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Research article

Preclinical safety profile of Siddha drug *Nerunji ver kudineer* through its toxicity studies

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Abstract

Background: The trial drug Nerunji ver kudineer poly herbal combination which is referenced from *Aathma ratchamirtham Ennum Vaidhya Sarasangiragam*, and primarily indicated for Kalladaippu (Urolithiasis). Even though it is from herbal origin, the safety profile such as acute and sub acute toxicity has to be evaluated for the purpose of safety concern.

Objective: To investigate the acute and sub-acute toxicity of Siddha poly herbal formulation *Nerunji ver kudineer* (NVK).

Methods : Acute oral toxicity and sub acute toxicity of *NVK* is carried out as per the OECD-423 guidelines after obtaining the animal ethical clearance from Institutional Animal Ethics Committee no IAEC/P.BERNATH/TNMGRMU/MD(S)/321611002/KMCP/24/2018. In the acute toxicity study female albino mice administered single oral dose (0,300,2000 mg/kg/bw) of *NVK*, while in sub-acute toxicity study male and female Wistar rats administered daily oral doses (50, 100,200 and 400 mg/kg/bw) of *NVK* for 28 days. At the end of the study, the animals were humanely sacrificed and assessed for the effect of *NVK* on body weight and relative organ weights and haematological, biochemical and histopathological parameters.

Results: In the acute toxicity study no mortality or behavioural changes were observed in mice treated with a single dose of *NVK upto* 2000 mg/kg/bw .So that dose is taken as LD_{50}. Similarly there is no such altered behaviours seen in Sub acute toxicity when trail drug is administered daily oral doses (50, 100,200 and 400 mg/kg/bw).

Conclusion: These results exhibit the absence of acute and sub-acute oral toxicity after treatment of *NVK* in female albino mice and wistar rats respectively. However, further clinical studies humans are needed in order to have sufficient safety evidence for its use in humans.

Keywords: *Nerunji ver kudineer*, Siddha Medicine, Toxicity studies, Aerva lanata

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INTRODUCTION

Nerunji ver kudineer is a poly herbal combination comprising 4 raw drugs. It is referenced from *Aathma ratchamirtham Ennum VaidhyaSarasangiragam*, and primarily indicated for Kalladaippu (Urolithiasis)(1). The ingredients such as *Tribulus terrestris*.L (Nerunjiver), *Aerva lanata*.L (Sirupeelaiver), *Amaranthus tricolor.L* (sirukeeraiver), *Cuminum cyminum.L* (seerakam) were used for the preparation of the drug. Each of the ingredients possess significant role in reducing renal calculi and several recent research has been reported on this. Urolithiasis also known as crystalluria is the condition where urinary calculi are formed in the urinary tract(3). As there is no single effective drug available for Urolithiasis today, surgery is considered to be the best alternative. However it is expensive and not affordable for common man. Hence the natural drugs are considered to be next alternative (2).

Tribulus terrestris is an annual (sometimes perennial in warm climates) herb with a long, slender, branched tap-root. The greenish-red stems are up to 2 m long, branched, radiating from a central axis and covered with fine hairs. *Tribulus terrestris* has been extensively studied and the occurrence of saponins, flavonoids, alkaloids, lignanamides and cinammic acid amides has been reported in *Tribulus terrestris*(3). This plant is extremely rich in substances having potential biological significance, including: saponins, flavonoids, alkaloids and other nutrients. The secondary diuretic ingredient was *Aerva lanata* (mountain knot-grass), which is a woody,prostrate or succulent, perennial herb in theAmaranthaceae family of the genus Aerva, nativeto Asia, Africa, and Australia. The second chief ingredient Aervalanata-comprises the alkaloids, flavonoids, phenol,tannin, proteins, amino acids and carbohydratesrespectively. *Aerva lanata* (L.) Juss. Schultz is herbal plant; itsexhibits significant therapeutic effects such as antihyperglycemic effect, Antiurolithiceffect, anthelmintic effect, antihyperlipidemiceffect, hepatoprotective activity, anti-oxidant, anti-microbial activity etc(2).

METHODS AND MATERIALS

Authentication of raw drug

The raw drugs required for the preparation of NVK(1) were purchased from traditional raw drug store and authenticated by Medicinal botanist of Govt. Siddha Medical College, Palayamkottai.

PREPARATION METHOD

All the raw drugs were purified as per literature and taken coarsely powdered to make kudineer

Tabel 1. Ingredients of the Nerunji ver Kudineer

Ingredients	Botanical name& family	Quantity
Nerunji ver	*Tribulus terrestris.L Zygophyllaceae*	5gm
Sirupeelaiver	*Aerva lanata.L Amaranthaceae*	5gm
Sirukeeraiver	*Amaranthus tricolor.L Amaranthaceae*	5gm
Seeragam	*Cuminum cyminum.LApiaceae*	5gm
Water	Quantity sufficient for decoction preparation	

ANIMAL STUDIES

Healthy adult albino mice and Wistar rat weighing between 170-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 + 2o C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study (4).

METHODS OF STUDY

Acute oral toxicity of *NVK* is carried out as per the guidelines Organization of Economic Co-operation and Development (OECD) - 423 guidelines. Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) (ShettyAkhila, *et al.,* 2007).

ACUTE ORAL TOXICITY OF NERUNJI VER KUDINEER

The adult albino mice models were fasted overnight and provided only water, after which the *NVK* is administered by gastric intubations to the relevant group of animals orally at the dose of 50 $mg.kg^{-1}$ body weight in Tween-80. The animals were then observed for 15 days and maintained with normal food. If the mortality rate of 2 or 3 animals in 15 days is recorded and that dose is said to be toxic dose. But when mortality of one animal is observed, then the same dose is repeated again for confirmation. However, if mortality is not observed, the procedure is repeated for further higher doses such as 300 and 2,000 $mg.kg^{-1}$ body weight. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality (Shah Ayub, 1997, Bürger, 2005)(5,6).

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. Special attention was directed for the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Body weight, food and water intake are recorded at two-day intervals from the initial day of treatment.

HISTO-PATHOLOGICAL EXAMINATION

Surviving animals were fasted overnight, weighed and humanely killed on the 15th day using anesthetic ether. All test animals were subjected to gross necropsy.

SUB-ACUTE TEST FOR NERUNJI VER KUDINEER

Male and female Wistar rats weighing 180 ± 10 g are used for the present study. The animals are divided into five groups of six animals each. The dose of the preparation is calculated based on the body weight of the animal. The animals in Group I were administered with a single daily dose of 0.5 ml of Tween 80 orally for 28 days. The animals in Group II were administered with 50 mg.kg^{-1}b.w. of the NVK orally once daily for 28 days. The animals in Group III were administered with 100 mg.kg^{-1} b.w. of the NVK orally once daily for 28 days. The animals in Group IV and V were administered once daily with 200 and 400 mg.kg^{-1}b.w. of the NVK respectively for 28 days orally (Pieme,*et al* 2006, Joshi, *et al* 2007, Mythilypriya, *et al.,* 2007) (7).

The animals are then weighed every seven days, from the start of the treatment, to record the weight variation. At the end of the treatment, blood samples were collected from eyes by puncturing retro orbital plexus after mild anesthesia for biochemical analysis. The collected blood sample is centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which is analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels, LDL-cholesterol, plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea (8,9).

RESULTS AND DISCUSSION

Acute toxicity study with NVK

The acute toxicity of *NVK* was evaluated using OECD-423 guidelines. There was no mortality or morbidity observed in animals through the 15-days period following single oral administration at all selected dose levels of the *NVK* (Table-1). The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose appeared normal. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal.

Table.2. Acute toxicity study of NVK on experimental mice using OECD-423 guidelines, where ST- sign of toxicity; NB- normal behaviour; D- died; S- survive. Values are expressed as number of animals (n=3).

	Dose(mg.kg^{1})	Sign of Toxicity (ST.NB^{-1})	Mortality (D.S^{-1})
Group I	0	0/3	0/3
Group II	300	0/3	0/3
Group III	2000	0/3	0/3

Effect of NVK in Sub- Acute Toxicity

The effect of NVK was observed for their effect on the body weight changes from the study it was observed that, there was significant increase ($p<0.05$) in body weight in all the animals observed. The results are shown in Table.2. The study on the effects of NVK on body weight changes in rats was carried out.. where, group I animals (GPI) were treated with normal saline (5 ml.kg^{-1}), group II animals (GPII) with 50 mg.kg^{-1} of NVK, group III animals (GPIII) with 100 mg.kg^{-1} of NVK , group IV animals (GPIV) with 200 mg.kg^{-1} of NVK, group V animals (GPV) with 400 mg.kg^{-1}NVK. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Effect of NVK on kidney,heart, liver and brain in rats

The effects of NVK on kidney, heart, liver and brainof the rats were observed.From the study it was clear that, significant ($p<0.01$) changes in the weights of various organs of the animals occurred with higher doses of the extract (400 mg.kg^{-1}bw), but macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control. The results are shown in Table.3.Statistical analysis are same as table.2.

Effect of NVK on biochemical profiles of rats

The effect of NVK on various biochemical parameters of the experimental animal 'rats' were tested .from the study it was evident that, there was significant decrease ($p<0.05$) in the plasma glucose level in treated rats especially at higher dose (400 mg.kg^{-1}) compared with control rats. The control rats were administered only with 5 ml of normal saline. Significant decrease ($p<0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels were observed. But a significant increase ($p<0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control animals. AST, ALT and ALP levels were also normal in the NVK treated animals. From the results of biochemical study there was no evidence of severe toxicity associated with the administration of higher concentration of NVK. The results are shown in Table.4.Statistical analysis are same as above table.

Table.3.The effects of NVK on body weight changes in rats.

Treatment	Day 1	Day 5	Day 10	Day 20
Control	185.25±6.11	187.47 ±6.25	196.15 ±6.40	196.7±6.60
NVK 50 mg.kg^{-1}	194.35 ±6.4	193.35 ±6.35	198.25 ±6.70	198.30±6.75*
NVK 100 mg.kg^{-1}	186.40 ±5.7	189.35 ±6.45	196.55 ±7.10	197.36±6.33*
NVK 200 mg.kg^{-1}	195.35 ±7.2	198.20±6.55	198.90 ±7.20**	206.45±7.31**
NVK 400 mg.kg^{-1}	187.70 ±6.05	192.15 ±5.65	195.60 ±6.35**	207.66±7438**

Table.4.The effects of Nerunji ver kudineer on kidney, heart, liver and brain of the rats.

Treatment	Heart (gms)	Kidney (gms)	Liver (gms)	Brain (gms)
Control	0.36 ± 0.07	0.66± 0.05	3.34± 0.07	0.67± 0.75
NVK 50 mg.kg^{-1}	0.37± 0.04	0.82± 0.05	3.44± 0.05	0.70± 0.5
NVK 100 mg.kg^{-1}	0.38± 0.08	0.80± 0.06	3.36±0.04	0.68± 0.4
NVK 200 mg.kg^{-1}	0.37± 0.06	0.75± 0.04	3.34± 0.04	0.75± 0.07
NVK 400 mg.kg^{-1}	0.36± 0.05	0.76± 0.05	3.37± 0.05	0.77± 0.07

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01.

Table.5.The effect on biochemical parameters

Treatment	Glucose (mg.dl^{-1})	Cholesterol (mg.dl^{-1})	Triglyceride (mg.dl^{-1})	HDL (mg.dl^{-1})	LDL (mg.dl^{-1})
Control	94.70± 0.67	39.62± 0.86	28.25± 0.45	135.25± 0.60	84.15±1.72
NVK@ 50 mg.kg^{-1}	92.55± 0.61	25.85± 0.75*	13.22± 0.23*	175.28± 0.70*	71.59±1.28
NVK@ 100 mg.kg^{-1}	89.50± 0.52	26.74± 0.86*	15.42± 0.28*	165.18±0.83*	69.84±1.10
NVK@ 200 mg.kg^{-1}	90.30± 0.60**	33.18± 0.20	17.84± 0.38*	184.30± 0.89*	48.60±1.30
NVK@ 400 mg.kg^{-1}	86.30± 0.509**	32.78± 0.30	19.28± 0.34*	182.2± 0.90-*	46.50±0.84

Table.6.The effects of biochemical parameters such as AST, ALT, ALP, TP and Albumin in rats.

Treatment	AST (IU.l^{-1})	ALT (IU.l^{-1})	ALP (IU.l^{-1})	TP (g.l^{-1})	ALBUMIN (g.l^{-1})
Control	328.5±12.40	71.5± 3.18	253.58± 8.80	69.85± 3.32	39.15±2.35
NVK@50 mg.kg^{-1}	317.0±9.50**	69.5± 2.20**	266.10± 2.75**	70.30± 2.32	36.30±2.65
NVK@ 100 mg.kg^{-1}	318.3±7.20**	67.1± 3.15**	260.18± 6.70**	80.15± 2.82	38.30±3.05
NVK@ 200 mg.kg^{-1}	313.4±7.95	64.4± 2.90	265.00± 5.20	69.25± 3.32	40.20±2.75
NVK@ 400 mg.kg^{-1}	323.2± 8.20	64.3± 3.52	269.40± 4.40	74.05± 2.58	39.48±2.70

Table.7.The effect of HB, Calcium, RBC and WBC in rats

Treatment	Haemoglobin ($mg.dl^{-1}$)	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium ($mg.dl^{-1}$)
Control	13.35± 0.27	9.15± 0.02	11.45± 0.05	9.45 ±0.02
NVK@ 50 $mg.kg^{-1}$	14.55± 0.28*	9.50± 0.04*	9.55± 0.01*	9.33 ±0.02
NVK@ 100 $mg.kg^{-1}$	14.35± 07*	9.55± 0.02*	8.35± 0.32*	9.21±0.20
NVK@ 200 $mg.kg^{-1}$	12.75± 0.22*	8.33± 0.12*	11.45± 0.03*	9.51 ±0.13
NVK@ 400 $mg.kg^{-1}$	13.5± 0.35*	8.46± 0.45*	10.5± 0.13*	9.73 ±0.02

Effect of NVK on biochemical parameters

The study on the effects of NVK on biochemical parameters such as AST, ALT, ALP, TP and Albumin rats was tested. where, group I animals were treated with normal saline (5ml.kg $^{-1}$), group II animals with 50 $mg.kg^{-1}$ of HAEBD group III animals with 100 $mg.kg^{-1}$ of NVK, group IV animals with 200 $mg.kg^{-1}$ of NVK, and group V animals with 400 $mg.kg^{-1}$NVK.

Effect of NVK on haematological parameters in rats

The effects of NVK were observed for its effect on haematological parameters on the experimental rats. From the study it was evident that, a significant increase ($p<0.01$) were observed in the haemoglobin contents and RBC count in the group treated with 200 $mg.kg^{-1}$ body weight of NVK and a significant decrease of the parameters occurred in the group treated with 400 $mg.kg^{-1}$ bw compared with the control. There was no significant change in the calcium level in all the treated animals compared to the control.

The study on the effect of NVK on haematological parameters such as Hb, RBC, WBC, Calcium in rats was tested. where, group I animals treated with normal saline (5 $ml.kg^{1}$), group II animals with 50 $mg.kg^{-1}$ of NVK group III animals with 100 $mg.kg^{-1}$ of NVK, group IV animals with 200 $mg.kg^{-1}$ of NVK, and group V animals with 400 $mg.kg^{-1}$NVK. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV and V. The statistical analysis was carried out using one way ANOVA method, where *P<0.05.

DISCUSSION

The acute toxicity study of NVK showed ,no mortality in both the animals of control group as well as animals treated with a maximum dose of 2000 $mg.kg^{-1}$. Hence, the sub acute toxicity started from 200 $mg.kg^{-1}$ of dose ($1/10^{th}$ of 2000 $mg.kg^{-1}$of acute dose i.e. hasn't shown any observed mortality during the period of study). There was no significant change in animal behaviour due to the absence of toxicity. Hence, this can be concluded that, the NVK has been taken as per the standard dosing protocol for the beneficial effect for suitable clinical conditions.

CONFLICT OF INTEREST: Nil

SOURCE OF FUNDING: Nil

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