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Acute and Sub Acute Toxicity study on "Neelakanda Kuligai"

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Abstract

Siddha system of medicine is a important traditional system of medicine practiced in India. In India Snakebite is a life threatening problem causing mortality from ancient period to till date. In our system of medicine Siddhar's mentioned many preparations for curing snake bite. Among the preparations Neelakanda kuligai is the important one. Here we concluded the safety dose of the Preparation by toxicity studies. Results showed a single oral dose of 2000mg/Kg b.Wt. was conclude as maximum tolerable dose in acute toxicity study. And the dose of 3.6mg/Kg b.Wt is safe in Sub acute toxicity study. Furthermore pharmacological studies and standardization studies are needed.

Keywords: Siddha medicine, Neelakanda kuligai, toxicity study.

Introduction

Among medicine system followed worldwide Siddha system is practiced since ancient days nearly goes back to BC 10,000 to BC 4000 ago. Siddharkal is founder of Siddha system. Siddhars are precise in the treatment of poisons. They found which are toxic substances and their antidote are documented in siddha literature. The astonishing knowledge of Siddhars in herbal, metal, mineral, animal products detoxification , purification methods increase the efficacy of drugs. The Signs, symptoms of poison in humans and the way of diagnosis tell their familiarity in Nanju Maruthuvam(toxicology). It contain general antidote for poisons. They also indicate specific antidote for particular poison. These are the beneficiation to the society.

"All things are poison, for there is nothing without poison qualities.

It is only the dose which makes a thing poison" - Paracelsus.

Most of the world‟s approximately 3,000 snake species are nonvenomous and only 200 are considered by the World Health Organization to be medically significant venomous species.. Snake bite is a life threatening problem causing mortality from ancient period to till date. About 94,000 snake bite deaths are recorded globally and 15000 in India per year[1].

Poor, rural areas that lack appropriate medical care and the correct anti venoms contribute to this high number of snakebite fatalities, and the World Health Organization considers snakebites to be a threat to public health in these areas. To overcome side effects and unavailability of anti venom.

The author select the drug "**Neelakanda kuligai**" (Ref:Nanju murivu nool)[2] a herbomineral preparation is indicated for all kind of poisonous bites to access its safety by toxicity study.

Materials and Methods

Preparation of Neelakanda Kuligai:

Ingrdients - Neervaalam-(Croton tiglium) 35 gram, Turusu-(Copper sulphate) 35 gram, Vengaram-(Borax) 10 gram, Navatcharam-(Ammonium chloride) 10 gram, Veeram-(Mercuric chloride) 10 gram, Pooram-(Mercurous chloride) 10 gram.

After purification of all ingredients, Mercury is first triturated with Sulphur, then veeram, pooram, thurisu, navachaaram, vengaram, seeds of croton tiglum was triturated for three days. When the triturated content reached mezhug padham consistent, made in to tablet form. Each tablet must have 4gram weight (1 varagan). It was stored in a clean dry container.

NEELAKANDA KULIGAI – NKK

Acute Oral Toxicity study (OECD guideline – 423)[3]:

All the animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. The control group received an equal amount of 2% CMC. 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. An oral dose of 50 mg/kg, 300 mg/kg, 1000 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rat were continuously monitored for 1 hr after dosing, periodically during the first 24 hr with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

Test Substance:

Neelakanda kuligai, is pale blue in colour, without taste and odour. It is partially soluble in water and suspended in 2% CMC (Vehicle) with constant vigorous mixing. In order to obtain and ensure the uniformity in drug distribution.

Repeated Dose 28-day oral toxicity study (OECD guideline – 407)[4]

As stated results of acute toxicity studies in wistar albino rat indicated that Neelakanda kuligai was toxic up to the dose of 2000mg/kg b.wt $LD_{50.}$ Toxic symptoms were observed after 4 hours of oral drug treatment. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Based on the acute toxicity study 1/10 of the LD_{50} dose was selected as the approximate therapeutic dose. The low dose was calculated from the therapeutic dose (200 mg) and body surface area of rat (0.018). Calculation of low dose – 200 × 0.018 = 3.6 mg/200 gm of animal.

Grouping of animals:

Repeated dose 28 day oral toxicity study was carried out at different dose levels. The animals in both sex were divided in four groups (group I,II, III & IV). Each group consist of 10 animals (5 males and 5 females). Group-I served as control and the other three groups II, III and IV for test drug of Low dose (X=3.6mg/kg b.wt), Mid dose (5X=18mg/kg b.wt) and High dose (10X=36mg/kg b.wt) respectively [X denotes rat therapeutic dose derived from therapeutic dose relating to body surface area].

Statistical analysis:

Findings such as clinical sings of intoxication, body weight changes, food consumption, hematology, and biochemical parameters were subjected to one-way ANOVA followed by Dunnet "t" test using a computer software programme INSTAT-V3.1.

Results

Acute Toxicity Study:

Acute Toxicity Study was done as per OECD Guideline-423 with dose levels of 50, 300, 1000, 2000 mg/kg b.wt. Throughout the 14 days of observation period, 3 mortality was observed in Neelakanda kuligai in 2000mg/kg b.wt treated groups. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups.

Table 1: Dose finding experiment and its behavioral Signs of Acute Toxicity study on rat

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	50	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	300	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1000	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
4	2000	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	+	+	3

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. Dyspnoea 20. Mortality

Repeated Dose Toxicity Studies:

Results are mentioned as Table,

Table-2: Body wt in (g) of albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study.

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	211±26.3	213.8±28	223.7±28.7	226.6±29.8	230.8±38.6
Low dose	213.8±28.1	219.4±26.7	224.7±34.6	238.5±44	240.1±49.7
Mid dose	215.4±28.5	224.6±26.8	227.8±26.7	242.3±33.8	248.7±53.2
High dose	208.8±31.6	215.3±32	224±36.1	238.7±50.32	244.6±51.02

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01.

Table-3. Food (g/day) intake of albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study.

Dose	Days(gm/rats)				
	1	7	14	21	28
Control	40±5.27	41±3.11	42±4.24	41.5±3.27	40±4.11
Low dose	38.4±1.98	38.4±2.27	38.13±2.77	38.2±3.12	39±2.53
Mid dose	39.3±2.65	40.1±3.11	40.2±3.25	41±4.2	41±4.51
High dose	43.04±3.55	43.5±3.01	42.8±2.78	43.3±2.40	43.1±3.83

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01

Table -4: Water (ml/day) intake of Albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study.

Dose (mg/kg/day)	Days (ml/rat)				
	1	7	14	21	28
Control	44.12±3.48	46.22±4.15	45.81±3.98	42.31±3.26	44.18±3.27
Low dose	41.20±3.24	42.52±3.21	45.14±4.57	44.20±3.12	46.54±4.50
Mid dose	41.12±3.27	42.46±3.80	48.10±3.00	44.18±3.44	44.20±4.23
High dose	42.20±4.70	45.68±4.30	44.28±3.56	46.74±3.98	47.10±3.88

Values are mean of 6 animals ± S.E.M. P<0.05.

Table-5:Hematological parameters of albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study.

Parameter	Control	Low dose	Mid dose	High dose
RBC (X10/mm3)	7.45±0.45	7.62 ± 0.50	7.93 ±0.59	7.71 ± 0.59
HB (g/dl)	12.20±1.27	11.02±1.13	9.10±0.88	8.09±0.62*
Leukocyte (x103/mm3)	7.9±0.88	7.0 ± 1.10	6.70 ±1.11	7.2 ± 1.9
Platelets (105/mm3)	3.16±0.25	2.5±90.40	2.84± 0.44	2.61 ±0.64
MCV (gl)	59.77±8.46	56.55±6.48	52.20±4.12	55.24±3.92
Neutrophil	25.56 ± 2.34	27.11 ± 1.69	27.50 ± 1.67	31.21 ± 10**
Lymphocyte	63.7 ±8.54	65.2±11.23	64.33± 5.88	60.66± 8.84
Monocyte	2.0±0.63	2.3±0.51	2.16±0.40	2.16±0.40
Eosinophil	2.49 ± 0.098	2.36 0.30	2.54 ± 0.055	2.48 ± 0.042
Basophil	1±00	1±00	2±00	2±00
ESR (mm)	1±00	3±00	4±00	4±00
PCV	42.11±2.80	38.10 ± 2.81	38.10 ± 1.48	38.40 ± 2.62
MCHC (g/dl)	32.54±2.90	32.03±0.31	32.96±0.52	33±0.44

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01

Table-6: Biochemical parameters- LFT of albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study

Dose (mg/kg)	Control	Low dose	Mid dose	High dose
Total Bilirubin (mg/dL)	0.7±0.1	0.7±0.2	0.7±0.1	0.7±0.2
Bilirubin direct (mg/dL)	0.4±0.1	0.4±0.1	0.5±0.3	0.3±0.1
Bilirubin indirect (mg/dL)	0.4±0.1	0.4±0.1	0.4±0.1	0.3±0.1
ALP (U/L)	201±6.77	197±14.16	186.24±23.06	182.23±13.56
SGOT (U/L)	19.5±1.37	20.65±2.43	23±4.01	20.92±4.68
SGPT (U/L)	23 ± 3.55	24.17 ± 3.01	24.86 ± 6.14	23.5± 4.68
Total Protein (g/dl)	6.5 ± 0.5	6.8± 0.5	7.7 ± 0.9	6.7± 0.5
Albumin (g/dl)	3.0±0.3	3.3±0.3	3.3±0.4	3.2±0.3
Globulin (g/dl)	2.95±0.70	3.03±0.350	3.36±0.242	2.62±0.463
GGT (U/L)	6.1±0.32	6.0±0.20	6.3±0.21	6.2±0.20

Values are mean of 6 animals ± S.E.M. (Dunnet's „t" test). *P<0.05; **P<0.01. vs control

Table -7: Biochemical parameters- RFT of albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study

Dose (mg/kg)	Control	Low dose	Mid dose	High dose
Urea (mg/dL)	31.33± 4.93	28.5 ± 6.3	30.66± 3.14	29.16± 5.07
Creatinine (mg/dL)	0.97 ± 0.20	0.95±0.14	0.99 ± 0.16	0.95± 0.09
Uric acid (mg/dL)	5.46±1.43	4.34±0.85	4.7±1.24	4.3±0.93
Na (m.mol)	140.55 ± 1.17	140.70 ± 1.14	140.15 ± 1.10	142.21 ± 1.23
K (m.mol)	21.61±2.28	19.25±2.00	20.02±1.20	19.25±2.18
Cl (m.mol)	96.10±3.18	102.12±4.02	102.10±4.82	100.30±5.20

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01. *vs. control*.

Table-8: Biochemical parameters- Lipid Profile of albino rats exposed to Neelakanda kuligai for 28days repeated Oral toxicity study

Dose (mg/kg)	Control	Low dose	Mid dose	High dose
Total cholesterol (mg/dL)	104.3 ± 36.8	106.3 ±14.6	113.47 ±11.38	113.5 ±11.38
HDL (mg/dL)	12.8±3.8	11.02±2.9	13.8±1.7	12.3±3.9
LDL (mg/dL)	61.5±18.3	69.7±12.11	68.9±11.3	61.4±15.08
VLDL (mg/dl)	30.05±15.98	31.8±4.76	34.55±4.12	35.3±389
Triglycerides (mg/dl)	150.25±79.94	159.5±23.89	172.75±20.59	176.4±19.47
Blood glucose (mg/dl)	115.25 ± 20.85	112.5 ± 25.15	118.75±27.86	103.75 ±6.88

Values are mean of 6 animals ± S.E.M *P<0.05; **P<0.01. vs. control

Table - 9: Urine analysis of albino rats exposed to Neelakanda kuligai for 28days repeated Oral toxicity study

Parameters	Control	Low dose	Mid dose	High dose
Colour	Yellow	Yellow	Yellow	Orange
Transparency	Clear	Slightly turbid	Slightly turbid	Cloudy
Specific gravity	1.010	1.010	1.010	1.010
PH	7.2	7	6.8	6.7
Protein	Nil	1+	1+	2+
Glucose	Nil	Nil	Nil	Trace
Bilirubin	-ve	-ve	-ve	+ve
Ketones	-ve	-ve	-ve	+ve
Blood	Absent	Absent	Absent	Present
Urobilinogen	Normal	Normal	Normal	Normal
Pus cells	Nil	0-cells/HPF	Nil	Nil
RBCs	Nil	Nil	0-1cells/HPF	0-2cells/HPF
Epithelialcells	Nil	Nil	Nil	Nil
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table-10. Organ weight of albino rats exposed to *Neelakanda kuligai* for 28days repeated Oral toxicity study

Dose (mg/kg)	Control	Low dose	Mid dose	High dose
Liver (g)	10.38 ± 0.8	9.25 ± 1.72	8.92± 1.15	9.82 ± 1.72
Heart (g)	0.66± 0.38	0.8 ± 0.16	0.92 ± 0.11	0.81± 0.325
Lung (g)	2.1 ± 0.5	2 ± 0.19	2± 0.2	2.2 ± 0.3
Spleen (g)	0.80 ± 0.13	0.61 ± 0.041	0.73 ± 0.22	0.75 ± 0.17
Ovary (g)	0.042 ± 0.007	0.047 ± 0.003	0.045 ± 0.002	0.044 ± 0.002
Testes (g)	1.550 ± 0.100	1.454 ± 0.099	1.502 ± 0.100	1.486 ± 0.080
Brain (g)	1.48±0.16	1.56±0.12	1.54±0.15	1.58±0.14
Kidney (g)	0.960 ± 0.092	1.045 ± 0.080	0.95 ± 0.19	0.702 ± 0.030*
Stomach (g)	1.40±0.12	1.37±0.14	1.40±0.18	1.46±0.14

Values are mean of 6 animals ± S.E.M. *P<0.05; **P<0.01 *vs control*

Discussion

In ACUTE TOXICITY STUDY, carried out as per OECD guidelines 423, there were no treatment related death or signs of toxicity developed in wistar albino rat at dosage levels of 50, 300 and 1000 mg/kg b.wt throughout the study period. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups. A single oral dose of 2000mg/kg b.wt. resulted in abnormal behavioral changes like severe diarrhoea, tremors, lethargy and writhings were observed (Table – 1).The acute toxicity study of Neelakanda kuligai indicated changes in the behavior and in the sensory nervous system responses in the animals. Also adverse gastrointestinal effects were observed in the rat after two hours of drug administrations. Mortality of the two animals occurred within five hour after the administration of the drug. The other one animals died within a day. Necropsy of the animals after mortality revealed gross pathological changes such as inflammation and congestion in Stomach, intestine. The rat received 1000mg/kg b.wt dose of NKK survived with mild gastro intestinal problem like diarrhea but all survived beyond the 14 days of observation.

REPEATED ORAL TOXICITY STUDY, was conducted for about 28 days as per the OECDguideline-407 in 4 doses control, The low dose was calculated from the approximate therapeutic dose (200 mg) and body surface area of rat (0.018). Calculation of low dose – 200 × 0.018 = 3.6 mg/200 gm of animal. low dose (3.6mg), mid dose (18mg), high dose (36mg). Animals were observed throughout the period. There was no significant change in body weight (Table 2), water (Table 4), and food intake (Table 3). After 28 days animals were sacrificed and blood samples were collected, investigated. The results revealed that there were significant changes in the haemoglobin level in 36mg/kg.b.wt, this may be to the binding capacity of haemoglobin with heavy metal reduce the count in hematological, Very significant changes in the Neutrophil count(Table 5), No changes in biochemical parameters (Table 6,7,8) , No changes in Urine analysis expect appearance of protein in drug treated animals and trace sugar, blood in high dose level (Table 9) . No changes in organ weight expect kidney in high dose level (Table 10) when compared to control group. The histopathological study on the organs such as brain, lungs, spleen, kidney, stomach, testis, and ovary was normal in control, low dose, mid dose and high dose groups; Expect the lymphocytic infiltration in liver and heart reciving high dose level.

Conclusion

A single oral dose of 2000mg/Kg b.Wt. was conclude as maximum tolerable dose from result. In Repeated oral 28 days toxicity study, there was no significant changes in haematological, biochemical parameter in 3.6mg/Kg b.Wt. of NKK treated group and the levels were within the physiological limit. The histopathology report also confirms that there is no remarkable cellular changes at this dose level. Based on these results it can be conclude that Neelakanda kuligai, the 120mg for a duration of 16 days (BD/day) is safer dose for human consumption. Further, pharmacological activity of Neelakanda kuligai (NKK) will take a scientific approval for this antidote for all kind of poisonous bite.

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