratory for 7 days prior to the start of the study.

Ethical considerations

Acute oral toxicity of Vembu Karpam is carried out as per the guidelines Organization of Economic Co-operation and Development (OECD) -423 guidelines after the animal ethical clearance from Institutional Animal Ethics Committee.

Cage Side Observations

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern.

Special attention is directed for the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Body Weight, Food and Water Intake

Body weight, food and water intake are recorded at two -day intervals.

Experimental Methods

The albino mice are fasted over night and provided only water, after which the Vembu Karpam is administered by gastric intubations to the relevant group of animals orally at the dose of 50 mg.kg^{-1} body weight in Tween-80. The animals are then observed for 14 days and maintained with normal food. A mortality rate of 2 or 3 animals in 14 days is recorded and the dose is said to be toxic dose. But when mortality of one animal is observed, then the same dose is repeated again for confirmation. However, if mortality is not observed, the procedure is repeated for further higher doses such as 300 and 2,000 mg.kg^{-1} body weight. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality (Shah Ayub, 1997, Bürger, 2005).(2,3).

Results

Acute toxicity study with Vembu Karpam

The acute toxicity of Vembu Karpam was evaluated using OECD- 423 guidelines. There was no mortality or morbidity observed in animals through the 15-days period following single oral administration at all selected dose levels of the Vembu Karpam (Table-1).

The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose appeared normal(Figure 1).

No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self mutilation, walking backward and so forth were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal.

	Dose (mg.kg^{-1})	Sign of Toxicity (ST.NB^{-1})	Mortality (D.S^{-1})
Group I	0	0/3	0/3
Group II	300	0/3	0/3
Group III	2000	0/3	3/3

Table.1. Acute toxicity study of Vembu Karpam on experimental mice. The acute toxicity of Vembu Karpam on experimental mice was tested using OECD-423 guidelines, where ST- sign of toxicity; NB- normal behaviour; D- died; S- survive. Values are expressed as number of animals (n=3).

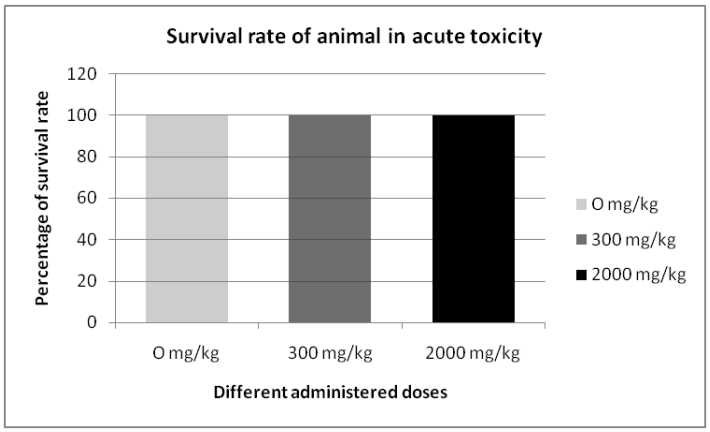


Figure 1. Survival rate in acute toxicity has showed in graphical representations

Histopathology studies

Macroscopic examination of the vital organs of treated animals revealed no abnormalities except liver. Histopathological representative images of liver are shown between Figure 1-5. In this Liver section of normal control rats showing normal liver lobular architecture with well brought out central vein and prominent nucleus and nucleolus.and in high dose range, it shows mild inflammation and which is in tolerable state.

Discussion

For centuries, natural products, such as medicinal plants have been the basis for the treatment of various ailments. However, many medicinal plants have also been reported to be toxic to both humans and animals.

Therefore, it should be emphasized that the traditional use of any plant for medicinal purposes, by no means, guarantees the safety of such plant.

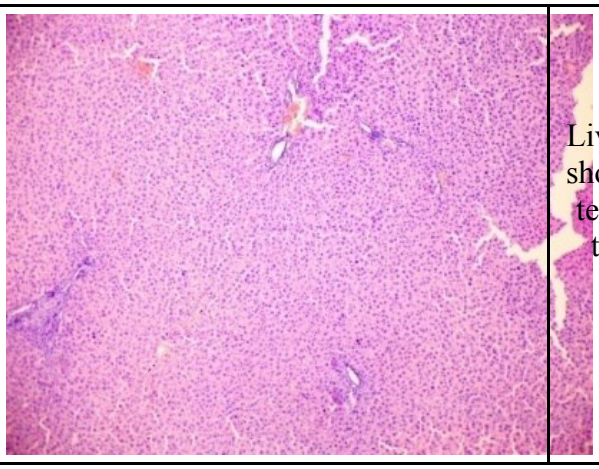
Furthermore, the data of the acute and subchronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in its safety to humans, particularly for use in the development of pharmaceuticals

The further evaluation of sub acute and sub-chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the Siddha drug *Vembu Karpam*.

Therefore from this study it is suggesting that, there is no significant toxic in acute oral toxicity studies animals.

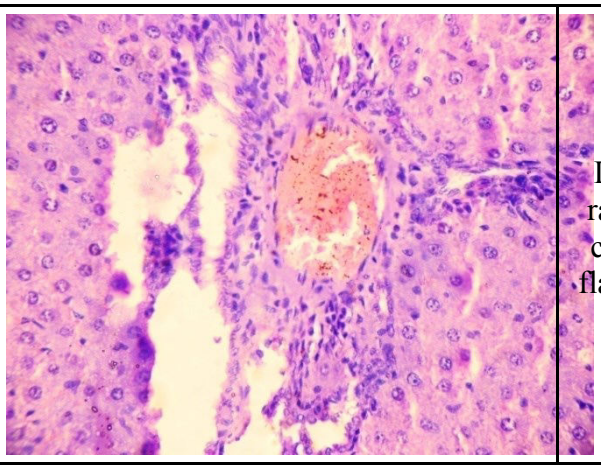
It is suggesting that, the drug has no such toxic effects. It doesn't produced any unwanted clinical signs for toxicity or mortality in selected animals.

Fig. 2 Histopathological Studies of liver tissue



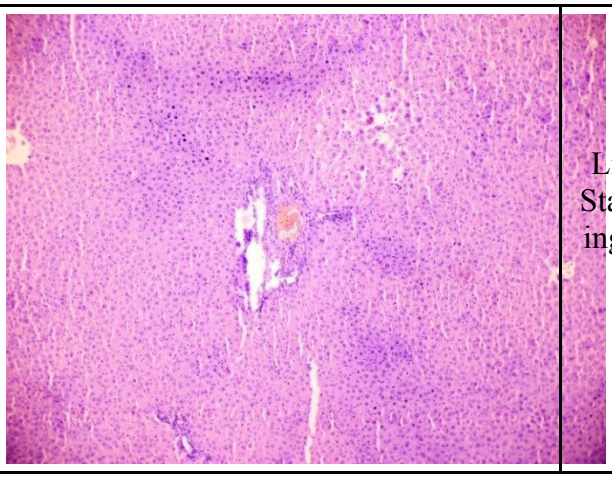
Liver section of normal control rats showing normal liver lobular architecture with well brought out central vein and prominent nucleus and nucleolus.

Fig. 3 Histopathological Studies of liver tissue



Liver section Chromium treated rats showing severe toxicity with congested blood vessels with inflammatory cell collection and endothelial cell swelling.

Fig. 4 Histopathological Studies of liver tissue



Liver section of rats treated with Standard drug + Chromium showing only a few inflammatory cells around portal tract.

Fig. 5 Histopathological Studies of liver tissue

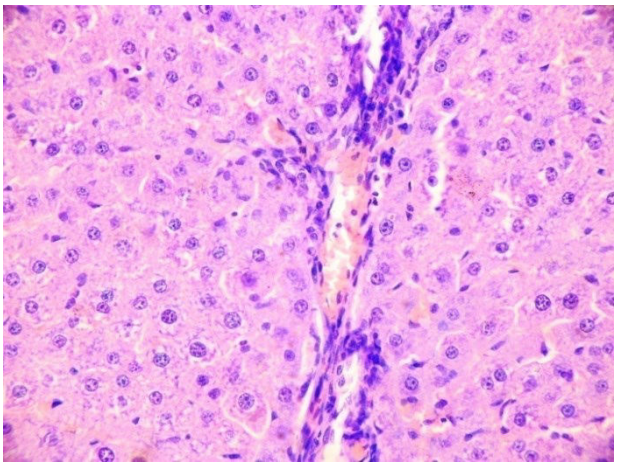
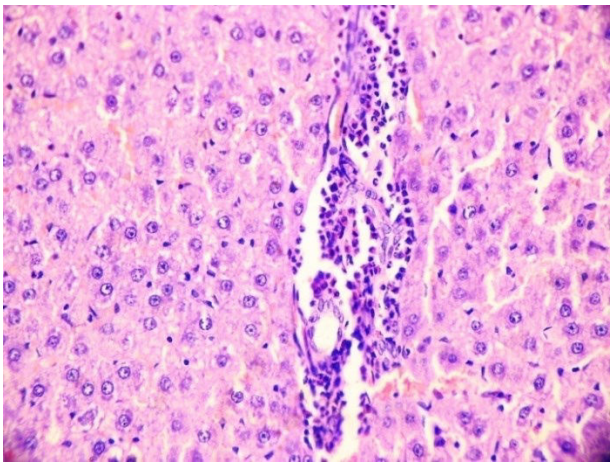
	Liver section of rats treated with siddha formulation vembu karpam (100mg/kg) + Chromium showing only slight inflammation.

Fig. 6 Histopathological Studies of liver tissue

	Liver Section of rats treated with siddha formulation vembu karpam+ Chromium showing only slight inflammation.

Discussion

For centuries, natural products, such as medicinal plants have been the basis for the treatment of various ailments. However, many medicinal plants have also been reported to be toxic to both humans and animals. Therefore, it should be emphasized that the traditional use of any plant for medicinal purposes, by no means, guarantees the safety of such plant. Furthermore, the data of the acute and subchronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in its safety to humans, particularly for use in the development of pharmaceuticals.

The further evaluation of sub acute and subchronic dosing in experimental animals may be more relevant in determining the overall toxicity of the Siddha drug *Vembu Karpam*. Therefore from this study it is suggesting that, there is no significant toxic in acute oral toxicity studies animals. It is suggesting that, the drug has no such toxic effects. It doesn't produced any unwanted clinical signs for toxicity or mortality in selected animals.

Conclusion

The preliminary results suggest promising this drug vembu karpam doesn't produce any toxic events and it is safe for the administration to the humans. The further evaluation of sub acute and sub-chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the Siddha drug *Vembu Karpam*

Conflict of Interest

None declared

Source of funding

Nil

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