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Original Article

Evaluation of Toxicity Profiles of Siddha Preparation of '*Kabasura Kudineer*' in Laboratory Animals

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ABSTRACT

Kabasura Kudineer' (KSK) is a poly Herbal decoction and well-known one in this series next to *Nilavembu kudineer* (NVK) from Siddha preparation. This system is most commonly practicing in India especially in southern regions his principle of medicine is proven to contain antiviral compounds it consist of Zingiber, Piper longum, Syzygium aromaticum, Justicia adathoda etc., The formulation was evaluated for acute and chronic toxicity in rats with reference to histopathological, haematological, and biochemical parameters from the various tissues of rats. During acute toxicity study, there were no any adverse effects found in the general behaviour and mortality at any dose level given. In chronic toxicity studies (90days) KSK in various dose level (0.15, 0.75, and 1.5 ml/kg B.wt) did not cause any changes in hematological and biochemical parameters with exception of a transient rise in uric acid, albumin, SGOT and lymphocyte level. The changes observed were significant only at the highest dosage of 1.5 ml/kg B.wt. Excepting albumin level, which increased with average dose (0.75ml), which was not significant when compared to control. Feed and water intake failed to reveal any marked changes in chronic toxicity studies. Histopathological lesions were non-specific in all organs between treated and control group except in liver revealed mild changes with highest dose level. In the present study the drug possessed no toxic effect up to 1.5 ml.

Keywords: Kabasura kudineer, haematology, histopathology, acute and chronic toxicity.

INTRODUCTION

The World health Organization (WHO) Predicted that 80% of the populations of developing countries rely on conventional medicines, mostly plant drugs for their primary health care needs. The curative Dimension revealed by the Siddhas is called as 'Siddha medicine'. In the Indian subcontinent, the systems of Siddha represent the ancient traditional systems of medicine. Siddha is nearly 10,000 years

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old, as recognized by the Indian government and other researchers of south Indian antiquity [1, 2]. There are thousand cases of swine flu in the present day. Due to the nature of respiratory virus, the transmission of this pathogenic virus is air borne transmission. Hence, the rapid spreading and difficulty in control of this infection can be expected [3]. The drug *Kapasura Kudineer(* KSK*)* has been quoted for kapasuram (fever), the symptoms of which is an analogue with swine flu, mentioned in Siddha Formulary of India It is used as first line therapy and general remedy for some types of fever caused by unidentified microbial infections. Like that "*Kapasura Kudineer*" *(*KSK*)* has taken the main role in the prevention of swine flu nowadays in a popular manner [4]. Prevention of disease by increasing endurance is improving the effectiveness of the immune system so that immune cells can continue to fight the cause of the disease and the body can be protected from various diseases. Since human beings are born with specific and non specific immune systems, the body's defense system is expected to offer protection from a variety of viruses, bacteria, fungi, and other foreign substances that can cause various diseases[5]. However, the information available on the safety profile of this preparation is insufficient. Hence, evaluate the safety of this preparation the present study was under taken with following objective. To evaluate the acute toxicity in rats, 90 days repeated dose administration of '*Kabasura Kudineer' (*KSK*)* in rats and estimate the levels of serum and various body organs after 90 days repeated dose administration in rats.

MATERIAL AND METHODS

Experimental Animals: Swiss albino Wister rats (150-200gm) of either sex and of approximate same age used in the present studies were procured from listed suppliers of Sri Venkateswara enterprises, Bangaluru, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangaluru) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.- P.Col/02/1676//12/2019/IAEC/ JSPC.) after scrutinization. The animals received the drug treatment by oral gavage tube.

Acute Toxicity: Twenty four (12 Male and 12 Female) Albino mice, weighing approximately 18-25g were used for the study. The acute toxicity study was carried out as per Guidelines for Ayurveda and siddha drugs. Healthy mice of either sex were selected and grouped into four comprising of six animals in each group. The animals were fasted for four hours prior to the study with free access to water. The test drug was suspended in the honey and given in graded doses up to maximum dose level. One group received honey only, which served as control. The animals were observed for any toxic manifestations and mortality for 72 hours. There after animals were observed for 14 days. [6]

Chronic Toxicity: The Wister rats ranging between 135-145 gms for males and 120-130 gms for females were selected for the present study. A total of twenty four Wistar rats (12 Males + 12 Females) were randomized and equally divided into four groups comprising six animals in each. The Group I, II & III received the KSK therapeutic dose, five times the therapeutic dose, and ten times the therapeutic dose (0.15ml/kg, 0.75ml/kg and 1.5ml/kg) respectively for 90 days daily Control group animal received honey. The animals were observed for pre-terminal morbidity and mortality and examined clinically. Clinical biochemical and hematological investigations were carried out. This was followed by euthanization and histopathological examination of various organs which includes liver, spleen, kidneys, lungs, heart, intestine, ovaries, testes, and stomach. [6]

Statistical methods: The statistical analysis was performed by the difference between the groups tested by one-way analysis of variance (ANOVA), $P< 0.05$ was considered to be statistically significant.

RESULTS

Acute Toxicity: The test drug and vehicle were administered to mice as per the guidelines, under close observation for 4 hrs and followed by daily observation for 14 days, produced neither behavioural changes nor mortality and morbidity in all the groups of animals. Hence further investigation planned

Chronic Toxicity

Body Weight and Feed Intake: No significant changes were observed in the body weight and feed in take in 90 days treatment in all the three dose level when compared to control. The growth rate was linear in all the treated groups when compared to control (Table 1.1-1'.4).

Table-1.1: Effect of '*Kabasura Kudineer' (*KSK) on feed in take (gm) in rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	9.15 ± 0.93[a]	10.31 ± 0.72[a]	11.00 ± 0.67[a]	9.74 ± 0.64[a]	10.50 ± 0.24[a]	12.60 ± 0.46[a]	11.10 ± 0.63[a]
Group I	9.18 ± 0.87[ab]	8.41 ± 0.48[b]	10.81 ± 0.65[a]	9.83 ± 0.79[a]	10.09 ± 0.89[a]	12.76 ± 1.16[a]	11.12 ± 1.39[a]
Group II	9.00 ± 1.16[b]	9.50 ± 0.38[a]	10.62 ± 0.41[a]	11.68 ± 0.60[a]	12.23 ± 0.99[a]	11.28 ± 1.38[a]	11.33 ± 0.93[a]
Group III	9.13 ± 0.87[a]	8.07 ± 0.40[b]	10.12 ± 0.27[a]	10.10 ± 0.36[a]	10.31 ± 0.50[a]	10.76 ± 0.70[a]	9.76 ± 0.39[a]

Means bearing different superscript differ significantly (P < 0.05)

Table-1.2: Effect of *Kabasura Kudineer' (*KSK*)* on feed in take (gm) in rats

Groups	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 91
Control	11.12 ± 0.31[a]	11.57 ± 0.23[a]	10.81 ± 0.64[a]	10.36 ± 0.33[a]	9.95 ± 0.24[a]	12.24 ± 0.51[a]	11.15 ± 0.52[a]
Group I	10.86 ± 1.41[a]	11.31 ± 1.19[a]	13.31 ± 1.10[a]	11.41 ± 0.90[a]	10.43 ±0.83[a]	13.00 ± 1.24[a]	12.31 ± 1.30[a]
Group II	11.05 ±0.70[a]	11.81 ± 0.75[a]	10.86 ± 0.57[a]	12.30 ± 1.02[a]	12.17 ± 0.94[a]	13.24 ± 1.01[a]	13.00 ±1.08[a]
Group III	10.17 ± 0.45[a]	10.45 ± 0.81[a]	12.36 ± 0.50[a]	11.33 ± 0.69[a]	10.30 ± 1.12[a]	13.05 ± 0.90[a]	12.14 ± 0.75[a]

Means bearing different superscript differ significantly (P < 0.05)

Table-1.3: Effect of '*Kabasura Kudineer' (*KSK) on body weight (gm) in rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	112.50 ± 3.59[a]	118.83 ± 2.71[a]	132.67± 6.04[a]	148.33 ± 7.38[a]	155.00 ± 8.85[a]	161.67 ± 9.28[a]	165.00 ± 10.08[a]
Group I	108.17 ± 7.47[a]	127.50 ± 8.04[a]	146.67 ± 10.22[a]	149.17 ± 14.23[a]	154.17 ± 13.13[a]	167.33 ± 15.99[a]	176.33 ± 17.67[a]
Group II	110.83 ± 3.75[a]	125.83 ± 4.55[a]	139.17 ± 5.69[a]	158.33 ± 7.49[a]	160.83 ± 10.28[a]	164.50 ± 11.87[a]	180.83 ± 13.32[a]
Group III	95.33 ± 1.67[a]	115.00 ± 3.16[a]	128.00 ± 4.58[a]	140.00 ± 3.87[a]	154.00 ± 5.87[a]	159.33 ± 7.61[a]	167.83 ± 7.79[a]

Means bearing same superscripts do not differ significantly (P < 0.05)

Table-1.4: Effect of '*Kabasura Kudineer' (*KSK) on body weight (gm) in rats

Groups	Day 49	Day 56	Day 63	Day 70	Day77	Day 84	Day 91
Control	163.33 ± 12.02[a]	170.00 ± 11.26[a]	172.50 ± 13.53[a]	178.33 ± 15.58[a]	181.67 ± 16.36[a]	182.50 ± 16.01[a]	189.17 ± 16.09[a]
Group I	188.33 ± 20.07[a]	193.00 ±20.36[a]	197.50 ± 21.98[a]	207.83 ± 22.75[a]	204.33 ± 23.48[a]	216.67± 26.92[a]	219.17 ± 27.33[a]
Group II	189.50 ±15.34[a]	205.00 ±16.07[a]	201.67 ± 17.97[a]	210.83 ± 17.53[a]	224.17 ± 20.18[a]	224.17 ± 21.66[a]	227.50 ± 22.20[a]
Group III	178.33 ± 8.43[a]	178.33 ± 9.10[a]	180.83 ± 10.04[a]	191.67 ± 10.85[a]	193.33 ± 16.26[a]	200.00 ± 15.44[a]	207.33 ± 16.43[a]

Means bearing same superscripts do not differ significantly (P < 0.05)

Clinical Biochemistry: Toxicity studies for 90 days with 1.5 ml dose level showed significant difference in uric acid level when compared to control. No significant changes were observed in Group I and II. Group II showed significant variation in albumin level compared to Group I and III but no significant to control. The serum glutamate level in Group III revealed significantly difference when compared to control, Group I and II. It is represented in the table1.5-1.6.

Table-1.5: Effect of '*Kabasura Kudineer' (*KSK) on Biochemical Parameters in rats

Groups	Sugar	Urea	Creatinine	Uric acid	T.P	Albumin
Control	70.00 ± 6.23[a]	42.81 ± 1.74[a]	0.80 ± 0.03[a]	0.75 ± 0.21[a]	7.03 ± 0.38[a]	3.13 ± 0.07[ab]
Group I	72.50 ± 4.31[a]	37.00 ± 2.10[a]	0.80 ± 0.03[a]	1.20 ± 0.21[ab]	7.11 ±0.25[a]	2.85 ± 0.13[a]
Group II	71.81 ± 7.82[a]	33.50 ± 2.39[a]	0.75 ± 0.02[a]	0.91 ± 0.13[ab]	7.45 ±0.46[a]	3.42 ± 0.11[b]
Group III	72.31 ± 4.75[a]	36.83 ± 3.77[a]	0.80 ± 0.03[a]	1.66 ± 0.23[b]	7.23 ± 0.45[a]	2.97 ± 0.11[a]

Means bearing different superscript differ significantly (P < 0.05)

Table-1.6: Effect of *'Kabasura Kudineer' (*KSK*)*on Biochemical Parameters in rats

Groups	Globulin	Bilirubin	A.L.P	SGOT	SGPT
Control	3.90 ± 0.35[a]	0.41 ± 0.09[a]	126.67 ± 13.87[a]	187.67 ± 11.02[b]	48.83 ± 2.39[a]
Group I	4.28 ± 0.25[a]	0.40 ± 0.02[a]	189.50 ± 20.81[a]	136.17 ± 15.40[a]	58.00 ± 3.33[a]
Group II	4.05 ± 0.57[a]	0.37 ± 0.06[a]	179.17 ± 18.49[a]	156.00 ± 8.41[ab]	58.83 ± 6.19[a]
Group III	4.45 ± 0.35[a]	0.37 ± 0.05[a]	188.67 ± 22.58[a]	250.67 ± 12.57[c]	66.67 ± 5.70[a]

Means bearing different superscript differ significantly (P < 0.05)

Hematology

Haematological data showed that the lymphocytes level was significant in between Group III and Group I, but these levels were found to be non significant when compared to control and Group II. And all other parameters remained normal without any significant difference in both control and treated Groups (Shown in table 1.7-1.8)

Table-1.7: Effect of '*Kabasura Kudineer' (*KSK*)* on Haematological Parameters in rats

Groups	PCV	Hb (g%)	MCHC (%)	TC (%)	Neutrophil (%)	Lymphocyte (%)
Control	44.18 ± 1.32[a]	15.52 ± 0.50[a]	35.10 ± 0.26[a]	5616.67 ± 423.81[a]	51.50 ± 1.98[a]	47.67 ± 2.25[ab]
Group I	41.95 ± 0.82[a]	14.76 ± 0.33[a]	35.26 ± 0.42[a]	5765.67 ± 494.76[a]	55.67 ± 4.90[a]	43.50 ± 5.20[a]
Group II	41.46 ± 1.10[a]	14.65 ± 0.30[a]	35.38 ± 0.30[a]	6314.67 ± 785.31[a]	39.83 ± 3.15[a]	54.83 ± 2.24[ab]
Group III	41.10 ± 1.04[a]	14.45 ± 0.34[a]	35.21 ± 0.15[a]	6314.67 ± 740.71[a]	43.17 ± 2.15[a]	58.17 ± 2.76[b]

Means bearing different superscript differ significantly (P < 0.05)

Table-1.8: Effect of '*Kabasura Kudineer' (*KSK*)* on Haematological Parameters in rats

Groups	Monocyte (%)	Eosinophil (%)	Basophil (%)	Platelets	RBC (10/mm)	MCV (Cu µ)	MCH (µg)
Control	0.33 ± 0.21[a]	0.33 ± 0.21[a]	0	3.97 ± 0.25[a]	5.15 ± 0.16[a]	85.82 ± 0.85[a]	29.95 ± 0.13[a]
Group 1	0.33 ± 0.21[a]	0.50 ± 0.22[a]	0	3.89 ± 0.32[a]	4.95 ± 0.12[a]	84.78 ± 0.92[a]	29.82 ± 0.14[a]
Group 2	0.17 ± 0.17[a]	0	0	4.49 ± 0.33[a]	4.88 ± 0.10[a]	85.47 ± 1.38[a]	30.00 ± 0.07[a]
Group 3	0.17 ± 0.17[a]	0.17 ± 0.17[a]	0	4.31 ± 0.27[a]	4.78 ±0.12[a]	85.88 ± 0.26[a]	30.20 ± 0.13[a]

Means bearing different superscript differ significantly (P < 0.05)

HISTOPATHALOGICAL STUDIES

The highest dose of '*Kabasura Kudineer' (*KSK) shows decongestion and degeneration of hepatocytes in liver, the all other organs histological studies shows normal archytexture between treated and control groups.

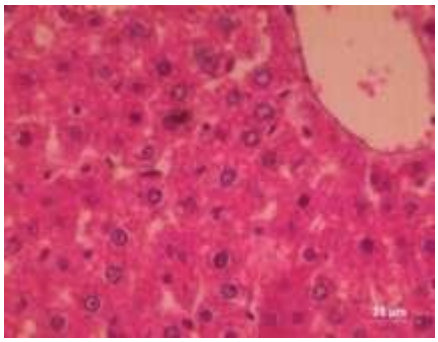

Fig-1: Photomicrograph of liver-Control

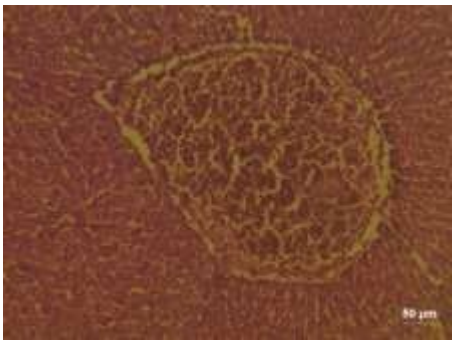

Fig-2: Photomicrograph of liver-treated with '*Kabasura Kudineer' (*KSK*)* (highest dose) showing congestion and degeneration of hepatocytes

DISCUSSION

In acute toxicity study, there was no mortality observed up to maximum dose level of KSK administered orally. Thus our test suggested that KSK does not cause any apparent acute toxicity. This test in rats was undertaken based on the report on non-lethality of animals in acute (mice) toxicity studies. The changes in body weight have been used as indicator of adverse effect of KSK [7]. Since there were no changes in animal behaviour and body weights at all the doses of treated groups when compared to control. The present result suggests that the oral administration of KSK is non toxic. There were no pre terminal deaths in animals receiving therapeutic dose, five times of the therapeutic dose and ten times of the therapeutic doses. The food intake were significantly different between groups exposed to test compound Group II, Group III and control on 0^{th} and 7^{th} day, but no gain in animal body weight at the same day, this may be due to normal physiological changes. The bio chemical parameter such as uric acid, SGOT, and albumin level in serum increased with Group III and Group II respectively, all other parameters remained normal. The SGOT is good indices of liver and kidney damage than SGPT [8]. Elevated levels of SGOT with Group III showed mild Histopathological changes in liver. The albumin is the one of the most important plasma protein, maintain the osmotic pressure of blood [9] and prevent the escape of fluids from blood to tissue

hence, indirectly maintain the blood volume and blood pressure and it is synthesized by liver. The decreased albumin level called Hypo albunaemia is also an indication of liver damage. The increased albumin level was significant between treated group and non significant to control. Our data revealed albumin level was normal in all treated and control groups [10]. And a histopathological examination of kidney in treated Groups shows normal compared to control animals. So from the above statement increased SGOT level does not caused marked changes in liver. The raised uric acid level has been observed with impairment of renal function [11]. In the present study significant increase in uric acid level at highest dose of KSK treated group manifest nonhazardous effect. In humans and higher primates, uric acid is the final oxidation (breakdown) product of purine metabolism and is excreted in urine. In most other mammals, the enzyme Uricase further oxidizes uric acid to allantoin [12]. So the increased uric acid level may be due to decreased uricase or high purine metabolism by the composition of formulation. Uric acid may be a marker of oxidative stress [13], and may have a potential therapeutic role as an antioxidant [14]. On the other hand, like other strong reducing substances such as ascorbate, uric acid can also act as a prooxidant.KSK increase the lymphocyte level in highest dose once but these level significant to Group I and non significant to control and Group II. The increased level of lymphocyte with high dose of KSK emphasizes the advantageous effect in immune system.

CONCLUSION

In conclusion, KSK can be considered safe with reference of above result of acute and 90 days toxicity studies with parameter of feed intake, body weight and biochemical and hematological. Further, the composition of formulation has some additional pharmacological properties these results substantiate the beneficial effect of formulation. From the above results proved that '*Kabasura Kudineer'* was safety for long term use at various dose levels.

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