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ACUTE AND SUB ACUTE TOXICITY STUDY ON SIDDHA DRUG *NERUNJIL VITHAI CHOORANAM* (NVC)

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ABSTRACT

The polyherbal formulation *Nerunjil vithai chooranam (NVC)* has been used for the treatment of Male infertility (MI). As a mandate, steps were taken to evaluate safety profile of NVC in rats following OECD guidelines. In acute oral toxicity study, a single dose of NVC was administered and observed for 14 days. In acute toxicity studies, NVC revealed no abnormal signs upto the dose level of 2000mg/kg body weight. Sub-acute toxicity studies were carried in four different groups in which NVC was administrated orally to rats once daily for 28 days in various doses ranging from 36, 180,360 mg/kg for rat respectively. Detailed hematological, biochemical, necropsy and histopathological evaluation of organs was performed for all animals. The NVC was well tolerated and no toxic manifestations were seen in any animal. Histopathological analysis revealed that Spleen, Testes, Pancreas, Lung, Intestine, Stomach, Liver, Brain, Heart, Ovary, Uterus and Kidney tissues of treated groups did not show any signs of toxicity. No toxic effect was observed in acute and sub-acute toxicity studies of *Nerunjil vithai chooranam.*

KEYWORDS

Nerunjil vithai Chooranam (NVC), Male infertility *(*MI), Acute toxicity and Sub-acute toxicity.

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INTRODUCTION

Infertility is a widespread problem. For about one in five infertile couples the problem lies solely in the male partner (male infertility)[1]. Current epidemiological evidence suggests that 15% of couples experience infertility in the world and half remain untreated or unresolved[2]. Infertility has increased as a problem over the last 30 years[3]. Some common causes of male infertility related to health

and lifestyle include alcohol and drugs, emotional stress, electromagnetic radiation, behavioural problems and psychological problems[4].

Moreover, fertility reflects a man's "overall" health. Men who live a healthy lifestyle are more likely to produce healthy sperm[4]. In Siddha system, this problem is noticed as *Aanmaladu.*

Siddha Literatures have described many medicines for the treatment of male infertility. So, the polyherbal formulation *Nerunjil vithai chooranam* in which most of ingredients have aphrodisiac action which will provide a very good solution for Aan maladu (male infertility)[5].

Conventional treatment for infertility usally involves invasive and expensive procedures. There are many alternative treatments available that can increase the chance of conception[6]. Herbs and minerals have been in use since long time to treat various diseases[7]. However, many issues related to a lack of scientific evidence about the efficacy and safety of the drugs remains unresolved[8-9]. The Pre-clinical toxicity studies were essential for determining a safe dose for human trials[10].

The interventional Siddha drug *Nerunjil vithai chooranam* (NVC) quoted in the siddha literature Gunapadam mooligai vaguppu has been used for the treatment of Aan maladu (Male infertility)[11]. Consequently an effort was made to evaluate acute and sub-acute toxicity of the polyherbal siddha formulation NVC in laboratory animals.

MATERIAL AND METHODS

Source of Drugs

The required raw drugs are procured from a well reputed indigenous drug shop. The raw drugs will be authenticated by the concerned pharmacognosist at SCRI, Chennai, Tamil Nadu, India.

Ingredients

Nerunjil vithai (*Tribulus terrestris*), Poonaikkali vithai (*Mucuna pruriens*), Kasakasa *(papaver sominiferum*), Neermulli vithai *(Hygrophila auriculata)*, Elavin verpattai *(Bombax ceiba*), Sirunaga poo *(Mesua nagassarium*), Nilapanai kilangu *(Curculigo orchioides*), Thanneervittan *(Asparagus racemosus)*,Sarkarai (*saccharum officinarum).*

Standard operating Procedure of NVC

All the drugs were dried well in shadow and made into fine powder. And they were put in a bottle and mixed thoroughly.

Experimental Animals

Wister albino Rats of either sex weighing 150-200gm were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai and maintained in the animal laboratory of Sairam Advanced Centre for Research. 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/1-5/2013). All the animals were kept under standard environmental condition (23±2°C), standard light cycle (12 h light, 12 h dark). The animals had free access to water and standard pellet diet. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute Toxicity Study-OECD423 guidelines[12]

Acute oral toxicity test for the *Nerunjil vithai chooranam* was carried out as per OECD Guidelines 423. 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 was administered in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing and weighed before the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours.

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. The animals were then observed daily for gross behavioral changes and any other signs of acute toxicity (Table No.1).

Sub-Acute Toxicity - OECD 407 Guidelines[13]

In a 28-days, sub-acute toxicity study, ten rats (Five Male and Five Female)were in each group divided into four groups. 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 *Nerunjil vithai chooranam*(p.o.) for 28 days at a dose of 36mg/kg(x), 180mg/kg(5x) and 360mg/kg(10x) respectively. The weight of each rat was recorded on day 0 and weekly throughout the course of the study (Table No.2).

At the end of the 28 days they were fasted overnight, each animal was anaesthetized with ether, following which they were then dissected 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

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 of **NVC** revealed no mortality, abnormal signs and behavioral changes except alertness, aggressiveness, touch response, muscle relaxant in rats at the dose of 2000 mg/kg body weight administered orally. The median lethal dose for **NVC** should be above 2000 mg/kg and it comes under unclassified (Table No.1).

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 of body weight indicates the effect of NVC in the body weight in the treated rats. There was no significant change in body weight upto 28 days treatment. All the treated groups of animals show gradually increase in the weight during the treatment period (Table No.2).

The result of haematological parameters indicates the effect of NVC in treated rats. There was no significant change in haematological parameters upto 28 days treatment (Table No.3).

The result of biochemical parameters indicates the effect of NVC in treated rats. There was no significant change in biochemical parameters upto 28 days treatment (Table No.4).

Urine analysis data (Table No.5) of control group and treated group of animals did not reveal major abnormalities

The result of organ weight indicates the effect of NVC in treated rats. There was no significant increase or decrease of weight of the organs such as Brain, Lungs, Heart, Spleen and Kidneys (Table No.6).

Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.

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 (Figure No.1 and Panel 1-4).

Preclinical toxicity studies of *Nerunjil vithai Chooranam* in Rat

Acute oral Toxicity

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 No.1: Dose finding experiment and its behavioral Signs of Toxicity for NVC formulation

S.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	2000	+	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-

SUB-ACUTE ORAL TOXICITY 28-DAY REPEATED DOSE STUDY IN RATS

Table No.2: Body weight (g) changes of rats exposed to NVC formulation

S.No	Dose (mg/kg/day)	*Days*				
		1	7	14	21	28
1	Control	122.37 ± 3.21	124.14 ± 4.09	128.21 ± 2.17	129.21 ± 5.11	133.32 ± 1.89
2	36	124.28±3.25	124.21 ±3.21	125.17 ±3.41	127.12 ±3.54	129.22 ±2.71
3	180	127.12 ±3.45	128.45 ±3.75	131.48 ±3.25	131.45 ±2.34	132.45 ±3.25
4	360	127.22 ±2.45	129.45 ±3.65	132.25 ±3.42	132.25 ±2.14	133.25 ±2.34

Values are mean of 10 animals ± S.E.M. N=10

Table No.3: Effect of NVC formulation on Haematological parameters in rats

S.No	Parameter	Control	36 mg/kg	180 mg/kg	360 mg/kg
1	RBC (x 10^6/mm^3)	7.51 ± 0.16	7.45±2.14	8.21±1.23	8.33±1.23
2	PCV (%)	48.2 ± 1.3	46.3±2.1	52.4±1.8	54.1±0.9
3	Hb (%)	15.6 ± 0.19	15.2±1.1	16.3±0.2	16.5±0.6
4	WBC(x 10^3/mm^3)	10.12 ± 1.2	9.6±1.6	11.3±0.8	10.6±1.4
5	Neutrophils (%)	22 ± 4	21±5.2	19±2.1	24±1.8
6	Mononuclear cells (%)	76 ± 2	77±4.2	78±3.1	76±2.4
7	Eosinophils (%)	2.4 ± 0.6	2.8±0.02	2.2±0.04	1.8±0.3
8	Platelets (x 10^3/mm^3)	423.2 ± 48.8	436.4±52.16	390±21.8	414.4±46.2

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 No.4: Effect of NVC formulation on biochemical parameters in rats

S.No	Parameters	Control	36 mg/kg	180 mg/kg	360 mg/kg
1	Protein (g/dl)	8.62 ± 1.3	8.2±2.6	7.2±0.96	8.4±2.18
2	Albumin (g/dl)	4.8 ± 0.6	4.2±1.2	3.6±1.12	3.8±0.8
3	BUN (mg/dl)	19.2 ± 1.2	15.4±1.34	15.6±0.89	18.4±1.6
4	Urea (mg/dl)	64.24 ± 3.11	66.4±1.2	52.34±1.34	68±2.6
5	Creatinine (mg/dl)	0.82 ± 0.16	0.42±0.05	0.7±0.12	0.85±0.14
6	Total Cholesterol (mg/dl)	91.24 ± 1.35	96.74±2.7	94.7±4.3	98.32±2.42
7	Triglycerides (mg/dl)	50.15 ± 3.21	54.32±1.32	53.34±2.69	56.34±3.46
8	Glucose (mg/dl)	110.16 ± 8.62	92.34±9.62	120.18±1.7	98.34±4.24
9	Total Bilirubin (mg/dl)	0.20 ± 0.04	0.26±0.08	0.22±0.06	0.29±0.03
10	SGOT (U/L)	73 ± 2.4	82±3.4	79.4±5.8	76.7±2.3
11	SGPT(U/L)	28.4 ± 1.2	32±1.3	30.46±2.6	26.37±4.3
12	Alkaline phosphatase (U/L)	102.4 ± 3.6	96.7±4.2	111.2±3.8	106.8±4.9
13	Sodium (mEq/L)	138.12 ± 3.14	122.6±1.24	128.4±2.2	138±4.32
14	Potassium (mEq/L)	7.2 ± 1.34	6.6±0.8	6.2±1.68	6.8±1.32

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

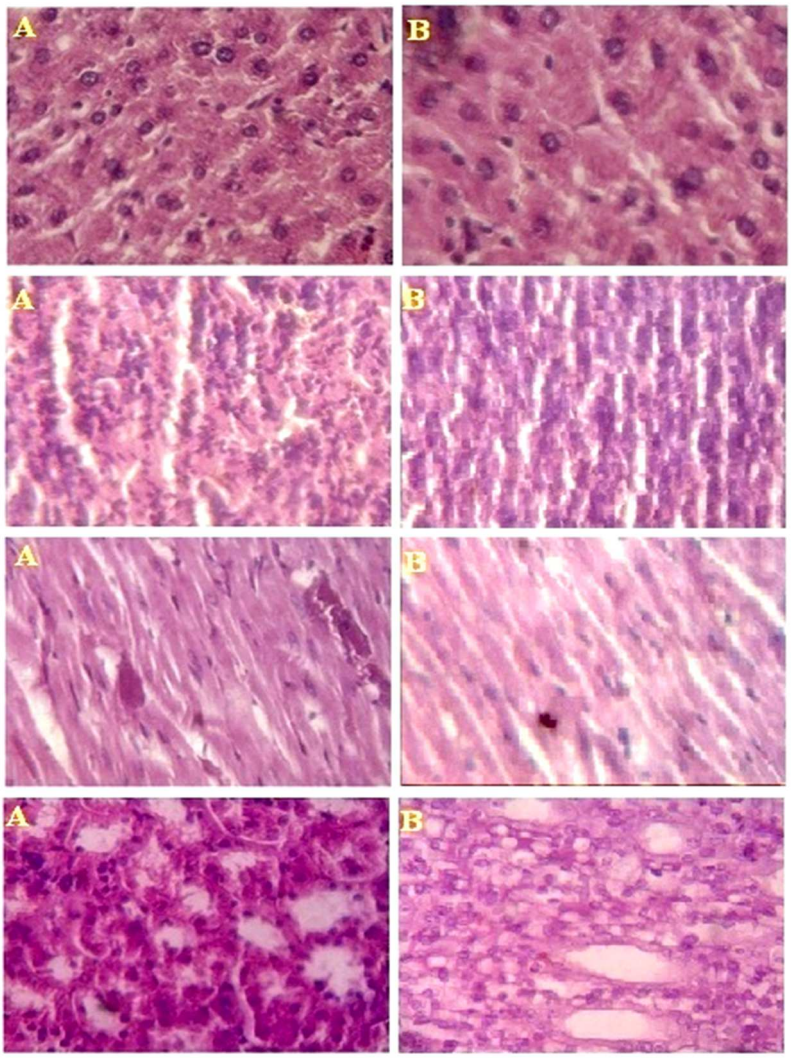
Table No.5: Effect of NVC formulation on Urine parameters in rats

S.No	Parameters	Control	36 mg/kg	180 mg/kg	360 mg/kg
1	Colour	Yellow	Yellow	Yellow	Yellow
2	Transparency	Clear	Clear	Clear	Clear
3	Specific gravity	1.010	1.02	1.04	1.04
4	Ph	7.2	7.4	7.4	7.5
5	Protein	Nil	Nil	Nil	Nil
6	Glucose	Nil	Nil	Nil	Nil
7	Bilirubin	-ve	-	-	-
8	Ketones	-ve	-	-	-
9	Blood	Absent	Absent	Absent	Absent
10	RBCs	Nil	Nil	Nil	Nil
11	Epithelial cells	Nil	-	-	-
12	Casts	Nil	Nil	Nil	Nil

Table No.6: Effect of NVC formulation on Organ weight in rats

S.No	Dose (mg/kg)	Control	36 mg/kg	180 mg/kg	360 mg/kg
1	Liver (g)	5.24±0.14	4.64±0.89	6.32±1.2	5.6±0.7
2	Heart (g)	0.70±0.05	0.69±0.06	0.82±0.07	0.74±0.02
3	Lung (g)	1.78±0.25	1.56±0.43	1.84±0.79	1.69±0.46
4	Spleen (g)	0.74 ±0.07	0.59±0.02	0.83±0.06	0.76±0.08
5	Brain (g)	1.43±0.18	1.3±0.24	1.69±0.46	1.46±0.09
6	Kidney (g)	0.70±0.05	0.56±0.04	0.82±0.16	0.76±0.07

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



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 Heart of a control rat
Figure A – Control
Figure B – Treated on high dose, no abnormality 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.

CONCLUSION

The acute and sub-acute toxicity study of *Nerunjil vithai chooranam* revealed no toxicity by oral route over a period of 28 days. So, it can be concluded that the *Nerunjil vithai chooranam* can be prescribed for therapeutic use in human with the dosage recommendations of upto 360mg/kg body weight p.o.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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