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ORIGINAL RESEARCH ARTICLE

Acute and 28 Days Repeated Oral Toxicity Studies of A Siddha Drug *Kalamega Narayana Chendooram* in *Kodiveli Verpattai Chooranam* on Wistar Albino Rats

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ABSTRACT

Kalamega Narayana Chendooram in Kodiveli Verpattai Chooranam (KMNC in KVC) a herbo mineral formulation has been employed as a traditional siddha remedy for seronegative polyarthritis since long time. Seronegative polyarthritis is more common causing more social burden to families, the symptoms are pain in multiple joints, joint swelling, tenderness, morning stiffness, restriction of movements etc. As a mandate, steps were taken to evaluate safety profile of KMNC in KVC in animal model under OECD guidelines. Acute toxicity studies done on female wistar albino rat under OECD guidelines 423 and 28 days repeated oral toxicity studies done on both sex of wistar albino rat under OECD guidelines 407. Acute oral toxicity study of KMNC in KVC revealed no mortality at the dosage of 2000 mg/kg body weight and the median lethal dosage of KMNC in KVC is estimated above 2000 mg/kg body weight. Repeated oral toxicity study of KMNC in KVC does not exhibit mortality at the high dosage of 100 mg/kg body weight given up to the period of 56 days including 28 days of drug administered. At the end of 28 days no specific changes are observed in hematological, hepatic, renal and other biochemical parameters. No gross morphological and histological changes are observed in the organs. The above studies recommend that KMNC in KVC is a safest drug under intended human adult dosage (565 mg) as illustrated in the literature.

Key words: *Kalamega Narayana Chendooram in Kodiveli Verpattai Chooranam*, Diarrhea, Acute toxicity, Sub-acute toxicity.

INTRODUCTION

The interventional Siddha drug *Kalamega Narayana Chendooram in Kodiveli Verpattai Chooranam* (KMNC in KVC) is a herbo mineral formulation of siddha medicine. It is the combination of a siddha herbal powder and a siddha poly mineral preparation. The herbal powder contains only purified and dried root bark powder of Kodiveli Verpattai (Plumbago zeylanica), the poly mineral preparation contains Rasam (Mercury), Gandhagam (Sulphur), Lingam (Redsulphide of Mercury), Thalagam (Arsenic trisulphidium) and Manosilai (Arsenic disulphide)[2]. The preparation has been noted in Sigitcha Rathna Deepam [1]. But the claim of the efficacy and safety is not yet to be tested. Previous pharmacological studies showed that the drug Kalamega narayana chendooram has significant antiarthritic, anti-inflammatory, antipyretic and analgesic activity at the dose of 400 mg/kg and there are no significant adverse effects [2].

Plumbago zeylanica contains a variety of important chemical compounds. Different plant parts of the plant possess naphthaquinones, alkaloids, glycosides, steroids, triterpenoids, tannins, phenolic compounds, flavanoids, saponins, coumarins, carbohydrates, fixed oil and fats and proteins [3] and useful for the treatment of arthritis [4].

In clinical aspect, KMNC in KVC is remedy for Seronegative polyarthritis. Seronegative poly arthritis is the inflammation of multiple joints which involves five or more joints simultaneously. The main cause of polyarthritis is Rheumatoid factor. A negative RF does not rule out RA; rather the arthritis is called **Seronegative**. This is the case in about 15% of patients. During first year of

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illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time. The common symptoms are joint pain, stiffness, tenderness and swelling [5]. The social burden of these musculo skeletal conditions increasing and it affects the quality of normal living [6]

However, a toxicity study of KMNC in KVC has not been studied. The preclinical toxicity studies are essential for determining a safe dose for human trials [10]. The present study has been planned to explore the real toxicity study of herbo-mineral siddha formulation of *KMNC in KVC in* Wistar albino rats.

MATERIALS AND METHODS

Preparation of the *KMNC in KVC*:

a) **Ingredients of KMNC:**
Rasam (Mercury), Gandhagam (Sulphur), Lingam (Redsulphide of Mercury), Thalagam (Arsenic trisulphidium) and Manosilai (Arsenic disulphide)

b) **Ingredient of KVC:**
Kodiveli Verpattai Chooranam (Root bark powder of Plumbago zyelanica)

c) **Procedure:**
The drugs of KMNC was purified as per text and made into fine powder form. First Rasam and Gandhagam was rubbed in Kalvam and made into a black colour powder and other three ingredients also added in it. Drugs are kept in Kalvam and rubbed with Sanga Dhiravagam[7] for 12 hours. Then it dried and kept in Kuppi. The Kuppi kept in Valuka endhiram and kept in fire. First 3 hours it fired in Dheebagini, next 6 hours as Kamalagini and last 24 hours as Kadagini. After it cooled the Kuppi opened and the Chendooram was taken out. Then it rubbed in Kalvam and made into powder. Then it is mixed in Kodiveli verpattai chooranam [1].

Animals:

Rat of either sex weighing more than 100 g were obtained from the animal house of King Institute of Preventive Medicine. The animals were used with the approval of the Institute animal ethics committee (IAEC) of Sairam Advanced Centre for Research, Chennai approval no. (1545/PO/a11/CPCSEA/1-1/2013). They were fed with a balanced standard pellet diet, maintained under standard laboratory condition providing 20 - 24^0 C temperature, standard light cycle (12 h light, 12 h dark) and water *ad libitum*.

All the animals were randomly selected and maintained under laboratory conditions for an acclimatization period of 5 days before performing the experiments.

Acute Toxicity Study – OECD 423 guidelines [8,9]:

Acute oral toxicity test for the *Kalamega Narayana Chendooram in Kodiveli Verpattai Chooranam* (KMNC in KVC) was carried out as per OECD Guidelines 423. Female wistar albino rat were fasted over night prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, to observe any death or changes in general behavior and other physiological activities. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. 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.

28 days repeated oral toxicity study [10]:

This study was carried out as per OECD Guidelines 407.In a 28 days repeated oral toxicity study, forty rats of either sex were divided into four groups. Each group consists of 10 rats

(5+5).Group I served as normal control and administered with 2 ml of distilled water (p.o) while group II, III and IV were administered daily with the Pirandai Uppu for 28 days at a dose of 20, 100, 200 mg/kg respectively (p.o).

The weight of each rat was recorded on day 0 and weekly intervals throughout the course of the study, food and water consumption per rat was calculated.

On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

RESULTS

Table 1: Dose finding experiment and its behavioral Signs of Toxicity for KMNC w KVC formulation

Dose (mg/kg)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2000	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-

1. Alertness, 2. Aggressiveness, 3. Pile erection, 4. Grooming, 5. Gripping, 6. Touch Response, 7. Decreased Motor Activity, 8. Tremors, 9. Convulsions, 10. Muscle Spasm, 11. Catatonia, 12. Muscle relaxant, 13. Hypnosis, 14. Analgesia, 15.Lacrimation, 16. Exophthalmos, 17. Diarrhoea, 18. Writhing 19. Respiration, 20. Mortality

Table 2: Body weight (g) changes of rats exposed to KMNC w KVC formulation

Dose (mg/kg/day)	*Days*				
	1	7	14	21	28
Control	122.37 ± 3.21	124.14 ± 4.09	128.21 ± 2.17	129.21 ± 5.11	133.32 ± 1.89
10	116.39±5.6	118.46±5.2	120.12±5.67	124.62±4.52	125.42±3.28
50	124.5±4.27	123.24±3.52	124.16±2.89	125.56±3.39	126.62±5.2
100	127.2±6.9	123.4±5.2	120.34±6.78	120.69±5.6	119.6±3.7

Values are expressed as mean ± S.E.M. N=10

Table 3: Effect of KMNC w KVC formulation on Organ weight in rats

Dose (mg/kg)	Control	10 mg/kg	50 mg/kg	100 mg/kg
Liver (g)	5.24±0.14	4.3±1.1	4.6±0.86	5.12±0.62
Heart (g)	0.70±0.05	0.52±0.08	0.63±0.04	0.69±0.06
Lung (g)	1.78±0.25	1.34±0.42	1.46±0.56	1.72±0.6
Spleen (g)	0.74 ±0.07	0.48±0.06	0.52±0.03	0.62±0.08
Brain (g)	1.43±0.18	1.24±0.14	1.36±0.24	1.39±0.46
Kidney (g)	0.70±0.05	0.62±0.02	0.52±0.08	0.66±0.04

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 *vs* control; N=10

Table 4: Effect of KMNC w KVC formulation on Haematological parameters in rats

Parameter	Control	10 mg/kg	50 mg/kg	100 mg/kg
RBC (x 106/mm3)	7.51 ± 0.16	6.6±1.32	6.4±1.36	6.2±0.89
PCV (%)	48.2 ± 1.3	42.4±3.2	39.2±1.32	39.8±2.67
Hb (%)	15.6 ± 0.19	14.6±2.12	14.2±1.6	14.5±1.26
WBC (x 103/mm3)	10.12 ± 1.2	12.4±2.6	11.8±3.1	13.6±2.1
Neutrophils (%)	22 ± 4	36.21±4.3**	38.4±2.2**	29.67±0.8
Mononuclear cells (%)	76 ± 2	62.6±2.7**	61.2±3.2**	69.3±1.2
Eosinophils (%)	2.4 ± 0.6	1.6±0.12	1.2±0.3*	0.8±0.05**
Platelets (x 103/mm3)	423.2 ± 48.8	414.16±26.4	433.14±32.6	404.6±48.34

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 *vs* control; N=10

Table 5: Effect of KMNC w KVC formulation on biochemical parameters in rats

Parameters	Control	10 mg/kg	50 mg/kg	100 mg/kg
Protein (g/dl)	8.62 ± 1.3	7.3±1.24	7.6±0.86	8.14±1.38
Albumin (g/dl)	4.8 ± 0.6	4.6±0.3	3.8±0.14	4.42±0.86
BUN (mg/dl)	19.2 ± 1.2	22.2±1.2	23.41±2.6	23.8±1.62
Urea (mg/dl)	64.24 ± 3.11	62.32±4.6	58.3±6.2	66.45±3.4
Creatinine (mg/dl)	0.82 ± 0.16	0.92±1.2	0.62±0.46	0.73±0.68
Total Cholesterol (mg/dl)	91.24 ± 1.35	86.5±6.9	97.14±7.2	113.4±5.2*
Triglycerides (mg/dl)	50.15 ± 3.21	49.3±3.4	59.34±4.2	60.38±6.3
Glucose (mg/dl)	110.16 ± 8.62	90.41±7.8	110.4±3.2	93.49±5.6
Total Bilirubin (mg/dl)	0.205 ± 0.04	0.352±0.04	0.423±0.06	0.6±0.18*
SGOT (U/L)	73 ± 2.4	62±3.4	56±5.4*	72±3.8
SGPT(U/L)	28.4 ± 1.2	37±2.6	39.4±3.9	40.6±4.2*
Alkaline phosphatase(U/L)	102.4 ± 3.6	103±4.6	119.4±3.9	116.4±4.3
Sodium (mEq/L)	138.12 ± 3.14	142.5±5.6	146.2±8.2	139.4±6.2
Potassium (mEq/L)	7.2 ± 1.34	4.6±0.68	4.2±0.16	5.3±0.79

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 *vs* control; N=10

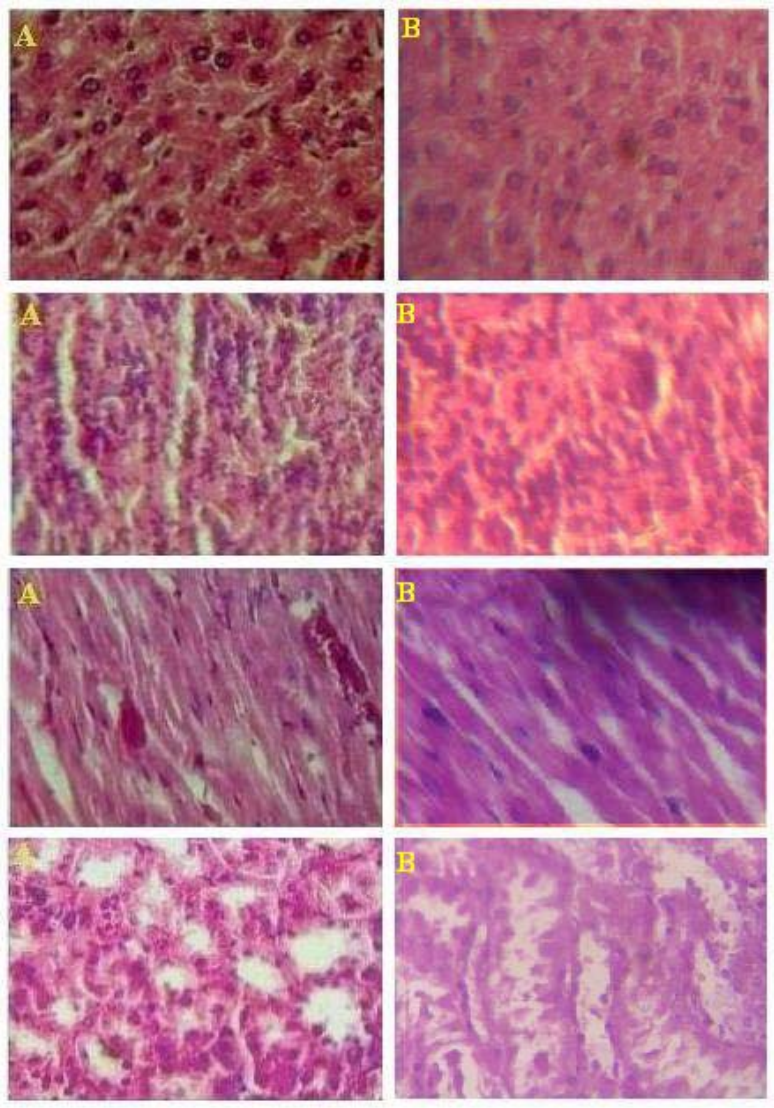
Table 6: Effect of KMNC w KVC formulation on Urine parameters in rats

Parameters	Control	10 mg/kg	50 mg/kg	100 mg/kg
Colour	Yellow	Yellow	yellow	yellow
Transparency	Clear	Clear	clear	Turbid
Specific gravity	1.01	1.02	1.01	0.9
pH	7.2	7.1	7.2	6.8
Protein	Nil	Nil	Nil	Trace
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	+
Ketones	-ve	-ve	-ve	-ve
Blood	Absent	Absent	Absent	Absent
RBCs	Nil	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Occasional

Histopathology:

The vital organs such as liver, heart, Spleen and kidneys were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Microscopically, these organs of the test groups revealed normal histological appearance when compared with the control group (Panel 1-4).

HISTOPATHOLOGY OF TOXICOLOGY STUDIES



Panel 1: Light photomicrography of liver of a control rat

Figure A – Control

Figure B – Treated on high dose, no abnormality is seen in hepatocytes, sinusoids.

Panel 2: Light photomicrography of Spleen of a control rat

Figure A – Control

Figure B – Treated on high dose, no abnormality is seen in trabeculae, capsule.

Panel 3: Light photomicrography of a Control

Figure A – Control

Figure B – treated on high dose, noAbnormality is seen in nuclei of Myocytes, Myocardium

Panel 4: Light photomicrography of Kidney of a control rat

Figure A – Control

Figure B – Treated on high dose, no abnormality is seen in glomeruli, Bowman's capsule, capillaries.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova followed by dunnet't' test using a computer software programme -INSTAT-V3 version.

DISCUSSION

The results of Acute toxicity study (**Table 1**) of *KMNC w KVC* revealed no mortality, abnormal signs and behavioral changes except Alertness, Grooming, Diarrhoea, Writhing in rats at the dose of 2000 mg kg-1 body weight administered orally. The median lethal dose for *KMNC w KVC* should be above 2000 mg/kg and it comes under unclassified.

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub-acute toxicity study.

The result Body weight (**Table 2**) indicates the effect of *KMNC w KVC* in body weight in the treated rats. There was no significant change in Body Weight up to 28 days of treatment period. All the treated group animals were gradually increase in the weight during the treatment period.

The result of Organ weight (**Table 3**) indicates the effect of *KMNC w KVC* in the organ weight in the treated rats. There was no significant increase or decrease of weight of the organs such as Brain, Lungs, Heart, Liver, Spleen and Kidneys.

The result of Hematological parameters (**Table 4**) indicates the effect of ***KMNC in KVC*** in hematological parameters of treated rats. The neutrophils of treated group at the dose level of 10mg/kg and 50mg/kg were significantly raised (P value <0.01), but it is within the normal reference value. The mononucleted cells of treated group at the dose level of 10mg/kg and 50mg/kg were significantly decreased (P value <0.01), but it is within the normal reference value. Eosinophils count were significantly decreased in the treated group of rats at the dose level of 50mg/kg and 100mg/kg (P value <0.05, and P value <0.001 respectively). The other hematological parameters show no significant changes comparable to the control group. More over all the hematological parameters in the treated group were within the normal limit.

The result of Biochemical parameters (**Table 5**) indicates the effect of ***KMNC in KVC*** in Biochemical parameters of the treated rats. The value of total Cholesterol, total Bilirubin and SGPT value were significantly increased in the treatment group at the dose level of 100mg/kg - High dose (P value <0.05). The value of SGOT seen significantly decreased (P value <0.05) in the treatment group at the dose level of 50mg/kg. the other Biochemical parameters where within the normal limit and statically not significant and comparable with the control even at the high dose.

CONCLUSION

The authors concluded based on the above findings, the median lethal dose of *KMNC in KVC* estimated as above 2000 mg/kg body weight, and results of 28 days repeated oral toxicity study revealed there is no significant changes found at 200 mg/kg body weight. It can be recommend that KMNC in KVC is a safest drug under intended human adult dosage (565 mg twice a day) as illustrated in the literature for diarrhea.

REFERENCES

1. Kannusami Pillai, Sigitcha Rathana Deepam (P : 238), B.Rathiana Nayakkar and sons, Chennai - 79, 1991.
2. Article id 13282, a study on kalamega narayana chendooram, http://ayushportal.nic.in/ShowDefault2.aspx.
3. Plumbago zeylanica l.: a mini review manu pant, ankita lal, swati rana, anju rani, international journal of pharmaceutical applications issn 0976- 639. Online issn 2278 – 6023 vol 3, issue 3, 2012, pp 399-405.
4. Alleviation of Collagen-induced Arthritis by Plumbago zeylanica. in Mice, Aparanji Poosarla, B. Veerendra Kumar, T. Raghava Rao, D.N. Rao, Rama Rao Athota, Pharmaceutical Biology, 2007, Vol. 45, No. 1 : Pages 54-59, http://www.bipublication.com.
5. Approach to Seronegative Arthritis, Rohini Handa, JIACM 2003; 4(3): 190-2.
6. Burden of major musculoskeletal conditions, Anthony D.Woolf1 & Bruce Pfleger2, Special Theme – Bone and Joint Decade 2000 – 2010,http://www.who.int/bulletin/volumes/81/9/Woolf.pdf.
7. Kannusami Pillai, Sigitcha Rathana Deepam (P : 299), B.Rathiana Nayakkar and sons, Chennai - 79, 1991.
8. Schlede E., Mischke U., Diener W. and Kayser D. The International Validation Study of the Acute-Toxic-Class Method (oral). Arch. Toxicol. 1994;69, 659-670.
9. Schlede E., Mischke U., Roll R. and Kayser D. A National Validation Study of the Acute-Toxic-Class Method – an alternative to the LD50 test. Arch. Toxicol. 1992;66: 455-470.
10. OECD Guidelines for the Testing of Chemicals (No. 407, Section 4: Health

Effects) "Repeated Dose 28-Day Oral Toxicity in Rodents" (Adopted on 12 May 198 1 and Updated on 27 July 1995).