

ORAL ACUTE AND SUB ACUTE TOXICITY STUDIES OF TWO SIDDHA FORMULATIONS VEDIKARA SILASATHU PAMPAM (VSP) AND NERUNJIL KUDINEER (NK) IN EXPERIMENTAL RATS

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ABSTRACT

The biological activity of the drug and its mechanism of action are obtained by conducting acute toxicity testing. Acute toxicity test is used in hazard identification and risk management of the drug. In this study, the acute and sub acute toxicity of Vedikara silasathu pampam(VSP) and Nerunjil kudineer(NK), the two siddha formulations given in combination for urinary tract infection was evaluated in Wistar albino rats. VSP contains, Potassium nitrate, Sodium nitrate, Silajith and NK contains Tribulus terrestris, Crataeva magna, Zingiber officinale. In acute toxicity study, both VSP and NK was administered orally at the maximum dose of 2000mg/kg/p.o. The toxic symptoms of the animals were observed up to 14 days and the results showed that there was no adverse effects found in the general behavior and mortality. VSP and NK was tested once daily at the dose of 500mg/kg/p.o for 28days in sub acute toxicity study. On the 29th day, the animals were sacrificed and the blood and biochemical parameters were measured. Vital organ's histopathological examination were studied further. When compared with the control group, the results of the test group did not showed any significant changes in food, water intake, body weight, liver and kidney function test and hematological parameters. Histopathological evidence of gross pathological changes were not showed in the vital organs. VSP and NK is safe for the treatment in urinary tract infection at the dose of 500mg/kg/p.o.

Keywords: Oral Toxicity, Vedikara silasathu pampam, Nerunjil kudineer, Urinary tract infection.

INTRODUCTION

Acute toxicity testing are conducted to get information on the biological activity and mechanism of action of the drug. The information generated by the test is used in hazard identification and risk management of the drugs¹.

Conventional drugs sometimes can have serious adverse effects. So traditional medicines are in worldwide search ² Traditional medicine-definition (WHO) 'A plant derived material with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants. Some traditions, materials of inorganic or animal origin may also be present.' The Indian traditional systems of medicine and folk medicines make use of thousands of plant-based formulations³ The principle underlying the use of more than one plant/plant product in these formulations is that they may produce synergistic and/or additive effects, or one may neutralize the toxic effect of another, which is otherwise therapeutic in the given context.⁴

After respiratory tract infection the most common infection is Urinary tract infection (UTI). Half of all women suffer from at least once in life time. 20–30% of women experience a recurrence.⁵ UTI reflects an infection of the urinary system causing an inflammatory response.⁶ In siddha system of medicine VSP and NK in combination is given for urinary tract infection. VSP contains Vediuppu (Potassium nitrate) ,Venkaram (Sodium tetraborate), Karpoora silasathu (Gypsum) & NK contains Nerunjil (Tribulus terrestris), Mavalingam (Crataeva magna) & Chukku (Zingiber officinale).

Shilajith reported to have anti-inflammatory and anti ulcerogenic activities. ⁷ It has been invariably effective in all the three phases of inflammation i.e. acute, sub-acute and chronic.⁸ Low toxicity mineral Borax (Na₂B₄O₇(H₂O)₁₀) has insecticidal, fungicidal and herbicidal properties. ⁹ For centuries Nitrates have been used as diuretics. In Thomas Willis' Pharmaceutice Rationalis of 1674, one of the first descriptions of the medical use of potassium nitrate was for the treatment of dropsy (edema).¹⁰ Potassium nitrate was used as a hypotensive and combats high blood pressure.¹¹ *T. terrestris* L. fruit is used for treating edema, cutaneous pruritus, tracheitis and inflammation.¹² *C.magna* is used as an antispasmodic, hypotensive, antiinflammatory, hypoglycemic, antiprotozoal, Anthelmintic, analgesic purposes.¹³ Ginger is believed to have anti-inflammatory, cholesterol-lowering and anti-thrombotic properties. ¹⁴

Preclinical studies of herbal drugs provide scientific justification for their traditional use and prove that they are safe and efficacious.¹⁵ When reviewed literature, so far no scientific evaluations were carried out in this drug for its toxicity profile pre-clinically. To establish evidence-based toxicity data for VSP & NK formulations, acute & subacute toxicity studies were conducted.

MATERIALS AND METHODS

Test Drugs

NK + VSP were prepared by the method described in Anubava Vaidhya devaragasiam ¹⁶ and kannuswamy Parambarai Vaidhiyam ¹⁷

Preparation of drug for dosing

All drugs used for the study was suspended each time with 1% (w/v) solution of sodium carboxyl methyl cellulose before administration.

Drugs and chemicals

Chemicals used in these experiments were obtained from Sigma Chemicals Company, U.S.A. Other analytical grade chemicals were obtained from S.d. Fine Chemicals Ltd., Mumbai. Raw drugs were obtained from local market, Chennai.

Preparation of trial drugs

Vedikara Silasathu Pampam

Equal parts of purified Vediuppu, Venkaram & Karpoora Silasathu were grounded together in kalvam with limewater for 3 samam (9 hours). This mass was made in to several small discs and dried in sun. Then these discs were kept in to a shallow earthen pan and covered with an identical pan inverted over it and edges were sealed with clay smeared cloth ribbon. This set up was dried and then placed and burnt in a kiln. 20, 25 cowdung cakes were used as fuel. Half the number of cowdung cakes was spread at the bottom of the kiln and the calcinations capsules were placed over this at the centre. The remaining cowdung cakes were arranged over these and were ignited all around. The calcinations capsules and the contents were taken only when the kiln had cooked down by itself. The product obtained by appropriate calcinations were finely ground in a mortar and taken.

Nerunjil Kudineer

Nerunjil, Mavilingam & chukku were purified and coarsely powdered, uniformly mixed and stored in an airtight container.

Experimental animals

Strains of wistar rats of both the sex weighing 140-160g were used for the toxicological studies. In standard conditions of 12:12 (day and night cycles) at 22^o C room temperature, in polypropylene cages the animals were kept. The animals were fed on standard pellet's (Hindustan Lever Pvt Ltd., Bangalore) and tap water ad libitum. To acclimatize to laboratory conditions the animals were housed in polypropylene cages prior to the experiments for one week. The experiment was conducted in C.L.Baid metha college of pharmacy and the protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Acute oral toxicity study

As per the OECD Guidelines 423 (acute Class toxic class method) Acute oral toxicity was conducted. It is a stepwise procedure with single sex of 3 animals per step. Based on the mortality status of the animals, on the average 2-4 steps will be necessary to allow judgment of the test substance's acute toxicity study. This stepwise procedure results in the minimal number of animal use and allow's acceptable data based scientific conclusion. 6 female sex wistar albino rats weighing 140-160 g(6-8wks),divided in to 2 groups with 3rats in each group. Animals were fasted overnight, but allowed water and libitum. Group 1 being the control group and received distilled water and group 2 was the test group and received VSP & NK at the dose level of 2000mg/kg/p.o. since the formulation is relatively non toxic in clinical practice the highest dose of 2000 mg/kg/p.o (as per OECD guidelines " unclassified") was used in the acute toxicity study. All the animals were observed at the time interval of 1,2,4,6 & 24 hr & twice from 2-14th day, then sacrificed 15th day for gross pathological examination of different organs including heart, lungs, livers, kidneys, sex organs and brain. The animals were observed closely for behavioral toxicity, if any by using FOB (functional observation battery).

Repeated oral toxicity study

Repeated oral toxicity studies can be used to get additional information regarding the toxicity profile of a chemical. Repeated oral toxicity studies are defined as those studies where the chemical is administered to the animal for the period of approximately 10% of the animals's expected life. Usually, the dose are lower than for acute studies and allow chemicals accumulation in the body before lethality occurs, if the chemical possess this ability. 20 Wistar albino

rats of both sex weighing 140-160 g, were divided in to 2 groups of 10 rats (5male,5female) in each group.

Group 1: Control animals received distilled water for 28 days.

Group II: Drugs suspended in NK+VSP was given at 500 mg/kg/p.o dose levels for 28 days.

The animals were observed for mortality, appearance and any signs of intoxicification. 29th day, the rats were fasted 12 hours and then anesthetized with ether. From each rat, blood sample was withdrawn from the jugular vein for hematological and biochemical analysis. Section of liver, kidney, and heart were dissected out and kept in 10% formalin to analyze the gross histopathological studies.

Biochemical studies

In non heparinized tubes the blood was collected and centrifuged for 10 min at 3000 rpm. To analyze the liver and kidney enzymes the serum was separated. Aspartate aminotransferase and Alanine aminotransferase was estimated using commercial AST kit (span diagnostics) by Reitman and Frankel (1957) method.¹⁸ Urea was assayed using the commercial kit (span diagnostics) by the method of Coulambe et al., (1965).¹⁹

Haematological studies

In the heparinized tubes the blood samples were collected. Erythrocyte count was estimated by Hemocytometer method of Ghai (1995).²⁰ Total leukocyte count was estimated by Hemocytometer method of john (1972)²¹, Hemoglobin was estimated by method of Ghai (1995).

Statistical Analysis

The data were expressed as means \pm standard deviation (S.D). Student's paired 't' test was applied to analyze the results. P <0.01 was considered significant.

RESULTS

Acute oral toxicity study

NK and VSP at the dose of 2000mg/kg/po did not exhibit any mortality in rats. As per OECD 423 guidelines the dose is said to be "Unclassified" under the toxicity scale. Hence further study with higher doses was not executed.

Repeated oral toxicity for 28 days

Test drug NK and VSP at the dose 500 mg/kg/po when administered for 28 days in rats orally did not show toxicity in hematological (Table 1) & biochemical (Table 2) parameter.

Table 1: Effect of Siddha Formulations (NK + VSP) on Haematological parameters after 28 days repeated oral dosing (500 mg/kg)

S.no	Groups	Drugs	Haemoglobin	RBC	WBC	Differential	Leucocyte count %
01.	Control	Distilled water	13.23 \pm 0.56	4.59 \pm 0.565	5650.08 \pm 9.43	76.06 \pm 3.27	5.610 \pm 1.27 19.84 \pm 4.647
02.	Test drug	NK+VSP	13.92 \pm 0.621 ^{ns}	4.68 \pm 0.267 ^{ns}	5786.66 \pm 3.823 ^{ns}	77.67 \pm 3.64 ^{ns}	6.16 \pm 1.87 ^{ns} 17.36 \pm 3.31 ^{ns}

n=6; Values are expressed as mean \pm S.D followed by Students paired 't' Test;ns - non significant

Table 2: Effect of Siddha formulation (NK+VSP) on Biochemical markers of liver and kidney after 28 days repeated oral dosing (500 mg/kg/po)

S.no	Groups	Drugs	AST (IU/L)	ALT (IU/L)	Cholesterol (mg/dl)	Urea(mg/100ml)	Uric acid
01.	Control	Distilled water	72.64 \pm 0.349	30.64 \pm 0.821	45.61 \pm 0.64	24.56 \pm 0.647	1.88 \pm 0.72
02.	Test drug	NK+VSP	81.45 \pm 0.36 ^{ns}	28.813 \pm 0.664 ^{ns}	51.23 \pm 0.56 ^{ns}	30.66 \pm 0.319 ^{ns}	2.41 \pm 0.64 ^{ns}

n=6; Values are expressed as mean \pm S.D followed by Students paired 't' Test; ns-non significant

DISCUSSION

Acute oral toxicity study for 14 days

There was no significant difference in the body weight of all the rats. Toxicity signs such as piloerection, salivation & lacrimation were not observed. VSP & NK at the dose of 2000mg/kg/po did not exhibit any mortality in rats.

Sub-Acute oral toxicity study for 28 day

There was no significant difference in the body weight of all the rats. Toxicity signs such as piloerection, salivation & lacrimation were not observed. The Hematological parameters like Hb, RBC, total WBC count of the treated rats were not significantly different Compared to the control group (Table 1). There were also no significant differences in the biochemical markers like AST, ALT, Cholesterol,

urea & uric acid of the test group when compared to that of the control group (Table 2).

Histopathological examination of different organs like heart, liver, kidneys from both control and treated groups showed no abnormal architecture, no detrimental changes and morphological disturbances caused due to the administration of VSP + NK for 28 days. The test drug VSP & NK at the dose of 500mg/kg/po when administered orally for 28 days in rats did not showed toxicity in liver and kidney.

CONCLUSION

From our observation, it can be concluded that VSP & NK at 2000mg/kg/p.o as a single dose orally & at 500mg/kg once orally for 28days is well tolerated by adult wistar rats. In conclusion, our results provide that VSP + NK is safe and is considered to be used widely at the clinical application

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