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Acute Toxicity Study of Siddha Varma Formulation Neerizhivu Kudineer in Albino Wister Rats

Dr. G. Thirunarayanan[1*], Dr. Binthuja G Dharan[2], Dr. S.Sujatha[3], Dr. A.Muneeswaran[4]
[1,2]PG Scholars, [3]Lecturer, [4]Head of the Department
[1, 3, 4]Department of Varma Maruthuvam, [2]Department of Siddhar Yoga Maruthuvam,
Government Siddha Medical College and Hospital, Palayamkottai, Tirunelveli,Tamilnadu, India

***Corresponding Author:**
Dr. G. Thirunarayanan
PG Scholar, Department of Varma Maruthuvam, Government Siddha Medical College and Hospital, Palayamkottai, Tirunelveli, Tamilnadu, India

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ABSTRACT
Varmam is a gem of Tamil culture which includes both internal medicine and external therapies and it is the part of Siddha system of medicine. People commonly use Siddha medicine and Varma medicine in their day to day life because of its non-toxicity effects. The Trail drug Neerizhivu Kudineer is an herbal formulation quoted in ancient Varma text for Neerizhivu (Diabetes mellitus). To ensure the non-toxic effect of herbal formulations this study aims to assess the acute toxicity of Siddha Varma formulation Neerizhivu Kudineer in albino wister rats. Neerizhivu Kudineer were analysed for its acute toxicity profile with reference to behavioural aspects, in Albino Wister rats. The limit test dose of 1000 and 2000mg/kg body weight was used following OECD guidelines. The results reveals there are no toxicity in Neerizhivu Kudineer.

Keywords: Neerizhivu Kudineer, Neerizhivu, Diabetes mellitus, Toxicity, Varma, Siddha

INTRODUCTION

Varmam is a part of Tamil culture and Siddha system of medicine. Varmam is being used to treat many illnesses that affect human beings from ancient times. Both internal medicines and external therapies are being used in Varma Maruthuvam. Siddha medicine and Varma medicines are safe and non-toxic, but it must be ensured before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one among the battery of toxicity tests that are used [(1)]. Varma Odivu murivu sara soothiram-1200 is an ancient Varmam text which indicates Neerizhuvu Kudineer for Neerizhivu (Diabetes mellitus). The toxic effect of Neerizhivu Kudineer is to be evaluated before it is used for therapeutic purpose in humans. Hence Neerizhivu Kudineer was analysed for its acute toxicity profile with reference to OECD guidelines [(2, 3)] in Albino Wister rats.

Materials and Methods:

Ingredients of Neerizhivu Kudineer

Table No. 1: Drug Description [6, 7]

S.No	Drugs	Botanical Name	Family	Parts Used
1	Alam Vithai	*Ficus benghalensis*	Moraceae	Seed
2	Vilam Pisin	*Limonia acidissima*	Rutaceae	Gums

Collection, Identification and Authentication of the Drug:

The drugs mentioned in the Table No.1 were collected from Tirunelveli and Kanyakumari district, Tamilnadu, India. Collected raw drugs were identified and authenticated by Botanist of Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu.

Purification and Preparation of the Drug:

The ingredients of this Neerizhivu Kudineer were purified according to the proper methods described in Siddha Classical Literature.

Experimental animals

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423[4]. The institutional ethical committee of K.M. College of Pharmacy, Madurai, Tamilnadu, India approved the protocol for these experiments.

Experiments were performed using healthy young adult female albino wister rats nulliparous, non-pregnant and weighing 25-30 g. Female rats were chosen because of their greater sensitivity to treatment[5].

Assignment of animals

The animals were randomly divided into two groups each containing five rats. They were identified by the markings using a yellow stain. One rat was unmarked and the others were marked on head to ease the observation.

Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2ºC). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets with water ad libitum.

Mode of administration

The test substance was administered in a single dose by gavage using specially designed rats oral needle. Animals were fasted 3 hr prior to dosing (only food was withheld for 3 hr but not water).

Administration Dose

Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 1000, and 2000 mg/kg. After the administration of test substance, food for the rats was withheld for 2 hr.

Test substance administration volume.

The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Observation period

Animals were observed individually after at-least once during the first 30 min, periodically during the first 24 hr, with special attention given during the first 4 hr, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

Signs recorded during acute toxicity studies

Direct observation parameters include tremors, convulsions, salivation, diarrhea, lethargy, sleep and

Volume 4, Issue 3; May-June 2021; Page No 387-391
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coma. Skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 hr. The number of survivors was noted after 24 hr and then these were maintained for a further 14 days with a daily observation.

Statistical Analysis

Data are presented as a mean ± SEM (Standard Error of the Mean). Comparisons were made between the treated groups by the use of single way Analysis Of Variance (ANOVA). All data were analyzed using newmann keuls multiple range tests. Graphpad instat version 3.1. $P< 0.05$ was considered as the level statistical significance.

RESULTS

The present study conducted as per the OECD guidelines 423 revealed that the Siddha formulation Neerizhivu Kudineer did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. The oral LD50 was indeterminable being in excess of 2000mg/kg body weight. So, testing the Siddha formulation Neerizhivu Kudineer at a higher dose may not be necessary and the Siddha formulation Neerizhivu Kudineer were practically non-toxic. Table 2 indicates the parameters observed after the administration of the test substance. The writhing reflux was observed immediately upto 15 min after administration of the test substance at all administered doses for the Siddha formulation Neerizhivu Kudineer. Whereas all the other parameters observed were normal even at the highest dosage of 2000mg/kg body weight of the test animal. This clearly indicated that the Siddha formulation Neerizhivu Kudineer do not produce oral toxicity. The medium lethal dose (LD50) of the Neerizhivu Kudineeris higher than 2000 mg/kg body weight and hence, in a single dose administration, the Siddha formulation Neerizhivu Kudineer had no adverse effect. From the statistical analysis of the dosage administered to the animals, it was found that the values are significant at 5%.

Table2: Effect of Siddha formulation Neerizhivu Kudineer on acute oral toxicity test in rats

S.NO	OBSERVATION	GROUP-1 Neerizhivu Kudineer (1000mg/kg)	GROUP-2 Neerizhivu Kudineer (2000Mg/kg)
1.	Alertness	Normal	Normal
2.	Grooming	Absent	Absent
3.	Touch response	Absent	Absent
4.	Torch response	Normal	Normal
5.	Pain response	Absent	Absent
6.	Tremors	Absent	Absent

Volume 4, Issue 3; May-June 2021; Page No 387-391
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7.	Convulsion	Absent	Absent
8.	Righting reflux	Present	Present
9.	Gripping strength	Normal	Normal
10.	Pinna reflux	Normal	Normal
11.	Corneal reflux	Present	Present
12.	Pupils	Normal	Normal
13.	Urination	Normal	Normal
14.	Salivation	Normal	Normal
15.	Skin colour	Normal	Normal
16.	Lacrimation	Normal	Normal
17.	Hyper activity	Absent	Absent

DISCUSSION:

The non-toxic nature of Varma medicine Neerizhivu Kudineer is evident by the absence of mortality of the test animals at oral treatment of 2000mg/ kg body weight. For any therapeutic and cosmetic application, compounds of the Siddha formulation used must be practically non-toxic. Hence to establish the non-toxic nature of Varma medicine Neerizhivu Kudineer, acute oral toxicity of Neerizhivu Kudineer was tested. The non-toxic nature of the Varma medicine Neerizhivu Kudineer is evident from the acute oral toxicity conducted as per OECD guidelines. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the foresaid Neerizhivu Kudineer. Hence Varma medicine Neerizhivu Kudineer could be safe up to the dose of 2000 mg/kg body weight of the animal.

CONCLUSION:

This study reveals the non-toxic nature of the foresaid phytochemicals at the tested dosage. Hence, the Varma medicine Neerizhivu Kudineer may be exploited for its use in product application like pharmaceuticals/ nutraceuticals/ cosmeceuticals. Further studies are warranted for determining chronic toxic symptoms.

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Volume 4, Issue 3; May-June 2021; Page No 387-391
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Volume 4, Issue 3; May-June 2021; Page No 387-391
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