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Preclinical Toxicological Evaluation of Siddha Formulation, Kabasura Kudineer by Sub-Acute Toxicity Studies

Parvatharajakumaran V.S.[1], Deepika Rani S.[1], Premkumar B.[1*], Leela Priyanka G.C.[1], Sruthy Jacob[1], Rabiyamma S.[1] and Syed Hussain Peeran S.[2]

[1]Department of Pharmacology, K.K. College of Pharmacy, India.
Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai (Tamil Nadu), India.
[2]Department of Zoology, RKM Vivekananda College, Affiliated to University of Madras (Tamil Nadu), India.

(Corresponding author: Premkumar B.)*
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ABSTRACT: Siddha system of medicines treats diseases using herbs, metals and minerals. One such gem of siddha formulation is Kabasura kudineer which efficient in treating respiratory disorders. Due to the lack of systemic data the current study conducted to evaluate the safety profile of the choornam in rats by OECD subacute toxicity. A total of 30 rats divided into 15 male and 15 female were grouped in 3 at three dose levels Kabasura kudineer 200mg, 400mg, 800mg. Body weight evaluated every week. Food consumption and water intake were noted every day. After 28 days the animals were sacrificed, blood and organ were collected haematological analysis, Biochemical parameters performed using the blood. The organs used for histopathological studies. The subacute toxicity proved that no toxicity or symptoms were seen in the rats and the study should be proceeded with non-rodent species.

Keywords: Kabasura kudineer, Subacute toxicity, Sprague Dawley rats, Haematology, Biochemistry.

INTRODUCTION

With its roots in Tamil Nadu, South India, the Siddha system of medicine is one of the oldest forms of traditional medicine. As it places a strong emphasis on both physical and spiritual welfare, it not only treats the body but also the mind and soul. According to Siddha medicine, disease is caused by an imbalance of the three biological components of each particular human being—Vatha, Pitha, and Kapha—and is treated using herbs, metals, and minerals. The classification of medications into internal and exterior uses was based on the method of application. On the basis of their form, mode of use, shelf life, and other factors, they were further divided into 32 groups (Mekala and Murthy 2020). The Government of India has recommended two of the 32 internal medications listed above, known as Kabasura Kudineer (KSK) for corona viral disease-19 (COVID-19) and swine flu (Kiran *et al*., 2022).

Given that the lungs are the primary organ of kapha, Kabasura Kudieer Choornam, a polyherbal Siddha formulation with 15 constituents, is advised for the efficient management of common respiratory disorders such colds, cough, difficulty breathing, and flu (Natarajan *et al*., 2020). Kaba stands for kapha dosha, which signifies fever brought on by an excessive build-up of kapha. (Mucus, phlegm) Asura represents symptom-relieving plants, kudineer means a decoction, and choornam is a powder (Maideen, 2021). According to reports, the preparation has antibacterial, antipyretic, and anti-inflammatory properties.

Despite the fact that KSK is often used in therapeutic settings, systematic data about its safety are lacking. As a result, the current study was undertaken with the following objective: to evaluate the safety of this preparation. KSK was administered to rats 28 times over the course of 28 days in order to measure the serum and other bodily organ levels and assess the acute toxicity in rats.

MATERIALS AND METHODS

Male and female Sprague Dawley rats that were six weeks old when they were acquired from Mass Biotech were provided. Before being kept in a room that was devoid of any known diseases, kept at a temperature of 24.0°C 1.0°C, a humidity range of 50%, and a light/dark cycle of 12 hours, the animals were given a week to acclimatise. This animal experiment was conducted in accordance with the guidelines for chemical testing. (Organization for Economic Cooperation and Development Guideline 407, entitled "Repeated Dose 28-day Oral Toxicity studies of drugs") (Toyoda *et al*., 2000). Institutional animal ethics committees (IAEC) approved this study in accordance with the standards set out by the Committee for the Control and Supervision of Experimental Animals (CPCSEA).

Rats were separated into three groups, each group containing a total of 10 rats (15 males and 15 females), for a total of 30 rats (Five males and five females).

Three distinct dose levels of KSK were administered orally: 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively. Six rats from the control group—three males and three females—were given 10 millilitres of distilled water for every kilogramme of body weight.

Body weight, food and water consumption. Each animal's body weight was monitored on a weekly basis during the trial. Based on the measured body weights, individual doses were calculated, and these doses were subsequently adjusted on a weekly basis to maintain accuracy. It was calculated and noted how much food each group consumed each week.

Haematology

All of the animals were put to sleep with isoflurane before having their hearts punctured to collect blood samples, which were then deposited in anticoagulant tubes. All of the samples were centrifuged for 15 minutes at a speed of 3000 revolutions per minute within an hour after blood collection to obtain plasma. Red blood cells, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were among the haematological parameters that were analysed using the fully automatic H360 cell counter.

Clinical Biochemistry. It was necessary to puncture the heart in order to get blood. All of the samples were centrifuged for 15 minutes at a speed of 3000 revolutions per minute within an hour of the blood being collected to obtain serum. ALP (Alkaline Phosphatase), BUN (Blood Urea Nitrogen), Cr (Creatinine), TP (Total Protein), Alb (Albumin), CK (Creatine Kinase), T-Chol (Total Cholesterol), and SGOT (AST-Aspartate Aminotransferase) are examples of other enzymes. (Erba, Transasia Biomedicals, Mumbai. Chemical 5 plus v2).

Necropsy. Animals were euthanized by exsanguination after blood samples were taken, and then necropsies were conducted on them. Each animal's entire gross post-mortem examination allowed for the observation of all of its interior and external surfaces. We weighed the heart, lungs, spleen, liver, kidneys, adrenal glands, ovaries, and testes among other internal organs. The fasted body weights served as a starting point for calculating the relative organ weights on the day of the necropsy.

Statistical analysis. To present the findings of the statistical study, GraphPad Prism was used (9.5 version). The t-test was used to evaluate whether there was a significant difference between each treated group and the control group for each gender after the data were subjected to either a one-way or a two-way analysis of variance (ANOVA). Here, the data's mean and standard deviation are displayed (Standard deviation). It was determined that a p value of 0.05 or less was statistically significant

RESULTS

After receiving 200, 400, or 800 mg/kg of KSK, rats of both sexes were observed for 28 days, and no deaths or negative clinical effects were discovered. Additionally, neither gender's clinical signs nor any deaths were noticed.

Body weight, feed, and water consumption. There was no appreciable difference in the amount of weight acquired or lost by either gender throughout the course of the 28-day administration period when compared to the controls in the major groups. The data showed that, except at the dose level of 400 mg/kg dose, there was an increase from the third week onwards in all treatment groups and normal controls. In the case of low dose KSK treatment, feeding was equivalent to normal control, and water intake was also decreased at 400 mg/kg dose levels (Fig. 1-3).

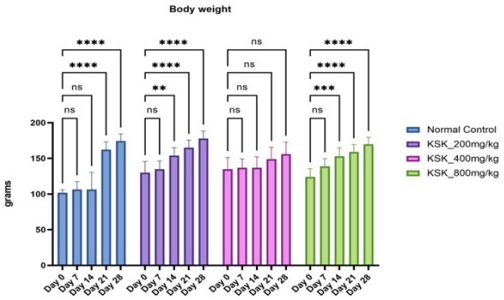

Fig. 1. Change in body weight rats treated with Kabasura Kudineer (KSK).

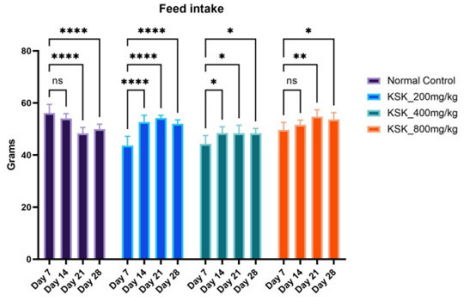

Fig. 2. Change in feed intake rats treated with Kabasura Kudineer (KSK).

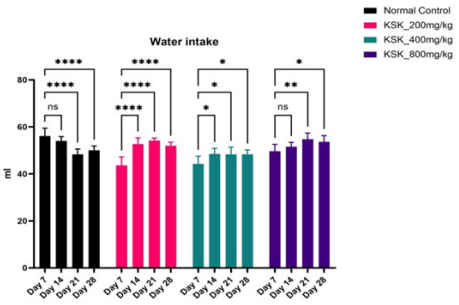

Fig. 3. Change in water intake for rats treated with Kabasura Kudineer (KSK).

Haematology. It was demonstrated that neither of the rats' haematology markers underwent any significant adverse changes. Hemoglobin and RBC count significantly dropped as compared to the control group. The 400 mg/kg KSK therapy significantly boosted the WBC count, and the concentration of neutrophils and lymphocytes also rose. When compared to control, there was a considerable rise in the MCV, MCH,

MCHC, and platelet counts. But these modifications were within the range of normal (Table 2).

Clinical chemistry. The SGOT and SGPT levels were identical to those of the control group, while the ALP levels significantly increased. The levels of urea increased at the KSK dose level at 400 mg/kg. Along with a decline in HDL levels, the levels of total cholesterol, LDL, and other lipids were noticeably lower in the lipid fractions. Importantly, when compared to control, creatinine levels were elevated at all dose levels of KSK. Other measurements, including albumin, VLDL, and triglycerides, were discovered to be comparable to normal. Male rats administered 800 mg/kg KSK did not show any significant changes after a 28-day treatment period. As a result, this amount of the test substance lacks any toxicological characteristics (Table 3).

Organ weight and necropsy. No obvious abnormalities in either the male or female bodies were discovered during the autopsy. The heart, brain, and spleen weights of the rats in the group that got 800 milligrams per kilogramme were considerably higher than those of the rats in the other groups. The absolute and relative organ weights of the female rats were not significantly different from those of the control group. It was demonstrated that neither the relative weights of the organs in male or female rats underwent any significant negative changes. (Table 4).

DISCUSSION

Since it's preferable to prevent illness than to treat it, Kabasura kudineer serves as a preventative treatment for the deadly condition known as COVID-19 (Sundaram and Ramasamy 2020). Kabasura kudineer, which is available in chooranam or powder form, was utilised during the pandemic that was swine flu. As such, Kabasura kudineer is utilised to treat all respiratory issues, including colds, fevers, excruciating phlegm, and the flu (Bala, 2021). Kabasura kudineer is effective in treating bacterial infections in addition to viral infections (Srivastava *et al*., 2021). Additionally, it aids in the treatment of digestive issues and lung irritation (Vincent *et al*., 2020). In the present study, the subacute toxicity study of KSK was undertaken, and at an oral dose level of 800 mg/kg of Kabasura Kudineer, there was no indication of repeated dose toxicity. There was not a single mortality during the administration or recovery phases in any of the KSK treatment groups. Similar to another trial in which KSK had been administered for 90 days, there were no appreciable alterations in body weight over the course of the medicine administration at dosages of 200, 400, and 800 mg/kg (Yessu, 2000). Water and food intake were decreased in the current study. Wistar rats were employed in earlier research to evaluate the long-term toxicity of KSK, but the Sprague Dawley strain was used in the current investigation. Regardless of the strain differences, the findings in both experiments' food and water consumption were comparable.

Table 1: Herbs, Phytoconstituents, and Therapeutic uses of Kabasura Kudineer (Kiran *et al*., 2022).

Sr. No.	Botanical name	Major Phytoconstituent	Parts Used	Therapeutic uses	Tamil Name
1.	*Zingiber officinale*	Zingiberene	Rhizome	Anemia, asthma, cough, dyspepsia, diarrhoea	Sukku
2.	*Piper longum*	Piperine	Fruit	Anemia, asthma, cough, headache, phlegm throat infection	Thippili
3.	*Syzygium aromaticum*	Eugenol	Flower bud	Diarrhea, dysentery, dyspepsia, ear/tooth ache, sinusitis, vomiting	Ilavankam
4.	*Tragia involucrate*	Costunolide	Root	Asthma, cough, eczema, fever, itching, skin diseases	Sirukanchori ver
5.	*Anacyclus pyrethrum*	Pyrethrin	Root	Arthritis, dental problem, epilepsy, fever, tonsillitis	Akkirakaram ver
6.	*Hygrophilla auriculata*	Apigenin	Root	Anemia, edema, sinusitis, urinary tract infection	Neermulli ver
7.	*Terminalia chebula*	Chebulic acid	Fruit rind	Diabetes, fistula, jaundice, leucorrhea, liver diseases, piles, stomatitis, vitiligo, vomiting	Kadukkai
8.	*Justicia adhatoda*	Vasicine	Leaves	Asthma, bleeding dysentery, cough, fever, throat infection	Adathodai
9.	*Coleus aromaticus*	Myrtenol	Leaves	Antibacterial, antimicrobial, insecticidal	Karpuravalli
10.	*Costus speciosus*	Costunolide	Root	Antifungal, antibacterial, antioxidant, antihyperglycemic	Koshtam
11.	*Tinospora cordifolia*	Cordifolioside A	Stem	Diabetes, diarrhoea, fever, hypertension, skin diseases	Seendhil
12.	*Clerodendrum serratum*	Carvacol	Root	Rheumatic fever, inflammatory disorders	Siruthekku
13.	*Andrographis paniculata*	Andrographalide	Whole plant	Arthritis, fever, sinusitis, syncope	Nilavembu
14.	*Sida acuta*	Carvacol	Root	Rheumatic infections, haemorrhoids, abdominal pain	Vattathiruppi ver
15.	*Cyperus rotundus*	Amentoflavone	Root tuber	Diarrhea, diabetes, inflammation, malaria, and bowel disorders	Korai kilangu

According to the haematological data, the hemoglobin, RBC, WBC, neutrophil, and lymphocyte counts were higher than those of normal rats, which were determined to be normal in earlier research using the Wistar strain of rats. Since the current investigation was conducted using the Sprague Dawley strain, the difference in strain could be the cause of the disparate haematological data (Hayakawa *et al*., 2013). In a prior investigation, there was a histological alteration in the liver and an increase in SGOT levels. The liver

enzymes were not elevated in the current investigation, and liver histopathology showed normal structural integrity. The strain difference in the current study may have contributed to the increase in ALP levels.

All other biochemical markers, as well as relative organ weights, were found to be within normal limits in the current investigation. Previous investigations have noted hypoalbuminemia, but the normal albumin levels in this study show minimal renal injury.

Therefore, the treatment with KSK showed no impact on the blood chemistry and haematological parameters, indicating that KSK is relatively safe for recurrent usage.

Table 2: Haematological values of rats treated with Kabasura Kudineer (KSK).

Parameters	Units	Normal	KSK 200mg/kg	KSK 400mg/kg	KSK 800 mg/kg
Hb	g/dl	14.27 ± 0.15	12.75 ± 0.45***	13.2 ± 0.3***	13.05 ± 0.35***
RBC	x 10^6 cells/µL	6.9 ±0.14	5.97 ± 0.5***	6.77 ± 0.09***	6.79 ± 0.15
WBC	x 10^3 cells/µL	8.517 ± 0.1	8.85 ± 0.25	9.55 ± 0.55	13.7 ± 3.6***
Neutrophils	%	31.5 ± 0.6	28.3 ± 0.1***	28.5 ± 1***	30.5 ± 0.5**
Lymphocytes	%	68.17 ± 1.4	67.5 ± 2.5	72.5 ± 2.5***	66.5 ± 0.5
PCV	%	48.5 ±0.76	38.75 ± 18.75	38.25 ± 1.05	37.4 ± 0.8
MCV	fL	55.83 ± 0.6	54.6 ± 0.1***	54.8 ± 0.7***	55.1 ± 0.1**
MCH	pg	20.17 ± 0.8	19.35 ± 0.25**	19.5 ± 0.7*	19.25 ± 0.05**
MCHC	g/dl	34 ± 0.93	34.95 ± 0.05**	35.4 ± 0.8***	34.9 ± 0.1*
PLT	x 10^3 cells/µL	562 ± 1.6	602 ± 30	805 ± 125***	614 ± 95

Hb – Haemoglobin, RBC – Red Blood Cells, WBC – White blood cells, PCV – Packed Cell Volume, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC - Mean Corpuscular Haemoglobin concentration, PLT – platelet. * Means significant differences at $p < 0.05$ compared with the control group, ** Means significant differences at $p < 0.01$ compared with the control group, *** Means significant differences at $p < 0.001$ compared with the control group

Table 3: Biochemical values of of rats treated with Kabasura Kudineer (KSK).

Parameters	Units	Normal	KSK 200mg/kg	KSK 400mg/kg	KSK 800 mg/kg
SGOT	IU/L	212 ± 2.121	212.2 ± 1.483	213.6 ± 2.302	212.8 ± 6.833
SGPT	IU/L	24.6 ± 3.049	25 ± 3.535	26.8 ± 2.387	24.8 ± 3.271
Albumin	g/dl	3.18 ± 0.01	3.01 ± 0.23*	3.15 ± 0.01	3.01 ± 0.06*
ALP	IU/L	425.5 ± 13.2	430 ± 48.7	216 ± 48.55***	312.7 ± 81.3***
Urea	mg/dl	24.4 ± 5.76	28.39 ± 0.02	32.98 ± 8.29**	29.33 ± 1.94
Creatinine	mg/dl	0.45 ± 0.09	0.98 ± 0.05**	0.89 ± 0.03***	0.97 ± 0.17***
Total cholesterol	mg/dl	79.5 ± 14.5	63.5 ± 12.5**	59.5 ± 4.5***	50.5 ± 0.5***
TGL	mg/dl	120 ± 66	74.5 ± 15.5*	119 ± 19	79 ± 1
Total protein	g/dl	7.18 ± 1.17	7.34 ± 0.27	7.21 ± 0.09	6.75 ± 0.15
HDL	mg/dl	31 ± 3	29.5± 2.5*	26 ± 2**	24.5± 6.5
LDL	mg/dl	56.9± 28.1	19.1 ± 6.9***	9.7 ± 2.7***	10.2 ± 6***
VLDL	mg/dl	24 ± 13.2	14.9 ± 3.1*	23.8 ± 3.8	15.8 ± 1

SGOT – Serum Glutamic Oxaloacetic Transaminase, SGPT – Serum Glutamate Pyruvate Transaminase, ALP –Alkaline Phosphatase, TGL – Triglycerides, HDL – High Density Lipoproteins, LDL – Low Density Lipoproteins, VLDL – Very Low Density Lipoproteins* Means significant differences at $p < 0.05$ compared with the control group, ** Means significant differences at $p < 0.01$ compared with the control group, *** Means significant differences at $p < 0.001$ compared with the control group

Table 4: Relative organ weights of rats treated with Kabasura Kudineer.

Organ	Normal	KSK 200mg/kg	KSK 400mg/kg	KSK 800 mg/kg
Brain	1.75 ± 0.01	1.66 ± 0.21	1.5 ± 0.21	1.35 ± 0.05*
Heart	0.85 ± 0.1	0.87 ± 0.07	0.93 ± 0.03	1.06 ± 0.01*
Kidney	1.102 ± 0.14	0.92 ± 0.03	0.78 ± 0.01**	0.99 ± 0.09
Liver	7.75 ± 0.19	7.73 ± 0.12	7.48 ± 0.57	8.3 ± 0.08
Lung	1.491 ± 0.084	1.483 ± 0.115	1.406 ± 0.082	1.363 ± 0.086
Spleen	0.062 ± 0.22	0.83 ± 0.09	0.8 ± 0.07	1.03 ± 0.13*
Testis	3.38 ± 0.4	3.29 ± 0.41	3.27 ± 0.54	3.65 ± 0.85
Ovary	0.072 ± 0.004	0.079 ± 0.004	0.071 ± 0.004	0.073 ± 0.002

Organ weights are represented in grams. . * Means significant differences at $p < 0.05$ compared with the control group, ** Means significant differences at $p < 0.01$ compared with the control group

CONCLUSIONS

In the subacute toxicity experiments, the Kabasura Kudineer did not result in any toxic effects or other symptoms. This suggests that Kabasura Kudineer is not particularly harmful. It is important to perform additional research on the toxicology of this product in non-rodent species or on humans.

The Kabasura Kudineer did not cause any signs of toxicity or any other symptoms in the subacute toxicity studies. That indicates that Kabasura Kudineer is relatively non-toxic. Further study regarding the toxicology of this preparation should be conducted in non-rodent species or in humans.

FUTURE SCOPE

This study gives an idea about the preliminary safety profile in rat species. Furthermore, to conclude its safety in human species, toxicity profiling in non-rodent species is essential to justify the safety of Kabasura kudineer.

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