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ANTI - INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITY OF TWO SIDDHA FORMULATIONS IN COMBINATION

B. Akila* and K. Manickavasakam

Department of Maruthuvam, National Institute of Siddha, Chennai-47, Tamil Nadu, India

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Correspondence to Author:

Dr. B. Akila

Ph. D Scholar, Department of Maruthuvam, National Institute of Siddha, Chennai-47, Tamil Nadu, India

E-mail: drakila10@gmail.com

ABSTRACT

In Siddha system of medicine, the two formulations Vedikara silasathu parpam and Nerunjil kudineer in combination is given for urinary tract infection. Inhibition in the synthesis of inflammatory mediators and prostaglandins owe to the anti- inflammatory and antinociceptive properties of the drugs. In this study, the two Siddha formulations VSP and NK in combination was evaluated for anti- inflammatory activity by means of carrageenan induced rat paw edema method and antinociceptive activity with tail flick method in Wistar albino rats. In the carrageenan induced paw edema technique, rats at the dosage of 500mg/kg/p.o of VSP and NK, significant ($p<0.001$) inhibition of inflammatory progression was observed than the control group. In the tail flick method, VSP and NK at dosage of 500 mg dose, increased the tail withdrawal time significantly ($p<0.001$) when compared to the control group. This study had established the significant anti- inflammatory and antinociceptive activity of VSP and NK.

INTRODUCTION: Urinary tract infection (UTI) is the most prevailing infection next to respiratory infection. Generally half of all women, at least once, during their existence suffer from UTI .There will be recurrence of UTI in 20–30% of women [1].

In UTI there will be infection in the urinary system which causes inflammatory reaction. In UTI the uropathogenic bacteria replaces normal flora of the periurethral area and then lead to cause a bacterial cystitis. Occasionally, this infection cause a bacterial pyelonephritis by ascending to the kidney [2].

There is a world wide trend in search for traditional medicines due to inadequate and serious adverse effects of the synthetic drugs [3]. It is a familiar fact that Traditional System of medicine plays an significant part in meeting the international health care needs. They are continuing at present and will play a major role in future also [4].

Thousands of plant-based formulations are used in Indian traditional system [5]. The theory in using these formulations is that they produce synergistic effect [6]. Siddha medicine formulations includes polyherbals, metals or animal products [7].

VSP and NK are combindely given for urinary tract infection in siddha system of medicine. VSP contains Vediuppu (Potassium nitrate), Venkaram (Sodium tetraborate), Karpoora silasathu (Gypsum) & NK contains Nerunjil (*Tribulus terrestris*), Mavalingam (*Crataeva magna*) & Chukku (*Zingiber officinale*).

Silajith have anti-inflammatory and anti ulcerogenic activities [8]. It is consistently effective in all the phases of inflammation i.e. acute, sub-acute and chronic [9]. The low toxicity mineral borax has fungicidal, herbicidal and insecticidal properties [10].

Potassium nitrate was given for the treatment of dropsy (edema) is found in Thomas Willis' Pharmaceutice Rationalis of 1674 [11]. Potassium nitrate lowers high blood pressure [12]. *T. terrestris* L. fruit is used for the treatment of edema, inflammation and tracheitis [13]. *C. magna* acquire antiinflammatory, analgesic, antiprotozoal, Anthelmintic antispasmodic, hypotensive and hypoglycemic activity [14]. *Zingiber officinale* have anti-inflammatory, cholesterol-lowering and anti-thrombotic properties [15].

In India, population more than 70% of the population makes use of traditional system of medicine. There is a empirical-data base for many of the natural drugs. [(16)] Preclinical studies of herbal drugs have proved that they are safe and efficacious [17]. Research has to be carried out on the medicinal plants and their formulations to show their significance.

In this study, it was aimed to evaluate the antinociceptive and antiinflammatory activities of VSP and NK and to expound the possible mechanism of its pharmacological activities.

MATERIALS AND METHODS:

Sample collection: The raw drugs for VSP and NK were collected from authorized dealer from Chennai, Tamil Nadu and were identified and authenticated by the head of the pharmacological department (Gunapadam) of Govt siddha medical college, Chennai, Tamilnadu. Instructions mentioned for purification of the ingredients in VSP and NK as per classic siddha text was followed [18, 19]. VSP and NK were prepared according to the method described in standard text books of siddha medicines [20, 21]. The process for the preparation of VSP and NK was monitored by the experienced officials of Govt Siddha medical college, Chennai, Tamilnadu.

Preparation of the formulations VSP and NK:

Vedikara Silasathu Parpam:

Ingredients:

- Purified Potassium nitrate - Vediuupu
- Purified Sodium tetraborate - Venkaram
- Purified Gypsum - Karpoora silasathu
- Lime water - Required quantity

All ingredients were taken in equal parts.

Purification of Raw drugs:

1. **Purification of Potassium nitrate:** Water was added to the salt and boiled on a hearth with mild flames. The white of eggs were added to the salt and the bubbles appeared with impure substances were removed with a wooden spoon. The Ingredients were transferred to another pot, sealed with mud pasted cloth, filtered and was kept in places without aeration. Next day the water was filtered and the salt was dried in sunshade. This process was repeated for seven times to get it purified.

2. **Purification of Sodium tetraborate:** It was fried till the moisture completely evaporated.

3. **Purification of Gypsum:** Gypsum was placed in milk and the milk was boiled. Then it was taken out after washing.

a. **Method:** 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 earthern pan and covered with an identical pan inverted over it and edges were sealed with clay smared 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 numbers of cow dung cakes were 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:

Ingredients:

- Purified Tribulus terrestris - Nerunjil
- Purified Crataeva magna - Mavalingam
- Purified Zingiber officinale – Chukku

All ingredients were taken in equal parts

Purification of Raw drugs:

1. **Purification of *Tribulus terrestris* and *Crataeva magna*:** The raw drugs were dried in sunshade and then the dust and foreign particles were removed.

2. **Purification of *Zingiber officinale*:** The outer skin was removed.

a. **Method:** Equal parts of purified Nerunjil, Mavilingam & chukku coarsely powdered, uniformly mixed and stored in an airtight container.

3. **Phyto chemical screening:** The presence of plant secondary metabolites like alkaloids, Tannins, flavonoids, saponins and glycosides were carried out using standard chemical test [22].

Studies in Rat:

Preparation of drug for dosing: All the drugs for the study were suspended each time with 1% (W/V) solution of sodium carboxyl methyl cellulose before administration.

Drug dose selection: Dose selection of the test drug VSP and NK was based on the acute toxicity test carried out at the dose of 500mg/kg/p.o

Drugs, chemicals and reagents: Raw drugs were purchased from local market (Chennai, Tamil Nadu, India). Analytical grade chemicals were purchased from S.D.Fine Chemicals Ltd., (Mumbai, India).Carrageenan was purchased from Sigma Chemicals Company, (U.S.A).

Experimental Rats: Wistar albino rats of either sex weighing 200-250g were used for the pharmacological study. The Standard conditions of 12:12 (day/night cycles) at 22° C room temperature were maintained and the rats were fed on standard pellet diet (Hindustan Lever Pvt Ltd., Bangalore) and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. Three groups were assigned for the experiment and each groups consisted of 6 animals/dose. All the rats were randomly selected and were used only once. The rats were allowed to fast over night before the experiments.

The experiments were conducted in the Department of Pharmacology, Baid Mehta College – TN, India and the protocol was approved by the institutional animal ethical committee (IAEC).

Repeated Oral Toxicity: As per OECD Guidelines 407 test drug NK and VSP at the dose of 500 mg/kg/po when administered orally for 28 days in rats which was lower than for acute studies (2000mg/kg/p.o) did not showed toxic effect [2.)] This end result served as a factor for dosage explanation in the experiments of anti-inflammatory and antinociceptive activities.

Anti-inflammatory activity - Acute model:

Carragenan induced hind paw edema: The modified method of carragennan – induced paw edema (winter et al 1962) was used [24]. The rats were divided in to 3 groups of 6 rats each. Group I received distilled water served as control. Group II received the doses of 500mg/kg/p.o of test drug VSP and NK. Group III received the standard drug, diclofenac sodium, 5mg/kg/p.o. Edema was induced by injecting 0.1 ml of 1% solution of carrageenan in saline into the plantar aponeurosis of the left hind paw of the rats. The test drug, reference drug and the control vehicle (distilled water) were administered 60 min prior to the injection of the carrageenan. The volumes of edema of the injected and contra lateral paws were measured at 0, 30mts, 1, 2, 3 and 4th hr after induction of inflammation using a plethysmometer (Bhatt et al., 1977). Paw diameters were measured by dividers and recorded. Reduction in the paw diameter's when compared to control groups was observed and was taken as the anti-inflammatory activity.

Antinociceptive activity:

Tail Flick method [25, 26]: Withdrawal of tail (Tail flick) for noxious thermal (radiant heat) was used for screening the test drugs for analgesic activity. Radiant heat was generated by passing electrical current through nichrome wire mounted in an analgesiometer (Inco, India). The base of the tail of the test rats was placed on a nichrome wire. The strength of the current passing through the nichrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5cm. The site of application of the radiant heat in the tail was maintained at 2.5cm, measured from the root of the tail.

The tail withdrawal for the radiant heat (flicking response) was taken as the end point. Normally the rats and mice withdraw their tails within 3-5 secs. A cut off time of 10-12 secs is used to prevent damage to the tail tissues [27]. Any animal failing to withdraw its tail in 3-5 secs was rejected from the study. Stop watch was used to determine the time. The experiment was conducted 1hr following the oral administration of the formulations.

The pre-screened animals (reaction time: 3- 5 secs) were divided in to 2 groups. Group I received distilled water served as control. Group II received the doses of 500mg/kg/p.o of test drug VSP and NK. The reaction time of test drug and control were taken at intervals of 30, 60 and 120mts. A reaction time (withdrawal time) increment of 2-5 secs more than the control animals was considerd for analgesic activity of the drug.

Stastical analysis: The datas were expressed as means ± Standard error of the mean (s.e.m). The obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by student's paired 't'test for differences among control and treated groups ; $P < 0.001$ was considerd significant [28].

RESULTS:

Phytochemical Screening: The phyochemical screening of VSP and NK used in the Pharmacological tests had exposed the presence of various phytoactive constiuents like flavonoids, Alkaloids, steroids, glycosides, aminoacid, starch in NK and tannins in both NK and VSP (**Table 1**).

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF VSP AND NK

Phytoconstiuents	NK	VSP
Alkaloids	+	-
Carbohydrates	+	+
Glycosides	+	-
Flavanoids	+	-
Tannins	+	+
Steroids	+	-
Phenols	-	-
Saponins	-	-
Proteins	+	-
Aminoacids	+	-

+ Present ; - Absent

Anti-Inflammatory activity: In acute inflammation model the administration of VSP and NK at the dose of 500mg/kg/p.o showed significant reduction in the paw edema volume at 3rd and 4th hr as compared to the control group (**Table 2**).

Antinociceptive activity: In tail flick method the test drug at the dose of 500mg/kg/p.o, produced increase in the reaction time at 30mts, 1 and 2nd hr when compared to the control group (**Table 3**).

TABLE 2: ANTI INFLAMMATORY ACTIVITY OF VSP + NK IN CARRAGEENAN INDUCED PAW EDEMA

Treatment (mg/kg/p.o)	Paw volume (ml) by mercury displacement at regular interval of time					
	0min	30min	60min	120 min	180min	240min
Control	1.233 ± 0.338	1.733 ±0.225	2.066±0.286	2.100±0.236	2.25±0.273	2.266±0.23
VSP+NK (500mg)	1.367±0.284^{ns}	1.730±0.236^{ns}	2.33±0.236 ^{ns}	2.466±0.316^{ns}	1.921±0.372***	1.591±0.62***
Dic.Sodium (5mg)	0.835±0.065^{ns}	1.315±0.069^{ns}	1.128±0.049***	1.011±0.056***	0.896±0.048***	0.85±0.054***

***P < 0.001 Vs Control, Ns- non significant as compared with control; Values are expressed as Mean± S.E., n=6 by students't' test

TABLE 3: ANTINOCICEPTIVE EFFECT OF VSP + NK BY TAIL FLICK METHOD

Treatment (mg/kg/p.o)	Paw licking response (sec)			
	0 min	30 min	60 min	120 min
Control	2.56 ±0.396	2.61 ±0.96	2.76 ± 0.67	2.46 ±0.53
VSP + NK (500mg)	2.96 ± 0.626 ^{ns}	3.133 ±0.258***	5.966 ±0.646***	5.33 ±1.734***

***P < 0.001 Vs Control, ns- non significant; Values are expressed as Mean± S.E., n=6 by students paired't'- test

The observed results established that VSP and NK have potent anti-inflammatory effect on acute edema induced by carrageenan and antinociceptive effect in opposition to thermal pain induced by nichrome wire.

DISCUSSION: The accepted inflammatory model to look at anti-inflammatory effect of compounds is Carragennan induced rat paw edema [29]. Inflammation induced by carrageenan involves three different phases of mediator release.

The first phase last between first to the second hour which involves the release of histamine and serotonin, the second phase involves the release of kinins and last from second to the third hour, whereas the third phase involves the release of prostaglandins which last after the third to the fifth hour (Surender and Mafumdar, 1995). Our outcome showed that the administration of VSP and NK inhibited the inflammation at the third phase that is 3rd to 4th hr and accordingly it can be directed that the mechanism all the way through which this combination exhibited its action is by the way of inhibition of the synthesis of kinins and prostaglandins.

The most widespread analysis to test narcotic drugs is the tail flick method. This experiment is based on phasic stimulus of elevated intensity. The substances efficient in tail flick put forth their effects chiefly via μ opioid receptors [30]. Analgesics usually well thought to act centrally, peripherally or both [31]. Analgesic acting peripherally perform by blocking the production of impulse at chemoreceptor location of pain and whereas when centrally acting not only elevate the threshold for pain, however in addition modify the physiological reaction to pain furthermore hold back the patient's nervousness and uneasiness [32].

Prostaglandins enclose two important events: they are intermediaries of swelling as well as sensitise nerve endings, lowering their threshold of reaction to stimulus, mechanical and chemical, allowing the further intermediaries of inflammation, e.g. histamine, serotonin, bradykinin, to strengthen the activation of the sensory endings [33].

Prostaglandins involved in the late phase of acute inflammation and pain perception is targeted by flavonoids [34]. A number of flavonoids by means of considerable antinociceptive and anti-inflammatory property have been isolated from medicinal plants [35]. Flavonoids additionally tannins previously been observed for Analgesic and anti-inflammatory effects (Ahmadiani *et al*., 1998; Ahmadiani *et al*., 2000). Phytochemical screening exposed the existence of flavonoids, alkaloids, steroids, glycosides, amino acid starch in NK and tannins in both NK and VSP. Hence it is likeley that the anti- nociceptive and anti-inflammatory effects with VSP and NK may be credited to its flavonoids and tannin contents.

CONCLUSION: At the end to conclude, VSP and NK acquire anti-inflammatory activity which was evident on acute inflammation and have obvious central analgesic action.The flavonoid content in VSP and NK known to act through inhibition of prostaglandin biosynthesis attribute's to its anti-inflammatory and analgesic activity.[(36)] The outcome hold up the traditional state to make use of VSP and NK in the management of pain and inflammation.

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