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REPEATED DOSE 90-DAY ORAL TOXICITY EVALUATION OF KUMAARA VEERIYA KAANTHA CHENDURAM, A SIDDHA HERBOMINERAL FORMULATION IN WISTER ALBINO RATS

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ABSTRACT

Aim: In siddha system of medicine, one of the typical kantham formulation is 'Kumaara veeriya kaantha chenduram' (KVKC) which is indicated for anaemia. The toxicological study of the drug was not reported. The objective of this study is to evaluate its safety by performing repeated dose 90-day oral toxicity studies as per OECD test guidelines 408 for the further clinical application in anaemia cases. Methods: In this study KVKC was administered in wister albino rats orally at low dose 100 mg/kg/ b.wt, Mid dose 200 mg/kg/b.wt, High dose 400 mg/ kg/ b.wt once a day for 90 days with honey. Animals were observed for toxic signs for 90 days. On 91st day cage side observations, hematological, biochemical and histopathological analysis were executed to recognize the toxic effects. Results: Results of the hematological, biochemical and histopathological analysis conducted on 91th day revealed that there were no significant changes in all the drug treated animals when compared with those of respective controls. According to these results, Kumaara veeriya kaantha chenduram could be concluded as no-observed-adverse-effect level (NOAEL). Conclusion: This study provides scientific validation for the drug's safety and also proved its effectiveness in long-term administration without harming the human being. And the drug KVKC is safe in the dose of 260 mg for human adult mentioned in the siddha literature.

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INTRODUCTION

Siddha medicines have been used for decades, and in a wide variety of diseases are valued for their various benefits. In siddha system of medicine, there are 32 types of internal medicines composed mainly by the four types of sources namely herbals, metals, minerals and animals.[1] Siddhars specialized in herbomineral, herbometallic preparations like Parpam, chendooram, chunnam, mezhugu and kattu. The advantages of these preparations over herbal formulations are longer shelf life (75 years in case of chenduram) and efficacy with very little dosage. Lack of assessment of toxicity is a lacuna in these siddha formulations that causes concern about their safety. Kumaara veeriya kaantha chenduram is one of the siddha herbomineral formulation includes two ingredients i.e. kaantham (Magnetic Oxide of iron) and thara leaves (*Mollugo oppositifolia* Linn.) and indicated for Iron deficiency anemia.[2] Since the drug KVKC is non sasthric and also it contains magnetic oxide of iron it is mandate to have a safety profile studies. Therefore, the drug was tested for safety profile as per OECD Guideline 408 in wistar albino rats.[3]

METERIALS AND METHODS

IAEC Approval & Purchasing animals

For the toxicological safety evaluation of Kumaara veeriya kaantha chenduram, Institutional Animal Ethical Committee (IAEC) approval (NIS/IAEC-VII/28082018/01) was obtained by submitting the duly filled Form B. The male & female albino rats were obtained from the authorized animal breeders of the animal laboratory in Tamil Nadu Veterinary & Animal Sciences University (TANUVAS), Madhavaram Chennai. The study (As per OECD Guideline - 408) was performed in Animal House, National Institute of Siddha, Chennai

Authentication of Raw Drugs & Preparation of KVKC

The raw drugs were authenticated by the Botanist, National Institute of Siddha and Associate Professor, Department of Gunapadam, National Institute of Siddha, Chennai. (Voucher No NISMB4002019) The raw drugs were purified and the medicine was prepared at Gunapadam lab of National Institute of Siddha.

Animal Care and Husbandry

Species : Wister albino Rats
Sex : Male and Female
Age / Weight : 8-12 weeks / 140 – 160 g b.wt.
Acclimatization Period : 7 Days prior to dosing.
Housing : Individually in polypropylene cages
Husbandry : 12-hour light/ 12 hour dark cycle.
Room temperature : 220 c (± 30)
Humidity : Relative 30 – 70 %
Feed and Water : Rodent pelleted feed, RO purified water *ad libitum.*

Animals were kept in Individual cages and numbered. The Male & Female albino rats were kept in the animal house at National Institute of Siddha, Chennai – 47. Animals were housed in a cage at $22^{o}c \pm 3^{o}$ and relative humidity 30 – 70 % and have free access to standard rat pellet diet which was purchased from Sai meera Feeds Pvt. Ltd, Bangalore.

Experimental details:

Animal selection and Identification:

The animals were randomly selected for each group. Each group contains ten male and ten female animals. They were marked on head, body, tail, Head body, Body tail with picric acid solution prepared in water for identification in each group.

Test guideline : OECD guidelines (408)
Length of exposure to test substance: 90 days
No of animals : 10Male +10 Female / group
Control group : Honey
Test groups : *Low Dose, Mid Dose, High Dose*
Route of Administration : Oral

Table 1: Groups No. of Rats:

S.NO	GROUPS	TREATMENT & DOSE	NUMBER OF RATS
1.	Group I	Control vehicle-honey	20 (10M+10F)
2.	Group II	KVKC 100mg/kg per oral	20 (10M+10F)
3.	Group III	KVKC 200mg/kg per oral	20 (10M+10F)
4.	Group IV	KVKC 400mg/kg per oral	20 (10M+10F)

Total 80 (40 male + 40 female)

*****KVKC - Test drug (*KUMAARA VEERIYA KAANTHA CHENDURAM*)

Experimental procedure:

The 40 male and 40 female Wister albino rats were used for 90 days repeated oral toxicity study. The animals were divided into four groups. Each group contains 20 animals (10 Female and 10 Male). The first group were treated as control and second, third, fourth groups were treated with Low dose 100 mg/kg/ b.wt, Mid dose 200 mg/kg/b.wt, High dose 400mg/ kg/ b.wt of test drug respectively for 90 days. The low dose, mid dose and high dose of test drug were calculated from human therapeutic dose based on by using the conversion table Paget and Barnes 1964.[4] The control animals were administered with honey solution. The administration was given by oral, once daily for 90 consecutive days. The animals were observed for the behavioural parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Feed & water intake were calculated daily. All the animals were sacrificed at the end of the study 91st day by using the intra peritoneal injection of Pentothal Sodium as prescribed dose level. Blood was collected from the anesthetized animals from the abdominal aorta for the following investigations like Haemotology and Biochemical analysis. Gross pathological changes were monitored in all the organs and then the vital organs were preserved and subjected to Histopathological examination.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

- All the animals were observed twice daily for 90 days
- Body weight were Calculated weekly once
- Feed & water intake were calculated daily

Cage side observation:

The animals were monitored for behavioural parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhoea, Writhing, Respiration, Mortality.

Laboratory Investigations:

On the 90th day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with Ethylenediamine tetra acetic acid (EDTA) for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

Hematological Investigations:

Blood samples of control and experimental rats were analysed for hemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto haematological analyser.

Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, blood urea nitrogen (BUN), Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of serum glutamate oxaloacetate transaminase/ Aspartate aminotransferase (SGOT/AST), serum glutamate pyruvate transaminase/ Alanine aminotransferase (SGPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 91st day. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, sex organs, of all animals were recorded.

Histopathology:

The organs included liver, kidneys, spleen, brain, heart, lungs of the animals were preserved, and they were subjected to histopathological examination. Histopathological investigation of the vital organs was done. The organ pieces (35µm thick) of all the animals (low, mid, high) were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Hematoxylin-eosin.

Statistical analysis:

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

RESULTS

Table 2: Signs of toxicity in repeated dose 90-day oral toxicity study (OECD Guideline-408):

Parameters Observed	Day- 1	Day- 15	Day- 30	Day- 45	Day- 60	Day- 75	Day- 90
Alertness	+	+	+	+	+	+	+
Aggressiveness	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-
Oedema	-	-	-	-	-	-	-
Touch Response	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-
Writing reflex	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-
Pile erection	-	-	-	-	-	-	-
Analgesia	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-
Hypnosis	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-

(+ indicates present and – indicates nil)

There was no toxic manifestations, behavioural alterations and mortality during the dosing period.

Table 3: Body weight (g) of albino rats(female) exposed to KVKC for 90 days.

DAYS	Control	Low dose	Mid dose	High dose
1	220 ± 3.5	222.9 ± 4.56	224 ± 5.23	225.8 ± 5.09
15	233.5 ± 4.06	236.5 ± 5.19	236.4 ± 6.06	237.4 ± 5.32
30	247.4 ± 4.38	248 ± 4.94	249.5 ± 6.17	252.7 ± 6.46
45	259.6 ± 7.63	260.3 ± 6.9	262.7 ± 8.18	267.6 ± 9.54
60	273.8 ± 10.06	275.3 ± 9.03	276.1 ± 10.96	281.9 ± 12.03
75	291.4 ± 11.64	294.4 ± 9.96	290.4 ± 11.81	296.3 ± 12.65
90	305.7 ± 9.14	312.4 ± 10.88	308.5 ± 10.62	310.7 ± 9.62

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01.

Table 4: Body weight (g) of albino rats(male) exposed to KVKC for 90 days.

DAYS	Control	Low dose	Mid dose	High dose
1	250.4 ± 6.33	251 ± 5.27	251.9 ± 6.84	254.9 ± 5.86
15	263.4 ± 6.47	263.8 ± 10.43	266.6 ± 7.31	268.5 ± 6.49
30	275.4 ± 6.13	277.2 ± 10.77	280.8 ± 10.09	282.4 ± 6.17
45	289.4 ± 6.83	290.2 ± 9.9	294.4 ± 8.69	296.2 ± 6.27
60	302.7 ± 9.36	305.5 ± 12.71	309.2 ± 6	309.7 ± 5.06
75	318.1 ± 8.71	315.5 ± 10.43	320.6 ± 8.66	322.2 ± 4.98
90	332.6 ± 9.31	329.7 ± 11.29	334.9 ± 9.31	335.2 ± 6.6

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Table 5: Water (ml/day) intake of albino rats (female) exposed to KVKC for 90 days.

DAYS	Control	Low dose	Mid dose	High dose
1	63 ± 2	61.7 ± 1.77	61.1 ± 1.45	61.8 ± 1.32
15	66.4 ± 1.9	65.8 ± 2.1	64.7 ± 1.7	65.5 ± 1.35
30	70.1 ± 1.6	69.4 ± 1.58	68.3 ± 1.25	69.1 ± 1.66
45	73.3 ± 1.7	73.2 ± 2.25	71.5 ± 1.78	72.5 ± 2.12
60	76.2 ± 1.55	77 ± 2.45	75.4 ± 1.71	76 ± 1.94
75	79 ± 1.63	79.8 ± 2.44	78.4 ± 2.01	79.3 ± 2
90	83.1 ± 1.37	83.5 ± 1.65	82.7 ± 1.57	83.6 ± 1.84

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01.

Table 6: Water (ml/day) intake of albino rats (male) exposed to KVKC for 90 days.

DAYS	Control	Low dose	Mid dose	High dose
1	78.4 ± 2.72	82.3 ± 3.16	81.7 ± 2.63	82.5 ± 2.76
15	84.1 ± 3.07	86.6 ± 2.67	86 ± 2	86.5 ± 2.46
30	89.4 ± 2.41	89.6 ± 3.89	88.1 ± 4.09	89.4 ± 3.72
45	93.5 ± 2.27	94.5 ± 2.27	93.8 ± 2.1	94.4 ± 2.27
60	96.2 ± 2.57	97.5 ± 2.12	97 ± 2	97.7 ± 1.89
75	99.1 ± 2.28	100.9 ± 2.38	100.2 ± 1.32	100.4 ± 1.84
90	102.3 ± 2.45	103.8 ± 2.25	103.3 ± 1.25	103.8 ± 2.1

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Table 7: Food (g/day) intake of albino rats (female) exposed to KVKC for 90 days.

DAYS	Control	Low dose	Mid dose	High dose
1	32.4 ± 1.58	33.3 ± 2.06	34 ± 2	33 ± 2.58
15	34.8 ± 1.32	36.3 ± 2.06	36.6 ± 1.71	36 ± 2.26
30	37.8 ± 1.32	39.2 ± 2.15	39.2 ± 1.32	38.4 ± 1.51
45	41.1 ± 1.91	42.5 ± 2.64	42.7 ± 1.49	41.7 ± 2.06
60	45.1 ± 1.85	45.9 ± 2.92	46.4 ± 1.71	45.5 ± 2.27
75	48.3 ± 2.54	49.1 ± 3.38	50 ± 2.49	48.6 ± 3.72
90	51.7 ± 2.36	52.1 ± 3.41	53.1 ± 2.23	51.9 ± 3.28

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01.

Table 8: Food (g/day) intake of albino rats(male) exposed to KVKC for 90 days.

DAYS	Control	Low dose	Mid dose	High dose
1	39.8 ± 1.32	40.3 ± 1.7	40.4 ± 1.51	41.1 ± 1.91
15	42.8 ± 1.48	43.6 ± 2.01	43.5 ± 1.35	44.3 ± 2.11
30	46.6 ± 1.35	46.7 ± 1.95	46.7 ± 1.49	48 ± 2.83
45	50.4 ± 1.9	50.2 ± 2.1	50.9 ± 1.66	52.1 ± 2.92
60	53.3 ± 1.57	53.4 ± 1.84	54.4 ± 1.43	55.6 ± 3.17
75	55.6 ± 2.12	55.4 ± 1.84	56.5 ± 1.65	57.8 ± 3.79
90	58 ± 2.4	58 ± 1.94	59 ± 1.56	61.2 ± 4.32

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Table 9: Hematological parameters after 90 days treatment with KVKC in female rats.

Parameters	Control	Low dose	Mid dose	High dose
Red blood cell ($x10^6$ ul)	6.3 ± 0.77	6.6 ± 0.85	6.67 ± 0.88	6.68 ± 0.62
Leukocyte ($x10^3$/ul)	12.39 ± 1.22	7.27 ± 0.74	6.77 ± 2.16	7.01 ± 0.74
N	1.93 ± 0.27	2.48 ± 0.39	2.531 ± 0.41	2.42 ± 0.64
E	1.65 ± 0.13	1.4 ± 0.25	1.55 ± 0.22	1.38 ± 0.16
B	0.92 ± 0.08	0 ± 0	0.1 ± 0.32	0 ± 0
L	76.06 ± 1.24	81.33 ± 3.69	81.41 ± 3.15	69.3 ± 6.14
M	3.03 ± 0.48	2.69 ± 0.7	2.5 ± 0.52	4.41 ± 0.72
Platelets ($x10^3$/ul)	555.3 ± 71.46	660.6 ± 54.7	708.9 ± 136.16	618.8 ± 74.93
HB (%)	12.13 ± 0.82	13.26 ± 1.06	13.81 ± 1.96	13.78 ± 1.55
MCH (fl)	15.97 ± 0.43	20.73 ± 2.26	19.88 ± 1.81	17.57 ± 0.57
MCV (pg)	65.49 ± 1.7	56.35 ± 3.39	62.42 ± 4.88	64.52 ± 3.39

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Table 10: Hematological parameters after 90 days treatment with KVKC in male rats.

Parameters	Control	Low dose	Mid dose	High dose
Red blood cell ($x10^6$ ul)	5.92 ± 0.53	6.32 ± 0.79	6.77 ± 0.84	6.31 ± 0.57
Leukocyte ($x10^3$/ul)	7.48 ± 0.73	7.5 ± 1.83	6.07 ± 0.56	7.28 ± 0.74
N	2.48 ± 0.81	2.4 ± 0.38	2.44 ± 0.46	2.54 ± 0.54
E	1.53 ± 0.18	1.49 ± 0.26	1.4 ± 0.2	1.38 ± 0.31
B	0.01 ± 0.03	0 ± 0	0 ± 0	0 ± 0
L	75.2 ± 1.38	78.45 ± 1.74	80.47 ± 2.99	77.12 ± 7.64
M	3.18 ± 0.44	2.95 ± 0.66	2.62 ± 0.64	1.75 ± 0.78
Platelets ($x10^3$/ul)	570 ± 68.14	531.8 ± 33.24	675.2 ± 51.3	635.8 ± 73.93
HB (%)	13.03 ± 1.44	13.06 ± 0.95	14.6 ± 1.95	14.44 ± 1.4
MCH (fl)	20.29 ± 2.03	19.47 ± 1.56	21.32 ± 2.23	17.37 ± 0.65
MCV (pg)	52.05 ± 1.3	64.29 ± 4.24	57.1 ± 4.5	61.64 ± 2.61

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01.

Table 11: Renal function test for female rats.

Dose (mg/kg)	Control	Low dose	Mid dose	High dose
BUN(mg/dl)	15.5 ± 2.07	15.8 ± 2.3	18.34 ± 10.16	15 ± 2.4
Creatinine(mg/dl)	0.75 ± 0.14	0.5 ± 0.19	0.51 ± 0.23	0.55 ± 0.2

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01.

Table 12: Liver function test for female rats.

Dose(mg/kg)	Control	Low dose	Mid dose	High dose
Total Bilirubin(mg/dl)	0.36 ± 0.14	0.48 ± 0.19	0.46 ± 0.21	0.62 ± 0.2
SGOT(U/L)	75.2 ± 2.04	46.6 ± 6.38	64.6 ± 3.37	67.5 ± 13.36
SGPT(U/L)	14.5 ± 1.78	29.8 ± 6.49	24.3 ± 4.72	25.7 ± 4.67

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Table 13: Lipid Profile for female rats.

Parameters	Control	Low dose	Mid dose	High dose
Total cholesterol (mg/kg)	104.3 ± 1.34	123.35 ± 10.11	119.76 ± 15.08	128.43 ± 16.5
HDL(mg/dl)	61.1 ± 2.23	49.9 ± 2.69	60.3 ± 6.5	62.9 ± 6.31
LDL(mg/dl)	30.6 ± 4.97	50.2 ± 3.22	48.4 ± 3.34	58.5 ± 7.69
VLDL (mg/dl)	14.89 ± 1.16	16.55 ± 0.79	17.67 ± 0.79	16.5 ± 1.37
Triglycerides(mg/kg)	26.9 ± 1.79	51.1 ± 7.69	58.9 ± 23.71	37.9 ± 7.05

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Table 14: Renal function test for male rats.

Dose (mg/kg)	Control	Low dose	Mid dose	High dose
BUN(mg/dl)	14.4 ± 2.91	14 ± 2.49	15.96 ± 1.56	16.5 ± 2.17
Creatinine(mg/dl)	0.59 ± 0.16	0.72 ± 0.16	0.55 ± 0.22	0.39 ± 0.2

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01.

Table 15: Liver function test for male rats.

Dose(mg/kg)	Control	Low dose	Mid dose	High dose
Total Bilirubin(mg/dl)	0.52 ± 0.22	0.32 ± 0.15	0.53 ± 0.25	0.43 ± 0.18
SGOT(U/L)	70.4 ± 5.91	71.4 ± 14.3	48 ± 3.94	67.6 ± 6.82
SGPT(U/L)	17.7 ± 4.92	25.1 ± 8.54	27.2 ± 2.94	23.1 ± 4.58

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant **indicates** that *P<0.05, **P<0.01.

Table 16: Lipid Profile for male rats.

Parameters	Control	Low dose	Mid dose	High dose
Total cholesterol (mg/kg)	105.73 ± 2.49	116.69 ± 28.55	119.32 ± 14.03	111.51 ± 8.78
HDL(mg/dl)	48.7 ± 4.52	52.6 ± 7.83	51.6 ± 3.53	66.3 ± 2.31
LDL(mg/dl)	43.8 ± 2.97	43.8 ± 11.71	50.8 ± 3.52	58.3 ± 17.49
VLDL (mg/dl)	15.61 ± 1.14	16.88 ± 2.16	17.13 ± 0.79	16.05 ± 1.76
Triglycerides(mg/kg)	25.2 ± 3.12	35.3 ± 11.48	68.5 ± 4.03	26.5 ± 3.63

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P< 0.01.

Histopathological results of vital organs

Figure 1: Histopathology of Brain

a) **b)**

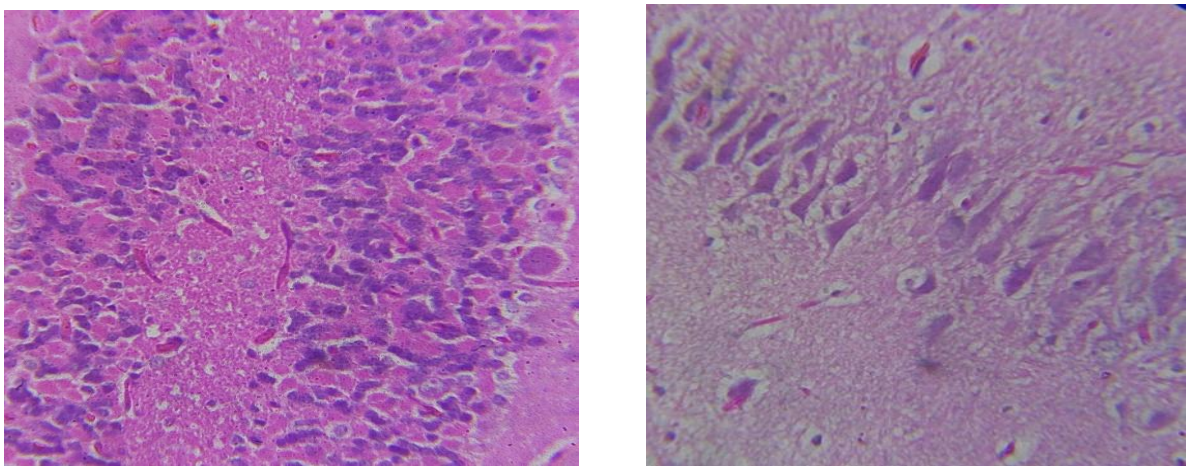

Figure 1: a) Histopathology of vehicle control male brain tissue b) Histopathology of high dose (400mg/kg bw) male brain tissue.

Observation: Granular and Purkinje cells appeared with distinct cytoplasm in control and high dose male.

Figure 2: Histopathology of Heart

a) **b)**

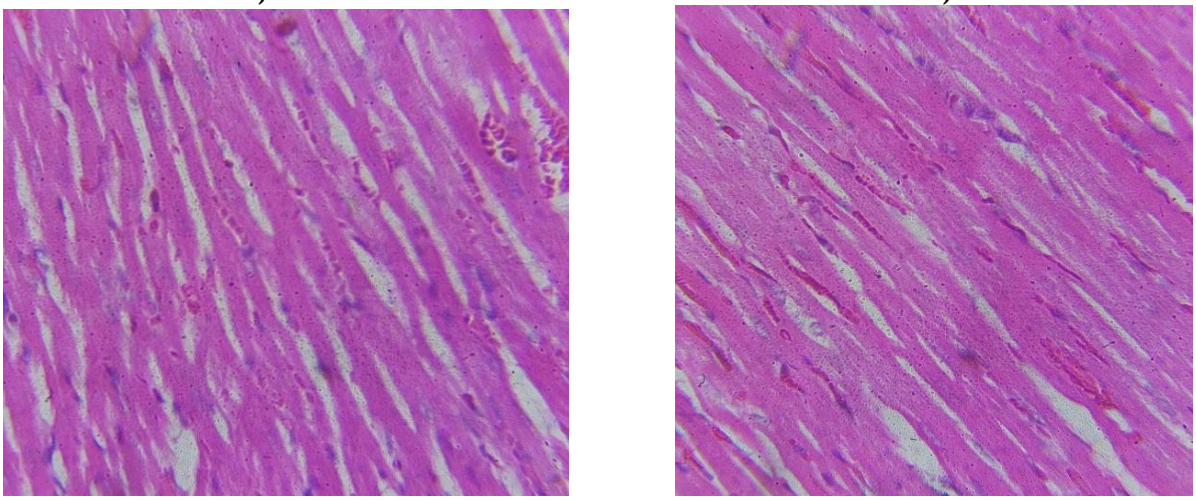

Figure 2: a) Histopathology of vehicle control male heart tissue b) Histopathology of high dose (400mg/kg bw) male heart tissue.

Observation: Showed the normal histological structure of myocardium in control and high dose male**.**

Figure 3: Histopathology of Lungs

a) **b)**

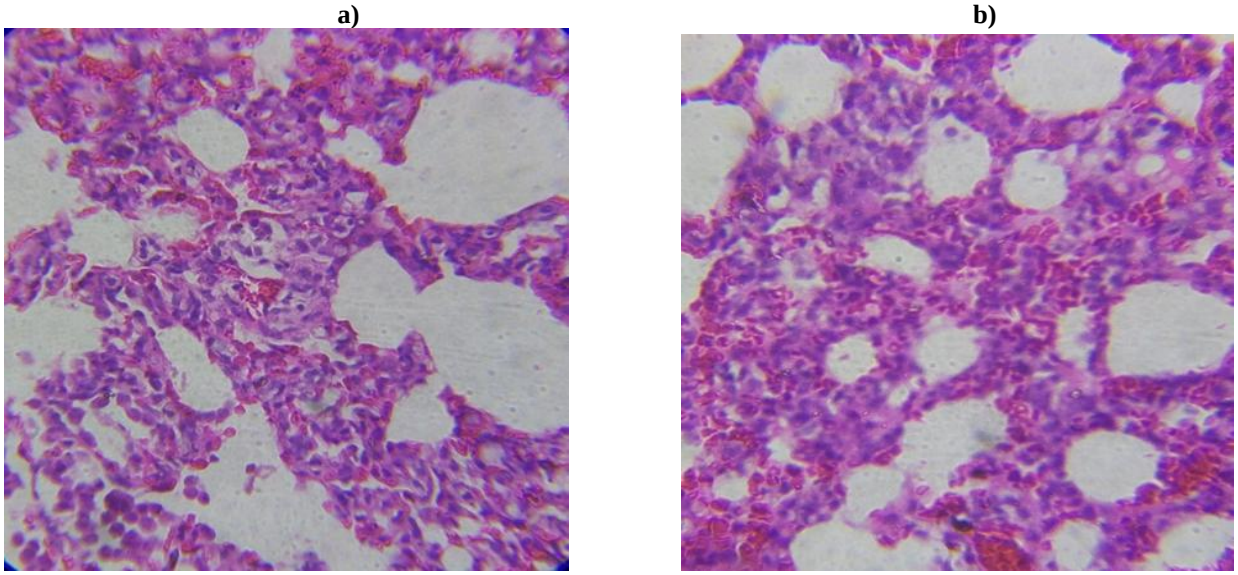

Figure 3: a) Histopathology of vehicle control male lung tissue b) Histopathology of high dose (400mg/kg bw) male lung tissue.

Observation Alveolar epithelium and capillaries appeared normal in control and high dose male.

Figure 4: Histopathology of Liver

a) **b)**

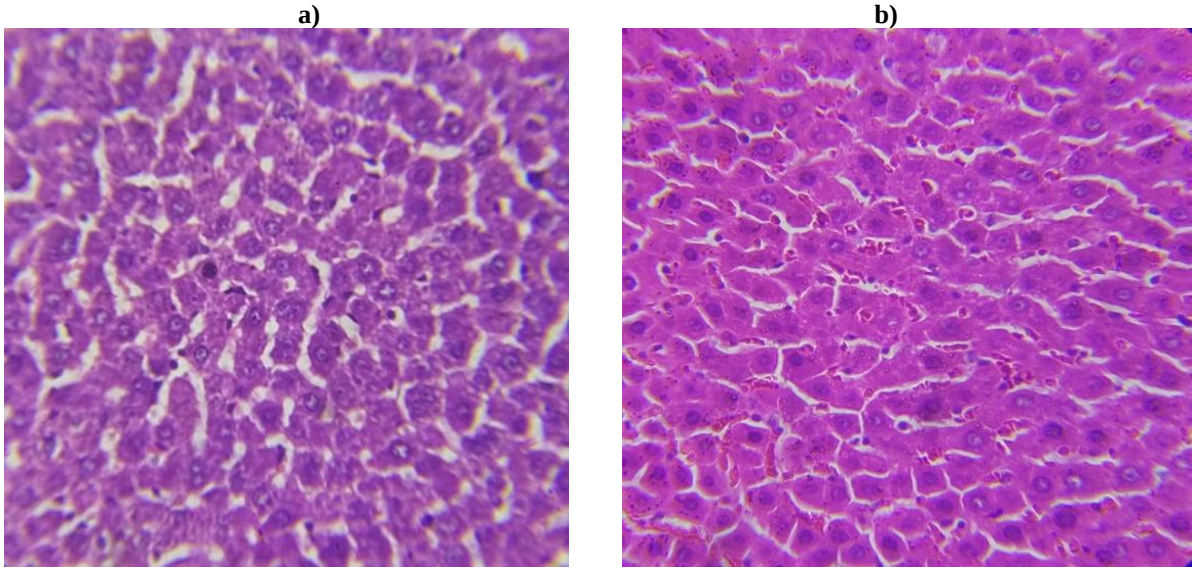

Figure 4: a) Histopathology of vehicle control male liver tissue b) Histopathology of high dose (400mg/kg bw) male liver tissue.

Observation: Hepatic cords appeared normal with radiating morphology in control and high dose male.

Figure 5: Histopathology of kidney

a) **b)**

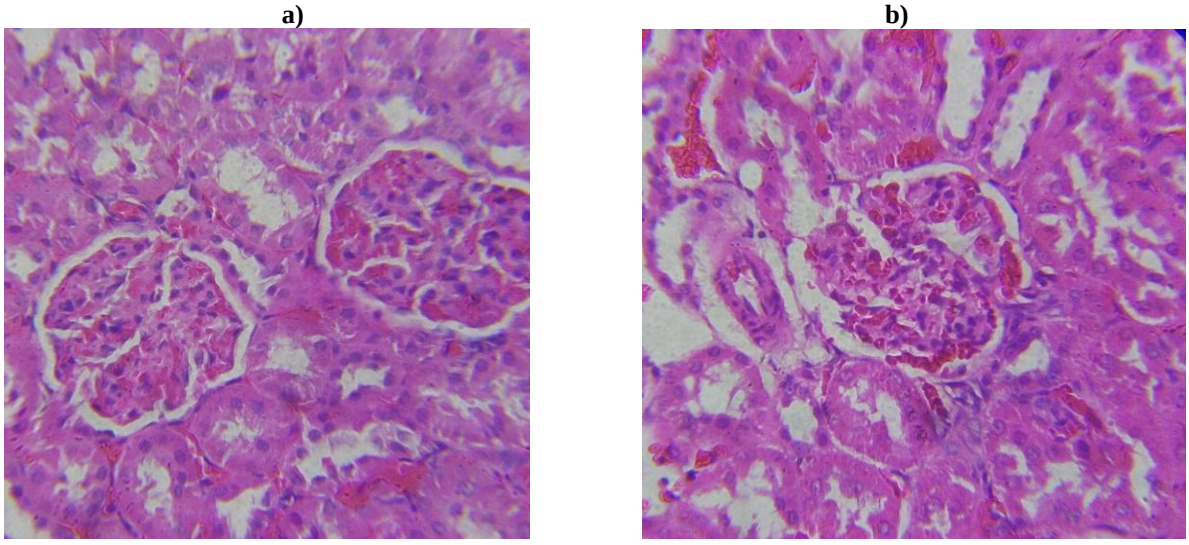

Figure 5: a) Histopathology of vehicle control male kidney tissue b) Histopathology of high dose (400mg/kg bw) male kidney tissue.

Observation: Appearance of proximal and distal convolutes tubules was normal in control and high dose male.

Figure 6: Histopathology of spleen

a) **b)**

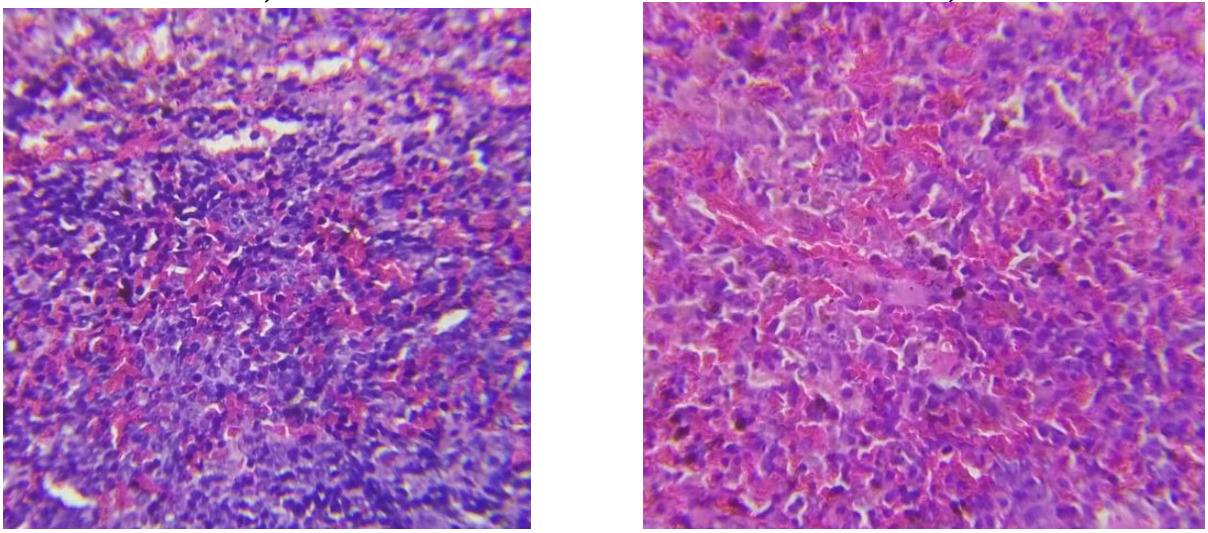

Figure 6: a) Histopathology of vehicle control male spleen tissue b) Histopathology of high dose (400mg/kg bw) male spleen tissue.

Observation: Marginal sinus (MS) of the spleen and its sinus lining cells appeared normal in control and high dose male.

DISCUSSION

Siddhars specialized in herbomineral preparations like Parpam, chendooram, chunnam, mezhugu and kattu. One of such herbomineral formulation is Kumaara veeriya kantha chenduram. It has haematinic, nervine tonic, and rejuvenation activity and is primarily given for anaemia with iron deficiency. Since there are many herbal formulations available for Iron deficiency anaemia these herbomineral preparations over herbal formulations are longer shelf life (75 years) and efficacy with very little dosage, easily palatable, odourless. Hence the herbomineral formulation Kumaara veeriya kaantha chenduram was selected to treat the Iron Deficiency Anaemia. Drugs intended for medical use should undergo toxicity evaluations before being considered safe for human use.

All animals from control and all the treated dose groups survived throughout the dosing period and there was no toxic manifestations, behavioural alterations and mortality during the dosing period of 90 days for chronic toxicity study. The results for body weight determination of animals from control and different dose groups showed comparable body weight gain throughout the dosing period of 90 days. During dosing period, the quantity of food and water consumed by animals from different dose groups was found to be comparable and normal with that by control animals.

Hematological investigations conducted on day 91st day revealed no significant changes in the hematological values when compared with those of respective controls.

Biochemical investigations conducted on 91th day revealed that there were no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT, SGPT, Bilirubin were within the limits. The other cardio vascular risk markers were also within normal ensured that Kumaara veeriya kantha chenduram did not influence the Cardio vascular system.

CONCLUSION

All animals from control and all the treated dose groups survived throughout the dosing period of 90 days in chronic toxicity study. No signs of toxicity were noted in all animals. Animals body weight, food and water intake of all animals were normal throughout the study period. Results of the hematological, biochemical and histopathological analysis conducted on 91th day revealed that there were no significant changes in all the drug treated group when compared with those of respective controls. According to these results, *Kumaara veeriya kantha chenduram* could be concluded as no-observed-adverse-effect level (NOAEL). It showed the drug 's safety upto the high dose of 400mg/kg b.wt that proved its effectiveness in long-term administration without harming the human being. And the drug KVKC is safe in the dose of 260 mg for human adult mentioned in the siddha literature.

ABREVIATIONS

OECD - The Organisation for Economic Co-operation and Development
KVKC - Kumaara veeriya kaantha chenduram
TANUVAS - Tamil Nadu Veterinary & Animal Sciences University
NOAEL - no-observed-adverse-effect level

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CONFLICT OF INTEREST

NIL

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