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ACUTE & SUB ACUTE TOXICITY OF A SIDDHA MEDICINE KARUNGALI VER KUDINEER IN ALBINO MICE AND RATS

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ABSTRACT
Karungali ver Kudineer (Acacia catechu root extract) – A Siddha drug act as central and peripheral analgesic. Prior to any study the safety is important. The acute and sub acute toxicity studies were carried out in animals. Under the strict standard laboratory condition the tests were performed. The dosage of the animals was calculated according to the fasted body weight of the animals. Data were analysed by using ANOVA. $P < 0.05$ is considered as significant. No toxic effect was identified up to 8ml /kg of karunkali ver Kudineer administered through orally for 28 days. Hence this study is a way for clinical trials will be carried in future.

KEYWORDS: Acute and Sub acute toxicity, Karunkali ver extract, Therapeutic dose.

INTRODUCTION
A Siddha drug Karungali ver Kudineer (Acacia catechu extract) is quoted as the treatment for both Neerizhivu (Diabetes) and Thimiru (neuropathy).[1] The karungali ver has central and peripheral acting analgesic activity.[2] The dosage of the drug is essential for treating the patient. For this purpose the acute and sub acute toxicity study is carried out in animals.

As per the OECD guidelines 425[3] the acute and sub acute toxicity done. The animals are selected in appropriate size and range. According to the fasted body weight of animals the doses are calculated. The appropriate doses are administered in animals from minimal to maximum range and observed for their behavioural changes and toxicity symptoms.

The blood samples of the animals are taken for analysis. After that the animals are sacrificed. The positions, shapes, sizes and colours of internal organs histopathological changes are evaluated.

Data are analyzed using one-way analysis of variance (ANOVA) and group means are compared using the Tukey-Kramer Multiple Comparison test using Graph Pad Instat-V3 software. $P < 0.05$ is considered significant.

No signs of significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days. Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality. The results of haematological investigations revealed following no significant changes in the values of different parameters investigated when compared with those of respective controls; Biochemical investigations revealed no statistically significant changes when compared with those of control. Histopathological examination did not reveal any abnormality.

Hence the Karungali Ver Kudineer can be used for therapeutic use in human with the dosage recommendations of up to 8ml/kg body weight p.o.

MATERIALS METHODS
Animals
Mice of either sex weighing 25-30g and rats weighing 210-240g were obtained from the animal house of Vels University. The animals were used with the approval of the Institute animal ethics committee and obtained from Vels University, Chennai. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28^{0}C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum.[5]

Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to

control and different treatment groups, six animals per group. The animals were acclimatized for one week under laboratory conditions.

ACUTE TOXICITY STUDY[3]

Acute oral toxicity test for the Karungali Ver Kudineer was carried out as per OECD Guidelines 425. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for a further 2 hours in mice.

The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behaviour and other physiological activities. Single animals are dosed in sequence usually at 48 h intervals. However, the time interval between dosing is determined by the onset, duration and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal. General behaviour, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity, and duration of these signs, if any, was recorded.[4]

SUB-ACUTE TOXICITY[7]

In a 28-days sub acute toxicity study, twenty four either sex rats were divided into four groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the Karungali Ver Kudineer (p.o.) for 28 days at a dose of 2, 4 and 8ml/kg respectively. The animals were then observed daily for gross behavioural changes and any other signs of sub acute toxicity.

The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethylether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinised tubes. The blood sample collected from each rat was centrifuged with 3000 X g at 4^{o}C for 10 min to separate the serum and used for the biochemical assays.

Hematological and blood biochemical analysis[5] [6]

After 4 weeks of the once daily treatment of Karungali Ver Kudineer, all animals were kept fasted for 16-18 h and then anesthetized with anesthetic ether on the 28th day. Blood samples for hematological and blood chemical analyses were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semi automated haematology analyzer.

The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis (glucose, creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were automatically determined using auto analyzer.[8]

Necropsy

All rats were sacrificed after the blood collection. The positions, shapes, sizes and colors of internal organs were evaluated. The Spleen, Testes, Pancrea, Lung, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovary, and Kidney tissues were excised from all rats to visually detect gross lesions, and weighed to determine relative organs' weights and preserved in 10% neutral formalin for histopathological assessment. The tissues were embedded in paraffin and then sectioned, stained with haematoxylin and eosin and were examined microscopically.[9]

Statistical analysis

Values were represented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparison test using Graph Pad Instat-V3 software. $P < 0.05$ was considered significant.

RESULTS

All the animals from control and all the treated dose groups up to 400 mg/kg survived throughout the dosing period of 28 days. No signs of significant intoxication were observed in animals from lower to higher dose groups during the dosing period of 28 days. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28days. Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality. The results of haematological investigations revealed following no significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits.

Results of Biochemical investigations revealed no statistically significant changes when compared with those of control. Functional observation tests conducted

at termination revealed no abnormalities. Urine analysis, conducted at the end of the dosing period in week 4 and at the end of recovery period in week 6, revealed no abnormality attributable to the treatment. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls. Gross pathological examination did not reveal any abnormality. Histopathological examination did not reveal any abnormality.

Table 1. Haematological parameters after 28days treatment with Karungali ver Kudineer

Parameter	Control	2ml/kg	4ml/kg	8ml/kg
RBC (mm^3)	7.20±0.33	7.25±0.31	7.18±0.24	7.22±0.28
HB (%)	14.62±0.27	14.55±0.23	14.72±0.25	14.66±0.32
Leukocyte($x10^6$/mL)	10.14±1.22	10.12±1.25	10.14±1.24	10.52±1.25
Platelets($X10^5$/µl)	1.33±0.15	1.30±0.14	1.32±0.12	1.30±0.14
MCV (g/l)	85.02±4.0	85.00±5.0	85.10±4.15	84.88±5.12
Neutrophil (%)	52.12±3.2	52.18 ±3.4	51.52±3.2	52.11±3.0
Lymphocytes (%)	44.14±2.22	45.10±3.0	45.25±2.8	45.21±3.2
Eosinophil's (%)	5.0±0.4	5.0±0.4	5±0.3	5±0.3
Monocytes (%)	3.0±0.02	3.0±0.03	3.0±0.03	3.0±0.02
Basophils (%)	0±0	0±0	0±0	0±0
ESR(mm)	1±00	1±00	1±00	1±00
PCV	45.42±3.12	44.00±3.33	43.00±3.21	43.26±3.15

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$; Vs Control N=6.

Table 2. Effect of treatment with Karunkali ver Kudineer biochemical parameters.

Dose (mg/kg)	Control	2ml/kg	4ml/kg	8ml/kg
Total Bilirubin (mg/dL)	0.211±0.06	0.214±0.04	0.215±0.05	0.215±0.05
Bilirubin direct (mg/dL)	0.1±0.04	0.1±0.05	0.1±0.04	0.1±0.05
ALP (U/L)	71.04±2.5	71.21±2.8	71.10±3.0	70.28±2.5
SGOT (U/L)	74.10±3.2	74.00±3.1	73.11±3.8	74.22 ± 3.4
SGPT(U/L)	81.1±3.0	82.00±3.2	81.50±2.5	80.14±2.9
Total Protein(g/dl)	9.00±1.22	9.10±0.23	8.15±0.22	8.21±0.25
Albumin(g/dl)	3.13±0.20	3.41±0.22	3.40±0.31	3.12±0.30
Globulin(g/dl)	5.00±0.28	4.78±0.26	4.90±0.24	4.88±0.26
Urea (mg/dL)	54.40±1.35	54.00±3.00	54.08±2.88	55.01±2.73
Creatinine (mg/dL)	29.56±3.4	29.11±3.0	28.04±3.14	27.22 ± 3.2
Uric acid (mg/dL)	1.6±0.18	1.5±0.16	1.7±0.12	1.6±0.15
Na m.mol	142.42±4.20	142.21±3.00	142.14±3.56	142.00±3.02
K m.mol	20.02±2.22	19.00±2.19	20.11±2.30	20.18±2.46
Cl m.mol	102.35±4.88	101.36±5.41	102.60±5.92	101.40±4.00

Values are mean ± S.E.M. (Dunnet't' test). $^{ns}P>0.05$ Vs Control.

Table-3. Lipid Profile

Dose (mg/kg)	Control	2ml/kg	4ml/kg	8ml/kg
Total cholesterol (mg/dL)	42.11±2.45	40.27±2.66	41.14±2.64	41.89±2.79
HDL(mg/dL)	14.22±2.28	14.42±1.84	14.11±1.82	14.00±2.36
LDL(mg/dL)	42.12±2.62	42.05±3.00	42.55±3.00	42.32±3.24
VLDL(mg/dl)	16.25±2.33	16.18±2.42	16.22±1.34	15.19±1.20
Triglycerides (mg/dl)	85.12±2.49	85.21±2.34	85.02±3.04	85.00±2.45
Blood glucose(mg/dl)	125.23±3.00	125.72±3.12	125.00±3.05	125.82±2.42

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$; Vs Control N=6.

Table-4 Urine Analysis

Parameters	Control	2ml/kg	4ml/kg	8ml/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly cloudy	Slightly turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	>7.2	>8.0	>8.0	>9.0

Protein	Nil	3+	3+	3+
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	+ve	+ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Abnormal	Abnormal	Abnormal
Pus cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	1-cell/HPF	Nil	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

DISCUSSION

The haematological parameters including RBC, HB, Leukocytes, Platelets, Neutrophil, Lymphocytes, Eosinophill, Monocytes, basophills, ESR, PCV were calculated in all the four groups. There were no significant changes observed in the four groups (Table-1).

The biochemical parameters such as Total Bilirubin, ALP,SGOT, SGPT, Total Protein, Albumin, Globulin, Urea, Creatinine, Uric acid and electrolytes such as Sodium (Na),Potassium (K), Chloride (Cl), were evaluated all the four groups. There were no significant changes observed. (Table-2).

The Total cholesterol, HDL, LDL, VLDL, Triglycerides, Blood glucose level were monitored. There were no significant changes in the above parameters. $p > 0.05$. (Table-3).

The complete urine analysis such as, colour, transparency, specific gravity, PH, proteins, glucose, Bilirubin, ketones, blood, urobilinogen, pus cells, RBC'S, epithelial cells, crystals, casts were seen. Protein and ketones were present in all the test groups (minimum to maximum). Abnormal urobilinogen were detected in all test groups. PH was elevated up to 9.0.

In the present toxicological investigation, no toxic effect was identified up to 8ml/kg of Karungali Ver Kudineer administered through oral route for 28 days. So, it can be concluded that the Karungali Ver Kudineer can be used for therapeutic use in human with the dosage recommendations of up to 8ml/kg body weight p.o. Further studies of the drug may lead the world to invent a single drug which acts against diabetes and its complication neuropathy.

REFERENCES

1. Murugesa Mudaliyar K.S., Siddha MateriaMedica (Medicinal Plants Division) 7th Edition, 2003, 232, Directorate of Indian Medicine and Homeopathy, Chennai 106.
2. S.Umera etal; Central and Peripheral Acting analgesic activity of karunkali ver (Acacia catechu); International Journal of Pharma Research and Review; May2013; 2(5): 13-17.
3. OECD guidelines 425 for the testing of chemicals Acute Oral toxicity up & down procedure (UDP) Adopted 3 october 2008.
4. Chan PK, O'Hara GP, Hayes AW. 1982. Principles and methods for acute and subchronic toxicity. In: Hayes AW, editor. Principles and Methods of Toxicology. New York: Raven Press. p 17-19.
5. Feldman BF, Zinkl JG, Jain NC, Moor DM. 2000. Schalm's Veterinary Hematology. 5th ed. Philadelphia: Lippincott Williams & Wilkins.
6. Gregg L, Voigt DVM. 2000. Anemias and Polychythenias. In*:* Hematology Techniques and Concepts for Veterinary Technicians. U.S.A: Iowa State University Press. p 95-101.
7. Itthipanichpong C, Ousavaplangchai L, Ramart S, Thamaree S, Tankeyoon M. 1987. Acute toxicity and subacute toxicity study of *Phyllanthus emblica*. Chula Med J 31(5): 367-376.
8. Sacher RA, McPherson RA. 2000. Test of liver function. In: Sacher RA, McPherson RA, Campos JM, Widmann FK, editors. Widmann's Clinical Interpretation of Laboratory Test. 11th ed. U.S.A: F.A. Davis Company. 562-599.
9. Woodman DD. 1996. Assessment of hepatotoxicity. In: Evans GO, editor. Animal Clinical Chemistry: a Primer for Toxicologist. London: Taylor & Francis. p 71-86.