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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SIDDHA FORMULATION NERUNJI VER KUDINEER IN CARRAGEENAN INDUCED ALBINO WISTAR RATS

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ABSTRACT

Background: ***Nerunji Ver Kudineer (NVK)*** is a siddha poly herbal formulation consisting of equal proportions of four ingredients. Siddha system of medicines possesses wide range of medicines with different sources of origin for treating various disease. Since a poly herbal formulation contains numerous bioactive principles it has ability to cure multiple clinical conditions. **Aim and Objective:** To determine the anti inflammatory action of *Nerunji Ver Kudineer (NVK*) by using Wistar Albino rats. **Method:** The concerned study was carried out by carrageenan induced paw edema method. Male adult albino wistar rats were selected and divided into 5 groups with each containing 5 rats. Beside negative control Group II-V were administered with carrageenan and produced paw edema. Group III (Indomethacin) was treated with standard medicine whereas Group IV and V where treated with **NVK** at dose of 100 mg/kg/bw and 200mg/kg/bw respectively. In order to measure the edema, linear paw circumference was measured using vernier caliper after 4 hours of carrageenan introduction. Similarly, carrageenan induced pleurisy was analysed. For this rat models were administered with carrageenan which caused pleurisy and treated with **NVK** at the dose of 100mg/kg/bw and 200mg/kg/BW. After 3 hours the rat models were sacrificed. Then total leucocyte count in exudates was calculated. **Result:** The siddha drug **NVK** showed marked reduction in paw edema and pleurisy in albino Wistar rat models. **Conclusion:** It can be concluded that **NVK** posses significant anti inflammatory activity in albino Wistar Rat Models.

KEYWORDS: Anti inflammation, Macrophages, *Nerunji Ver Kudineer.*

1. INTRODUCTION

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants and finaly damaged cells. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. The standard signs of inflammation are expressed by increased blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular influx.[8] Upon the presence of the inflammatory agent, cell membranes induce the activation of phospholipase A2 followed by release of arachidonic acid and inflammatory mediators such ascytokines, serotonin, histamine, prostaglandin and leukotriene's that increase vascular permeability, thus facilitating the migration of leukocytes to the site of inflammation.[15]

Inflammation induced by carrageenan is acute, non immune, well researched, and highly reproducible. Cardinal signs of inflammation is severe pain, edema, hyperalgesia, and erythema resulting from action of pro inflammatory agents—bradykinin, histamine, tachykinins, complement and reactive oxygen, and nitrogen species. Saponins have displayed significant anti nociceptive, anti-inflammatory and antipyretic activities possibly due to their non-glycosides moiety, the sapogenin, but also many diverse activities have also been reported such as anti-allergic, anti-fungal, analgesic and others.[2,7,11] More over a variety of siddha formulation preparation have proved to be useful in animal models of inflammation.[1,4,9,10] Paw swelling, or footpad edema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. Typically, test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of Carrageenan-induced paw swelling. In the study attempts are made to validate the claims of *Nerunji Ver Kudineer (NVK*) regarding the anti-inflammatory activities of this siddha preparation.

2. MATERIALS AND METHODS

2.1. a.Nerunji Ver Kudineer Ingredients

Drug	Botanical name & family	Quantity	Important phytochemicals	Action
Nerunji ver	***Tribulus terrestris.L*** **Zygophyllaceae**	5gm	Dioscin Protodioscin Diosgenin	**Diuretic** Refrigerant Astringent
Sirupeelai ver	***Aerva lanata.L*** **Amaranthaceae**	5gm	Apigetrin Rutin Myricetin	**Diuretic Lithotriptic**
Sirukeerai ver	***Amaranthus tricolor.L*** **Amaranthaceae**	5gm	Amaranthin Isoamaranthin	**Diuretic**
Seeragam	***Cuminum cyminum.L*** **Apiaceae**	5gm	Carvacrol Carvone Pinene	Carminative
Water		400 ml		

2.2. Methods

Male albino rats (180 ± 5 g) were obtained from animal house,K.M.College of pharmacy,madurai and maintained in standard laboratory conditions. They were given standard laboratory diet and water ad libitum. All animal experiments are approved by the Institutional Animal Ethics Committee, and were in accordance with the guidelines of the committee for the purpose of Control and Supervision of Experiments on Animal (**IAEC/P.BERNATH/TNMGRMU /MD(S)/321611002 / KMCP/24/2018**), KM College, Tamilnadu,

Acute inflammation

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti inflammatory activity of the siddha formulation Nerunji Ver Kudineer was evaluated by carrageenan-induced rat paw edema method[3] Albino Wistar rats (180 ± 5 g) were used. Anti inflammatory activity was measured using carrageenan induced rat paw edema assay. The rats were divided into 5 groups of 5 animals each. Group I. were given normal saline and treated as negative control. Rats of Group II was treated with carrageenan (1%w/v) in saline in the sub planter region of the right hind paw Rats in Group III were administered Indomethacin (10 mg/kg, bw) and considered as standard. Rats from Group IV and V were given two doses siddha formulation (100 and 200 mg/kg bw). Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution, pre-pared in normal saline. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference will be measured at hourly interval for 4 h. The perimeter of paw was measured by using vernier calipers. Measurements were taken at 0–4 h after the administration of the carrageenan.

Carrageenan Induced Pleurisy In Rats

The animals were divided into five groups of five rats each as described in the carrageenan induced paw edema model[3][12] and each were pretreated with siddha formulation (100 and 200 mg/kg, p.o.), Indomethacin (10 mg/kg, p.o.) or normal saline (0.1 ml).One hour later all the animals were received 0.25 ml of an intra pleural injection of 1 % carrageenan on the right side of the thorax.

The animals were sacrificed 3 h after carrageenan injection by ether inhalation. One ml of heparinized Hank's solution was injected into the pleural cavity and gently massaged to mix its contents. The fluid was aspirated out of the cavity and the exudates were collected. The number of migrating leukocytes in the exudates was determined with Neubauer chamber.

The values of each experimental group were expressed as mean ± SEM and compared with the control group.

3. RESULTS AND DISSCUSSION

Table 3.1: Effect of siddha formulation NVK on Carrageenan Induced Pleurisy in rat models.

Treatment	Dose (mg/kg, p.o.)	Pleural exudates (ml)	Leukocytes (×103 cells/ml)
Normal control	10ml / kg saline	0.12±0.04	0.39±0.05
Toxic control	ml, 1% carrageenan	0.46±0.18*a	4.22±0.38*a
Standard control	10mg / kg Indomethacin	0.15±0.06*b	0.47±0.07*b
Treatment control	100mg / kg NVK	0.22±0.09*b	0.53±0.09*b
Treatment control	200mg / kg NVK	0.7±0.07*b	0.50±0.08*b

Table 3.2: Effect of siddha formulation NVK on Carrageenan Induced Rat Paw Edema.

Normal control	10ml/kg saline	1.12 ± 0.10	
Toxic control	0.1 ml, 1% carrageenan	3.48 ± 0.28*a	
Standard control	10mg/kg Indomethacin	1.17 ± 0.11*b	66.37%
Treatment control	100mg/kg NVK	1.39 ± 0.17*b	60.05%
Treatment control	200mg/kg NVK	1.30 ± 0.13*b	62.64%

Values are expressed as mean ± SEM.
Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
* (a) Values are significantly different from normal control G1 at P<0.01.
* (b) Values are significantly different from Toxic control G2 at P<0.01.

Statistical analysis
Results in table I &2 showed, the anti inflammatory activity were expressed as Mean increase in paw diameter ± SD. Results were analyzed using one way ANOVA. Differences were considered as statistically significant at P < 0.05 are compared to control.

3.1. a. Anti-inflammatory Activity of siddha formulation Nerunji Ver Kudineer
a) Carrageenan-induced pleurisy in rat models
The result of carrageenan-induced pleurisy in rats is shown in Table 1. volume of pleural exudates in the toxic control group was0.46±0.18 ml. Animals treated with the **NVK** (100 and 200 mg/kg, p.o.) decreased the pleural exudates to 0.22±0.09 ml and 0.17±0.07.Treatment with Indomethacin(10 mg/kg, p.o.) produced the exudates of 0.15±0.06 ml. The leukocyte count for the control group was found to be $4.22\pm0.38\times10^3$ cells/ml. Animals treated with the **NVK** and standard produced a leukocyte migration of $0.53\pm0.09\times10^3$, $0.50\pm0.08X10^3$ and $0.47\pm0.07\times10^3$ cells/ml, respectively.

b) Carrageenan-induced paw edema in rat models
The effect of **siddha formulation NVK** on carrageenan-induced edema in rats is shown in Table 3.2. The results obtained indicate that the **siddha formulation NVK** had significant anti-inflammatory activity in rats. The **siddha formulation NVK** reduced the edema induced by carrageenan by 60.05% and 62.64% on oral administration of 100 and 200 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 66.37%.

DISCUSSION

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is need to focus on the scientific exploration of siddha formulation drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. Carrageenan induced inflammation is a biphasic phenomenon.[13]

The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action.The tests performed with the **NVK** in the pleurisy model showed that the **NVK** behaves as an inhibitor of leukocyte migration and the formation of pleural exudates when given orally, as reported earlier.[13] Thus it can be concluded that the **NVK** possess significant anti-inflammatory activity in rats. Further studies involving the purification of the preparation and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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