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Research article

Safety studies of Siddha Medicine *Karunjeeraga chooranam* in acute and subacute toxicity wistar rat models

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Abstract

Background

Herbal formulations are frequently recommended in day to day clinical practice for therapeutic purposes by many Siddha practitioners. However, the safety of Siddha herbal drugs remains a challenge when communicating to the scientific community because of the high variability of phyto-chemical components involved.

Objective

To investigate the acute and subacute oral toxicity of *Karunjeeraga chooranam* in adult female wistar rat models.

Materials and Methods

In acute oral toxicity study, *Karumjeeraga chooranam* were administered orally initially at the dose of 50 mg/kg/body weight which was increased up to 2000mg/kg/body weight and animals were observed for toxic symptoms till 14 days as per the OECD-423 guidelines.

For sub-acute toxicity study, the *Karunjeeraga chooranam* were administered for 20 days, with the doses of 50 mg, 100 mg, 200 mg and 400 mg/kg/ body weight for different groups. At the end of 20th day, the animals were sacrificed and toxicity parameters were assessed. Biochemical analysis on blood samples and histo-pathological evaluation of different organs were also performed to assess any toxicity.

Results

In acute toxicity study, no mortality was found at a dose of 2000 mg/kg .So the dose is taken as oral LD50 of *Karunjeeraga chooranam*. The administration of drug at varied doses upto 400mg/kg/body weightfor20 days did not produce any significant change in haematological and biochemical parameters of wistar rats as compared to normal control group. No pathological changes were observed in histology of various organs of treated rats as compared to normal control animals.

Conclusion

The *Karunjeeraga chooranam* was found to be safe when administered to adult wistar rat models for longer duration and in maximal LD50 doses.

Keywords

Siddha medicine, Nannari ver Kudineer, acute & sub acute studies.

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Introduction

The trial drug *Karunjeeragachooranam*(4), primarily composed of seed *Karunjeeragam* (*Nigella sativa*), which belongs to the Ranunculaceae family. It is an annual herb with many pharmacological properties. The seeds of *Karunjeeragam* are used in the treatment of various diseases like bronchitis, diarrhea, rheumatism, asthma and skin disorders. It contains many active components, such as thymo-quinone (TQ),(1,2) alkaloids (nigellicines and nigelledine), saponins (alpha-hederin), flavonoids, proteins, fatty acids, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, tanethol, sesquiterpene, longifolene, α-pineneand thymoletc that yields positive effects in the treatment of patients with different diseases &has been extensively studied for its biological activities and shown to possess wide spectrum of activities such as diuretic, hypolipidemic, antihypertensive, bronchodilator, gastroprotective, hepatoprotective, hypoglycemic, anticancer and Immunomodulatory, analgesic, antimicrobial, anti-inflammatory, spasmolytic, renal protective and antioxidant properties(3).

Methods and materials

Maintenance of Test Animals

The study was carried out in the Department of Pharmacologywith the approval of the InstitutionalAnimalEthicsCommittee,IAEC/D.ARIVOLI/*TNMGRMU/MD (S)/321611001/KMCP/23/2018* KM College, Tamilnadu. Adult female Wistar albino rats (150–200 g) were used in the study. Animals werehoused under standard laboratory conditions at 25 ± 2°C, and humidity levels were in the range of 30 - 70% in groups with free access to food and water ad libitum. They were acclimatized to the laboratory conditions for a period of 2 days before the study(5).

Drug for the Toxicity study

The raw drug was authenticated by Botanists of Government Siddha Medical college, Palayamkottai, Tirunelveli. Then it was subjected to purification. The proposed drug *Karunjeeraga chooranam*was prepared as per Gunapaadam – Mooligai vaguppu text book (page no:464) and followed the Standard Operating Procedure for the preparatory process.

Acute toxicity study

Acute oral toxicity test was performed as per the OECD 423 guidelines (OECD, 2001). All the animals were randomly distributed into three different treatment groups. The female Wistar rats (n = 3) were weighed, and the dose was calculated in reference to the body weight. The rat models were fasted overnight and provided only with water, after which the *Karunjeeragachooranam*(4,6)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. If the mortality rate of 2 or 3 animals in 14 days is recorded and the dose is considered 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(7).

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 was directed towards the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma(8).Body weight, food and water intake were recorded at two-day intervals. Surviving animals were fasted overnight, weighed and euthanized on the 15th day using anesthetic ether. All test animals were subjected to gross necropsy.

Sub-acute test for *Karunjeeraga chooranam*

This experiment evaluates the toxicity potential of *Karunjeeraga chooranam* for more than 14 days. Male and Female Wistar rats weighing 180 ± 10 g were used for this study(9). The animals were divided into five groups of six animals each. The dose of the drug administered was calculated on the basis of body weight of the animal. The animals in Group I were administered with a single daily dose of 0.5 ml of Tween 80orally for 20 days. The animals in Group II were administered with 50 $mg.kg^{-1}$b.w. of the *Karunjeeraga chooranam*orally once in a day for 20 days. The animals in Group III were administered with 100 $mg.kg^{-1}$b.w. of the *Karunjeeraga chooranam* orally once daily for 20 days. The animals in Group IV and V were administered once daily with 200 and 400 $mg.kg^{-1}$b.w. of the *Karumjeeraga chooranam* respectively for 20 days orally (Pieme,*et al* 2006, Joshi, *et al* 2007, Mythilypriya, *et al.,* 2007).

The animals were weighed during every five days, from the initial day of the treatment, to record the weight variation. At the end of the treatment, blood samples were collected from eyes by puncturing retro orbital plexus after mild anesthesia for biochemical analysis. The collected blood sample was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma. Itis then analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels, LDL-cholesterol, plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea(10).

Results

Acute toxicity study with *Karunjeeraga chooranam*

In acute toxicity, there was no mortality or morbidity observed in animals through the 14-days period following single oral administration at all selected dose levels of the *Karunjeeragachooranam* (Table-1). The animals did not show any changes in the general appearance during the observation period.

	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	0/3

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

Table.2.The effects of Karunjeeraga chooranam on body weight changes in rats

Treatment	Day 1	Day 5	Day 10	Day 20
Control	187.16±6.13	187.45 ±6.20	196.14 ±6.35	196.74±6.24
Karunjeeragachooranam 50 mg.kg^{-1}	196.30 ±6.4	194.30 ±6.30	199.25 ±6.70	199.35±6.72*
Karunjeeragachooranam 100 mg.kg^{-1}	187.35 ±5.7	190.30 ±6.40	197.55 ±7.10	198.36±6.40*
Karunjeeragachooranam 200 mg.kg^{-1}	196.35 ±7.2	199.15±6.50	199.90 ±7.20**	207.41±7.22**
Karunjeeragachooranam 400 mg.kg^{-1}	189.67 ±6.05	193.15 ±5.60	196.68 ±6.35**	208.65±7.38**

Table: 3 The effects of Karunjeeraga chooranam on kidney, heart, liver and brain of the rats

Treatment	Heart (gms)	Kidney (gms)	Liver (gms)	Brain (gms)
Control	0.39 ± 0.07	0.69± 0.06	3.35± 0.08	0.70± 0.08
Karunjeeraga chooranam@50 mg.kg^{-1}	0.40± 0.02	0.85± 0.03	3.47± 0.03	0.73± 0.33
Karunjeeraga chooranam@100 mg.kg^{-1}	0.41± 0.06	0.83± 0.04	3.39±0.06	0.71± 0.22
Karunjeeraga chooranam@200 mg.kg^{-1}	0.40± 0.08	0.78± 0.06	3.37± 0.07	0.78± 0.08
Karunjeeraga chooranam@400 mg.kg^{-1}	0.39± 0.07	0.79± 0.08	3.39± 0.05	0.80± 0.08

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01.

Table.4.The effect of Karunjeeraga chooranam on biochemical parameters

Treatment	Glucose (mg.dl^{-1})	Cholesterol (mg.dl^{-1})	Triglyceride (mg.dl^{-1})	HDL (mg.dl^{-1})	LDL (mg.dl^{-1})
Control	92.65± 0.62	37.62± 0.56	26.25± 0.45	133.25± 0.55	82.15±1.72
Karunjeeraga chooranam@ 50 mg.kg^{-1}	90.50± 0.56	23.85± 0.25*	11.22± 0.23*	173.28± 0.65*	69.59±1.28
Karunjeeraga chooranam@ 100 mg.kg^{-1}	89.50± 0.42	26.79± 0.28*	15.47± 0.30*	165.82±0.81*	69.89±1.12
Karunjeeraga chooranam@ 200 mg.kg^{-1}	90.30± 0.57**	33.23± 0.32	17.89± 0.40*	184.35± 0.83*	42.65±1.60
Karunjeeraga chooranam@ 400 mg.kg^{-1}	86.30± 0.47**	32.83± 0.31	17.28± 0.34*	182.7± 0.87*	46.55±0.86

Table.5.The effects of Karunjeeraga chooranam on biochemical parameters such as AST, ALT, ALP, TP and Albumin in rats.

Treatment	AST (IU.l^{-1})	ALT (IU.l^{-1})	ALP (IU.l^{-1})	TP (g.l^{-1})	ALBUMIN (g.l^{-1})
Control	328.10±12.45	71.10± 3.21	253.58± 8.82	69.90± 3.37	39.20±2.49
Karunjeeraga chooranam@50 mg.kg^{-1}	317.0±9.55**	69.5± 2.22**	266.15± 2.77**	70.35± 2.35	36.35±2.67
Karunjeeraga chooranam@ 100 mg.kg^{-1}	318.8±7.25**	67.6± 3.20**	260.19± 6.76**	80.20± 2.82	38.35±3.08
Karunjeeraga chooranam@ 200 mg.kg^{-1}	331.4±7.97	62.4± 2.96	265.00± 5.22	69.25± 3.34	40.25±2.78
Karunjeeraga chooranam@ 400 mg.kg^{-1}	323.2± 8.25	64.3± 3.57	269.40± 4.45	74.05± 2.63	39.48±2.75

Table.6.The effect of HB, Calcium, RBC and WBC in rats

Treatment	Haemoglobin ($mg.dl^{-1}$)	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium ($mg.dl^{-1}$)
Control	15.3± 0.30	11.15± 0.02	13.45± 0.05	11.40 ±0.08
Karunjeeragachooranam@ 50 $mg.kg^{-1}$	16.55± 0.31*	11.45± 0.06*	11.5± 0.01*	11.21 ±0.03
Karunjeeragachooranam@ 100 $mg.kg^{-1}$	16.35± 0.15*	11.55± 0.02*	10.3± 0.32*	11.27 ±0.20
Karunjeeragachooranam@ 200 $mg.kg^{-1}$	14.27± 0.20*	10.32± 0.12*	13.4± 0.03*	11.56 ±0.13
Karunjeeragachooranam@ 400	15.5± 0.35*	10.46± 0.45*	12.5± 0.13*	11.70 ±0.02

The **Karunjeeraga chooranam** was also evaluated for subacute toxicity .The effect of Karunjeeragachooranam on the body weight changes shows that, there was significant increase ($p<0.05$) in body weight in all the animals observed. The results are shown in Table.2.

Effect of *Karunjeeraga chooranam* on kidney, heart, liver and brain in rats

The effects of *Karunjeeraga chooranam*on kidney, heart, liver and brain of the rats were observed. From the study it was clear that, significant ($p<0.01$) changes in the weights of various organs of the animals occurred with higher doses of the extract (400 $mg.kg^{-1}$bwt), but macroscopic examinations did not show any changes in colour of the organs of the treated animals when compared with the control. The results are shown in Table.3. Table.3.The effects of *Karunjeeraga chooranam* on kidney, heart, liver and brain of the rats. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01.

Effect of *Karunjeeraga chooranam*on biochemical profiles of rats

The effect of *Karunjeeraga chooranam*on various biochemical parameters of the experimental animal 'rats' were tested. From the study it was evident that, there was significant decrease ($p<0.05$) in the plasma glucose level in treated rats especially at higher dose (400 $mg.kg^{-1}$) when compared with control rats. The control rats were administered only with 5 ml of normal saline. Significant decrease ($p<0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels were observed.

But a significant increase ($p<0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control animals. AST, ALT and ALP levels were also normal in the *Karunjeeraga chooranam* treated animals. From the results of biochemical study, there was no evidence of severe toxicity associated with the administration of higher concentration of *Karunjeeraga chooranam*. The results are shown in Table.4.

In Table.4.The effect of *Karunjeeraga chooranam*on biochemical parameters such as glucose, cholesterol, triglyceride, HDL and LDL. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05

Table.5.The effects of *Karunjeeraga chooranam*on biochemical parameters such as AST, ALT, ALP, TP and Albumin in rats. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Effect of *Karunjeeraga chooranam*on haematological parameters in rats

The effects of *Karunjeeraga chooranam*were observed for its effect on haematological parameters on the experimental rats. A significant increase ($p<0.01$) were observed in the haemoglobin contents and RBC count in the group treated with 400 $mg.kg^{-1}$ body weight of *Karunjeeraga chooranam*. There was no significant change in the calcium level in all the treated animals compared to the control. Table.6.The effect of *Karunjeeraga chooranam*on haematological parameters such as HB, Calcium, RBC and WBC in rats. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV and V. The statistical analysis was carried out using one way ANOVA method, where *P<0.05.

Discussion

The acute toxicity study of *Karunjeeraga chooranam* was carried out as per OECD-423 guidelines, no mortality was observed in both the animals of control group as well as animals treated with a maximum dose of 2000 $mg.kg^{-1}$. Hence, $1/10^{th}$ of 2000 $mg.kg^{-1}$ i.e. 200 $mg.kg^{-1}$ of dose was selected as a minimum dose for Chronic toxicity study. The results of sub-acute toxicity study shows that there was no significant change in animal behavior due to the absence of toxicity.

There was a slight decrease in plasma glucose level when higher doses of *Karunjeeragachooranam*(400 $mg.kg^{-1}$) was administered in the treated rats. Interestingly, significant increase in the levels of hemoglobin was found in treatment with *Karunjeeraga chooranam*with a higher dose of 400 $mg.kg^{-1}$.

The possible reason could be that one of the constituents *Karunjeeraga chooranam* may increase absorption of iron. The overall results suggest that *Karunjeeraga chooranam* is non toxic to the haaematopoietic and leucopoietic system.

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Conflict of Interest :

None Declared

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