Acute And Long Term Toxicity Study Of Siddha Poly Herbal Formulation Kiranthi Mega Chooranam

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Abstract- The siddha system of medicine uses a fascinating combination of herbs, minerals and metals and to promote good health and longevity. ***Kiranthi mega chooranam* (KMC)** is one of the Siddha formulations which are indicated as an effective drug for various diseases. Acute Oral Toxicity study and Long term toxicity study in vivo toxicity studies were carried out on Kiranthi Mega Chooranam (KMC) by World Health Organization (WHO) guideline for testing traditional medicines. In Acute toxicity study there was no abnormal signs reported at the dose level of (2000 mg/kg b.wt) within 24-72 hours in wistar albino rats. Long term toxicity study was conducted for about 90 days as per WHO guideline in 3 doses Low dose (270mg/kg b.wt), Mid dose (540mg/kg b.wt), High dose (1080mg/kg b.wt). Animals were observed throughout the period. The histopathological study on the organs such as lung, brain, kidney, liver, spleen, heart, stomach, ovary, testis, uterus was normal in Control and High dose group of the animals. Analyses of these results with the information of signs, behavior, and health monitoring could lead to the conclusion that Acute and Long term oral administration of **Kiranthi mega chooranam (KMC)** for 90 days does not cause toxicity.

Index Terms- Kiranthi mega chooranam, Toxicity study, Siddha medicine, WHO guideline

I. INTRODUCTION

Siddha Medical system is a special, significant and scientific system, being in practice, since time immemorial. It is one of the ancient systems of medicine contemporaneous with Grecian, Egyptian, Mesopotamian, Chinese medicines. It is a unique system which dwelt among the Tamil people of South India rendering service to humanity for more than five thousand years BC era in combating diseases and in maintaining physical, mental, social and spiritual health Traditional Medicine has played an important role in meeting the demands of primary health care in many developing countries and its use has expanded widely in many developed countries[1]. Siddha system of medicine is one among them, which has flourished in the Southern India especially Tamilnadu [2].In Siddha system of medicine, Drugs are prepared with ingredients of herbs, minerals, metals and animal products. Siddhars use single or combinations in their medicine preparation in addition of herbs to increase its potency, efficacy, therapeutic index because of the long shelf life. Siddhars are highly intellectual and spiritual combined with supernatural power their works involve high order of chemistry (Rasavatham). Siddha medicine uses herbal formulations as a first line drug of choice, which emphasizes to use roots of the plants as medicine primarily and then to use leaves and other parts of the plant and finally to use herbo-mineral preparations in its oxide form, sulphide forms etc [3].

Kiranthi mega chooranam (KMC) is one of the traditional Siddha formulation which is indicated as a best drug for Kiranthi (Syphilis), kadividam (Poisonous bites), kuttam (Skin diseases), Thadippu (Urticaria), megasoolai (syphilitic arthritis) in Siddha text *Anuboga vaithiya navaneetham*. Therefore, an endeavor has been made to reveal the facts about the poly herbal Siddha drug Kiranthi mega chooranam, from the literature by scientific analysis of its purification and preparation process by evaluating the Physico chemical characters, Pharmacological actions and toxicological analysis.

There is no report available on the toxicological profile of above mentioned drugs; therefore, present studies were conducted to evaluate the acute and long term toxic effects on the test drug *Kiranthi mega chooranam* (KMC) on rodents(Rats).

Sub chronic toxicity studies are generally designed to examine the adverse effects resulting from repeated exposure over a portion of the average life span of experimental animals. This study gives valuable information on the cumulative toxicity of substances on target organs and on physiological and metabolic tolerance of a compound at two dose levels for prolonged exposure[4].

II. MATERIALS AND METHODS

2.1 Preparation of test drug

The herbal drugs such as *Nilavaarai ilai* (Cassia senna) ,*Thavasi murungai samoolam*(Justicia tranquebariensis),*Sivanaar vembu samoolam*(Indigofera aspalathoides) *Vellarugu samoolam* (Enicostemma littorale),*Thoothuvalai samoolam (*Solanum trilobatum), *Kottai karanthai samoolam* (Sphaeranthus indicus) The Ingredients were purified as per siddha literature. After purification of all the above ingredients are dried well and equal quantity the plant leaves and Whole plants were taken make as fine powder and filtered well(Vasthira kayam) mix chooranam and stored in the air tight container [5].

Sample Solubility

S.No	Solvent Used	Solubility
1.	Water	Soluble
2.	Methanol	Soluble
3.	Ethanol	Soluble
4.	Hydrogen Peroxide	Soluble

2.2 Toxicological evaluation of Kiranthi Mega Chooranam

The following in vivo toxicity studies were carried out on Kiranthi Mega Chooranam (KMC) by World Health Organization (WHO) guideline for testing traditional medicines [6] Acute Oral Toxicity study and Long term toxicity studies were carried out at National Institute of Siddha. The study was done after getting permission from the Institutional Animal Ethics Committee IAEC Approved No: **NIS/IAEC-II /28082018/19.**

For Acute and Long-term toxicity studies test animals were obtained from Tamil Nadu Veterinary and Animal Sciences University, Madhavaram. Animals were kept in animal house, National Institute of Siddha, Chennai.

a) Selection of the animals:

Animals were selected as per WHO guideline. Healthy adult animals of Wistar albino rat, both male and female rats were used for acute oral toxicity study and Long term toxicity study. The female animals used in the studies were nulliparous and non-pregnant.

b) Housing and feeding conditions:

Temperature: In the experimental animal room: 22ºC (± 3ºC)
Humidity: 60 ± 10 %
Lighting: Artificial, the sequence being 12 hours light, 12 hours dark.
The animals were housed in polypropylene cages provided with bedding of husk. The animals had free access to RO water.
For feeding, Standard pellet diet (bought from Sai Meera foods pvt. Ltd, Bangalore) was used.

c) Preparation of animals:

The animals were randomly selected, to permit individual identification by cage number and individual marking on the fur of each animal was made with picric acid. The animals were kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The principles of laboratory animal care were followed.

d) Test Substance:

Kiranthi Mega Chooranam (KMC) was brown in color, without taste and odour. The drug was dissolved in hot water to obtain and ensure the uniformity in drug distribution.

e) Route of administration:

Oral route was selected, because it is the normal route of clinical administration.

f) Preparation of doses:

The stock solution was prepared freshly as dose per animal suspended in 1ml hot water.

2.3 Acute oral toxicity

Acute oral toxicity of the test drug will be evaluated in rats following WHO guideline. Animals will be divided into 2 groups by randomization method, each group containing 10 animals (5 females and 5 males). One group as control and the other as test group. Control group is treated with hot water and test group were treated with the test drug Kiranthi Mega Chooranam ten times more than the therapeutic dose (2000mg per kg b.wt)

No of animals used for long term toxicity study:

GROUP	NO OF RATS
Group-1 Control (Hot water)	5 Male, 5 Female
Group-2 Test drug (2000 mg /kg b.wt)	5 Male, 5 Female

a)Administration of doses:

The test drug was administered in a single dose by using oral gavage.
Animals were fasted prior to drug administration. Following the period of fasting, the animals were weighed and test drug was administered. The control groups received equal volume hot water. The test drug was administered at 10 times the therapeutic dose (2000 mg / kg b.wt). The food was withheld for 3-4 hours after dosing the animal.

b) Cage side observations:

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convulsion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection, reactivity of touch, salivation, scratching, sedation, stereotypes (chewing), stereotypes (head movements), stereotypes (sniffing), tremor and writhes, diarrhoea, leathery, sleep and coma.

c) Gross necropsy:

At the end of 14th day animals will be sacrificed for gross necropsy. It includes examination of the external surface of the body, all orifices, and organs like brain, thymus, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals. If there will any occurrence of mortality during the trial period, the vital organs will be subjected to Necropsy.

2.4 Long term toxicity study : (WHO guidelines)

Long term toxicity study was carried out at different dose levels. The animals in both sex was divided in four groups (group I, II, III & IV). Each group consists of 20 animals (10 males and 10 females). Group-I served as control and the other three groups II, III and IV for test drug of Low dose (108 mg/kg/b.wt), Mid dose (540 mg/kg/b.wt) and High dose (1080 mg/kg b.wt) respectively The low dose was calculated from the therapeutic dose (6g) and body surface area of rats.

No of animals used for long term toxicity study:

GROUPS	NO OF RATS
Control (Hot water)	(10 Male, 10 Female)
Low Dose	(10 Male, 10 Female)
Mid Dose	(10 Male, 10 Female)
High Dose	(10 Male, 10 Female)

Total 80 (40 Female + 40 Male)

a) Administration of doses:

The animals were dosed with the test drug daily for a period of 90 days. The test drug mixed with ghee and was administered by oral gavage, and this was done in a single dose to the animals once in daily for 90 days.

b) Observations:

During the study, body weight of the animals, water and food consumption
were evaluated weekly; mortality events were evaluated daily. By the end of 90 days, on 91st day animals were sacrificed by excessive aesthesia. Blood were collected in all overnight (12 hours) fasted rats through abdominal aorta and it were processed for investigations like Complete heamogram, Renal function test, Liver function test, Lipid profile. Vital organs were collected from the animals and subjected to histopathology.

c) Statistical analysis:

Findings such as body weight changes, food consumption, water intake, haematology and biochemical analysis were subjected to One - way ANOVA Dunnet‟s test using a computer software program followed by D Graph Pad Instat - 3.

III. Results and Discussion

3.1 Acute oral toxicity study of Kiranthi Mega Chooranam

Acute toxicity study carried out as per WHO guidelines, there were no treatment related death or signs of toxicity developed in Wistar albino rats at dosage of 10 times the therapeutic dose (2000 mg/kg b.wt) throughout the study period. Further, No abnormal behavior and mortality was observed during study and observational period after drug treatment in any experimental group. no gross pathological changes have been seen in the internal organs of both control and treated groups.

3.2 Long term toxicity study of Kiranthi Mega Chooranam

Long term toxicity study carried out as per WHO guideline for a period of 90 days. The changes in the food intake, water intake, body weight changes, haematological and biochemical parameters were observed and noted in consideration for assessing the toxicity profile of the test drug Kiranthi Mega Chooranam **(KMC).**

a) Body weight:

The mean body weight of male and female rats changed after administration 10 times higher than recommended dose are depicted in Table 1 (a and b) respectively. There was no significant difference abnormality in body weight gain of KMC treated rats compared to control rats during the entire period of study.

b) Food and water consumption:

The average daily food and water intake consumption of KMC treated rats was comparable with control animals. Data of food and water intake is presented in Table 2 and Table 3 respectively. There is no significant changes in Food and water intake.

c) Effect of on haematology:

The 90 days oral administration of test drug KMC did not depress the haemoglobin and blood cell values. There was no major shift in white blood corpuscles of drugs treated rats compared to control animals. There was no leukocytosis or leucopenia for 10 times higher than recommended dose respectively. The result of haematology is presented in Table 4.

d) Effect on Liver:

The test drug KMC did not cause any disorder in hepatic functions as compared to control group rats. The excretory function of liver in the test drug KMC treated animals was not disturbed because alkaline phosphatase values (ALP) were not elevated in comparison to control rats. The alanine transaminase (ALT) and aspartate transaminase (AST) values are more important in assessing and monitoring the degree of liver cell damage, inflammation and necrosis[7]. In present study, it is found that there is no statistically significant increase in values of these enzymes even when the rats were administered up to ten times higher than the recommended dose. Further, biochemical parameters showed that drugs taken for the study did not cause any disturbances in synthesis of albumin even at high dose. The total serum cholesterol values, which are statistically insignificant, suggest that the test drug KMC devoid of any metabolic adverse effects. The results are presented in Table (5 and 6)

e) Effect on Kidney:

There was no any adverse effect on renal function after the administration of the test drug KMC. Biochemical parameters revealed that excretory function of kidney was well maintained, as there was no rise in the values of blood urea nitrogen (BUN), and serum creatinine levels in plasma of rats which treated with above-mentioned drug. (Table 7).

TABLE 1.a.Effect of Kiranthi Mega Chooranam on Body weight changes of Male Wistar albino rats in long term toxicity study.

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
0th day	183.7±11.4	181.5±8.6	192.5±8.6	194.4±6.1
15th day	198.7±10.1	196.5±12.5	209.2±4.1	209.2±5.1
30th day	218.2±13.6	221.8±17.6	231.6±5.3	229.2±6.4
45th day	239.6±17.7	241.6±16.5	253.8±5.9	256 ± 8.6 *
60th day	264.2±15.8	265.6±17.6	285.2± 9.2	280.2±8.6*
75th day	288.2±16.0	287.6±12.9	308±9.2**	312±8.8**
90th day	316.2±18.2	308±15.5	335±12.9*	340 ±6.5**

TABLE 1.b.Effect of Kiranthi Mega Chooranam on Body weight changes of Female Wistar albino rats in long term toxicity study

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
0th day	151.3±12	157.2±15.7	167.4±16.4	170±16.2
15th day	164±11.6	172.6±16.2	182.2±22.1	178.3±16.0
30th day	173.4±12.8	186.6±17.1	193±26.5	195.2±19.0
45th day	193.6±6.8	194.6±15.2	199.4±18.6	205.2±20.0
60th day	214.4±6.0	215.6±11.5	214.6±20.8	219.8±18.1
75th day	241.6±10.1	240.4±15.0	247.2±25.0	262.8±26.1
90th day	261.2±12.6	272.8±19.6	270.6±24.5	284.8±26.9*

Values are mean± S.D. (Dunnett‟s test).*P<0.05,**P<0.01,N=10.

TABLE 2: Effect of Kiranthi Mega Chooranam on feed intake of Wistar albino rats in long term toxicity study.

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st day	41.5±2.53	31±6.07	35±14.1	56±14.6
15th day	40.2±12.8	36.8±5.87	42.3±9.44	45.4±13.2
30th day	35.2±8.5	39.5±7.01	43.5±10.6	45.8±12.1
45th day	36.6±7.9	39.1±10.5	43.9±12.5	51.5±14.1*

60th day	39.5±9.12	42.5±10.0	45.5±17.3	50.5±12.0
75th day	43.4±12.6	47.9±13.4	47.8±13.6	48.8±15.5
90th day	44.5±11.5	46.4±18.5	55.5±13.4*	57.7±15.5

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

TABLE 3: Effect of Kiranthi Mega Chooranam on water intake of Wistar albino rats in long term toxicity study

GROUPS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
1st day	86±7.07	62±14.1	71±28.2	89±20.2
15th day	60±17.4	55.4±16	73.3±21.8	80.9±25.1
30th day	66±18.7	74.1±22.1	75.6±25.2	81±27.3
45th day	68±22.5	79.5±23.1	82.1±21.6	87.4±28.8
60th day	70±20.8	81.2±24.9	87.6±32.9	84.3±25.2
75th day	71.3±22.5	84.2±28.1	88.2±32.5	88.8±31.1
90th day	82.3±23.5	89.3±32.1	91.3±33.8	93±29.8

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

TABLE 4: Effect of Kiranthi Mega Chooranam Haematological parameters of Wistar albino rat in long term toxicity study

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
RBC (X 10^6µl)	5.5±1.32	6±1.07	6.1±0.9	6.9±1.53
WBC (X10^3/µl)	10±2.08	9.3±2.48	5.4±0.96**	9.04±1.35
Platelet(X10^3/µl)	752.8±172.6	642.9±164.5	762.6±113.7	723±138.4
Haemoglobin(g/dl)	12±2.03	13.2±1.43	12.1±2.38	13.5±2
MCH(pg)	18.2±1.57	17.5±1.64	19.10	17.3±1.14
MCV(fl)	58±5.10	58.1±4.24	58.5±6.4	62.1±6.11
Neutrophils 10^3/mm3	22.3±0.72	22.6±0.78	22..1±0.75	21.6±0.43
Eosinophils (%)	1.3±0.27	1.3±0.29	1.4±0.30	1.4±0.32
Basophils (%)	0.3±0.38	0.1±0.32	0.3±0.40	0.3±0.38
Lymphocytes (%)	73.7±12.2	74.2±12.1	75.8±10.66	75.1±8.89
Monocytes (%)	4.2±0.74	3.1±0.81	3.2±1.50	3.9±0.96

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

TABLE 5: Effect of Kiranthi Mega Chooranam biochemical parameters-Liver function test of wistar albino rat in long term toxicity study

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
TOTAL BILIRUBIN (mg/dl)	0.4±0.13	0.3±0.14	0.5±0.13	0.6±0.14
SGOT(U/dl)	99±16.4	89±16.9	98±16.4	101±16.9
SGPT(U/dl)	26±12.20	28±1.50	32±0,12	35 ±12.0

Values are mean± S.D. (Dunnett's test).*P<0.05,**P<0.01,N=10

TABLE 6: Effect of Kiranthi Mega Chooranam on biochemical parameters- Lipid profile of wistar albino rat in long term toxicity study

PARAMETERS (mg/dl)	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
TOTAL CHOLESTROL (mg/dl)	125±18.13	132.6±15.8	137±27.15	139.8±25.3
HDL(mg/dl)	59.3±5.86	62.8±10.8	68.2±6.05	115±27**
LDL(mg/dl)	50.3±21.0	50.1±20.8	50.8±21.4	49.2±20.3
VLDL(mg/dl)	15.7±2.25	15.9±2.15	16.1±4.02	16.3±4.12
TGL(mg/dl)	39.1±8.21	37.7±10.6	40.1±7.88	38.1±11.6

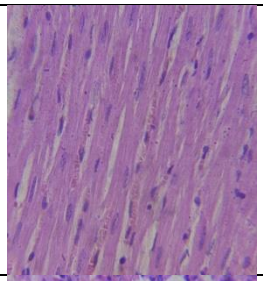
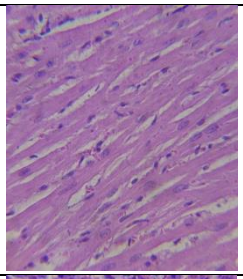
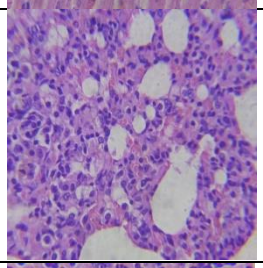
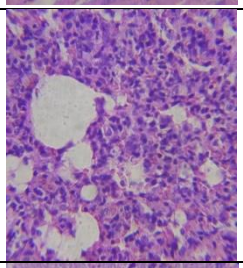
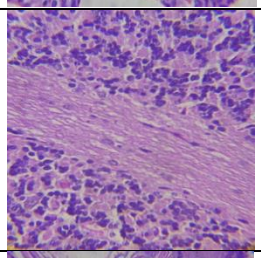
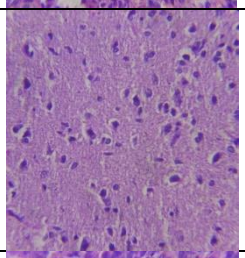
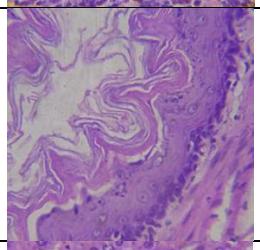
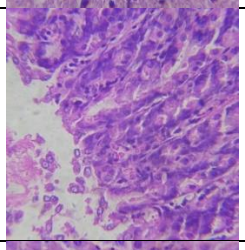
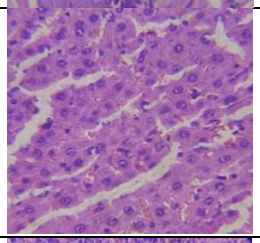
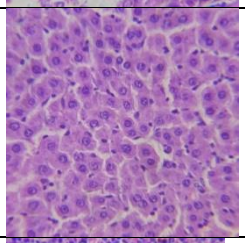
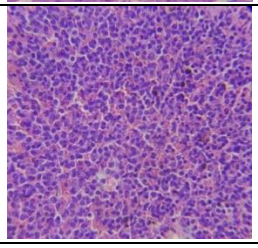
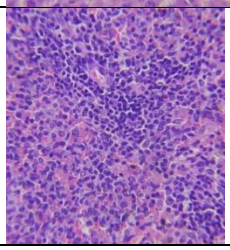
Values are mean± S.D. (Dunnett"s test).*P<0.05, **P<0.01, N=10

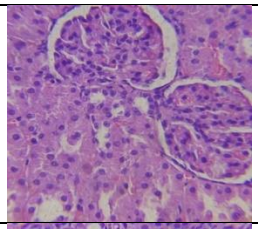
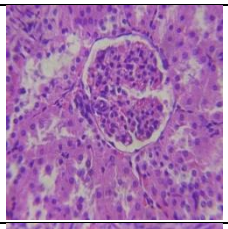
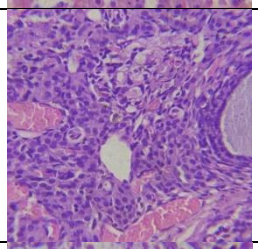
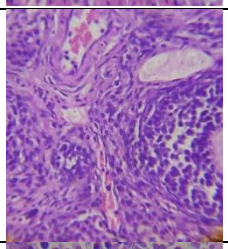
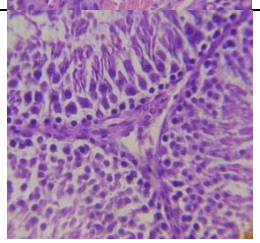
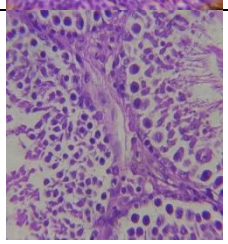
TABLE 7: Effect of Kiranthi mega Chooranam on biochemical parameters- Renal function test of Wistar albino rat in long term toxicity study

Parameters	Control	Low dose	Mid dose	High dose
BUN (mg/dl)	15.8±3.32	16.1±2.54	15.1±3.15	14.9±3.27
Serum Creatinine(mg/dl)	0.7±0.12	0.69±0.17	0.71±0.22	0.73±0.16

Values are mean± S.D. (Dunnett"s test).*P<0.05,**P<0.01,N=10.

Table 8: Histopathology of High power magnification Vital organs 40X (Albino Rats)

ORGANS	LOW DOSE	HIGH DOSE	RESULTS
HEART			No abnormality
LUNGS			No abnormality
BRAIN			No abnormality
STOMACH			No abnormality
LIVER			No abnormality
SPLEEN			No abnormality

KIDNEY			No abnormality
UTERUS			No abnormality
TESTIS			No abnormality

3.3 Histopathological study:

Histological sections of vital organs of control and test group show normal architecture(Table 8). Liver biopsy of treated rats show normal lobular architecture of liver with normal central portal vein, radiating plates of hepatocytes and peripheral portal tracts composed of hepatic artery, bile duct and distal portal vein. No focal or diffuse foci of necrosis of hepatocytes, and infiltration of chronic inflammatory cells were observed. Kidney biopsy of KMC treated rats show normal architecture composed of normal renal glomeruli, collecting tubules, interstitial tissue and blood vessels. There were no foci of necrosis, degeneration or fibrosis in the interstitium. Histological study of sections of stomach shows normal architecture. No any epithelial damage and ulceration was noted in the stomach sections of test drug treated groups. Submucosa, muscularis propria and serosa were all unremarkable.

IV. Conclusion

In vivo toxicity study reveals that there was no mortality and signs of toxicity observed for acute oral administration of Kiranthi Mega Chooranam till the dose of ten times the therapeutic dose of 2000mg/kg b.wt in prescribed manner. In long term toxicity study there was no significant changes in haematological and biochemical parameters in test drug treated groups when compare to control group. The histopathological report are also confirms that there was no cellular changes at all doses level. It clearly demonstrate that No Observed Adverse Effect Level (NOAEL) is up to the high dose level (1080mg/kg b.wt), which is ten times of that therapeutic dose. Acute and Long term toxicity studies of drugs used in the study clearly showed the non-toxic nature and high safety profile of Kiranthi Mega Chooranam in Rodents.

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