

EFFECT OF *NIGELLA SATIVA* LINN (RANUNCULACEAE) GROUND SEED EXTRACT ON CARRAGEENAN INDUCED INFLAMMATION IN RATS

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Abstract

Nigella sativa Linn (Family: Ranunculaceae) Bengali name “kalo jera” is used as spice in Bengali foods. Native to Western Asia, Turkey, Iraq and Egypt, the black seed oil has been valued for its health benefits for centuries. This plant has been used in traditional medicine for the treatment of stomach aches, asthma, bronchitis, coughs, fevers, tumour and as a tonic. The dried and grounded seed was extracted with ethanol and the extract was evaluated for anti-inflammatory activity in carrageenan induced rat paw edema model. The extracts were administered orally at the doses of 250 and 500 mg/kg body weight, and statistically significant ($p < 0.05$) anti-inflammatory effects were observed in a dose dependant manner. The extract showed 28.75% and 43.79% inhibition of inflammation at the doses of 250 and 500 mg/kg body weight after first hour of the carrageenan administration which was comparable to that of standard drugs aspirin 40.52% and hydrocortisone 47.71% respectively. The result of this study supported the traditional medicinal uses of this seed.

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Introduction

Inflammation is a protective response intended to eliminate the initial cause of cell injury as well as the necrotic cells and tissues resulting from the original insult.¹ The anti-inflammatory drugs which are currently available are a heterogeneous group of compounds, often chemically unrelated, which nevertheless share certain unwanted effects. The most common is a propensity to induce ulceration. Therefore, the present trend is to find out more acceptable agents which will be devoid of the potential adverse effect. Use of herbal medicine throughout the world is increasing. Plants are the primary source of supply of many important drugs used in modern medicine. Our chosen herb *Nigella sativa* Linn (Family: Ranunculaceae) is a common spice of south east Asia. *N. sativa* (locally called Kalajira) has been in use in Bangladesh, India & many Middle Eastern communities as natural remedy of many acute

conditions for two thousand years. Various research works proved previously that thymoquinone- an active component of *N. sativa* is a potent inhibitor of prostaglandins (PGs), histamine, 5HT, leucotrienes polymorphonuclear leucocytes.²

The objective of the study was to determine the effects of ethanol extract of *N. sativa* on acute inflammation induced by 1% Carrageenan injection in rat paw edema model. The effect of *N. sativa* was compared with that of aspirin and hydrocortisone.

Materials and Methods

Plant Material

The seed of *N. sativa* Linn was collected from local market and was taxonomically identified by Department of Botany, University of Dhaka.

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Extraction

The collected seeds (250gm) were crushed in a mortar and pestle. The crushed seeds were soaked in 600ml ethanol for 72 hours with occasional shaking. The material was filtered and the ethanolic filtrate was collected. The extract was then evaporated to dryness in a vacuum rotatory evaporator followed by a lyophilizer. After removal of all water present in the extract under lyophilizer a deep brownish oily material (60gm) was obtained.

Animal

Long Evans Norwegian rats of either sex (weighing 200-250gm) were collected from BSMMU research division. The animals were kept in polyvinyl cages under controlled room temperature ($25 \pm 2^\circ\text{C}$) in the laboratory environment. The rats were kept 12 h in dark and 12 h light cycle for seven days. The ICDDR, B formulated food pellets were supplied to the animals along with water *ad libitum*. Animals were fasted overnight and weighed before the experiment. The study involving rats was approved by the Bangladesh Medical Research Council, and the experiments were carried out strictly in accordance with the guidelines provided by the World Health Organization.

Preparation of the samples

The oily extract 0.625gm and 1.25gm was taken in separate test tube. A suspension of the extract was prepared with Tween 80 and water in six ml each. Each 0.6ml contained 62.5mg and 125mg for a dose level of 250mg and 500mg per kg body weight respectively. Aspirin 75mg tablet and hydrocortisone injection available in the local market were collected as reference standard.

Anti-inflammatory Activity: Carrageenan induced

The anti-inflammatory activity of the extracts of *N. sativa* was measured by the carrageenan-induced rat paw oedema model.³ Experimental animals were randomly selected, irrespective of sexes, and divided into five groups consisting of 6 rats in each group. Group I: Received 0.6ml normal saline administered orally and served as control. Group-II: received ethanol extract of *N. sativa* 250mg/kg (0.6ml) body weight administered orally. Group-III: received ethanol extract of *Nigella sativa* 500mg/kg (0.6ml) body weight administered orally. Group-IV: received aspirin

100mg/kg body weight administered orally. Group-V: received hydrocortisone 2mg/kg body weight administered subcutaneously. After 1 h of treatment, acute inflammation was produced by sub-planter injection of 0.1 ml of 1% suspension of carrageenan in sterile water in the right hind paw of the rats. The paw volume was measured plethysmometrically (Ugo Basile, Italy) after one hour of carrageenan injection. Results were expressed as percentage of inhibition of oedema calculated by the formula- $(1 - V_t/V_c) \times 100$, where V_t and V_c are the mean paw volume in the treated and controlled groups, respectively.

Statistical Analysis

All the results have been expressed as mean plus/minus standard error of mean (mean \pm SEM). Significance of difference between groups was assessed by using ANOVA Test.

Results

The mean initial (0 hr.) paw volume of group-I, II, III, IV and V were 117.25 ± 1.28 , 121.05 ± 3.32 , 133.69 ± 2.48 , 128.63 ± 5.16 , 131.59 ± 4.63 respectively. Simultaneously the mean paw volume after 1 hour of Carrageenan injection pretreated with test drugs were 193.75 ± 2.14 , 175.55 ± 2.10 , 176.69 ± 1.17 , 174.13 ± 1.68 , 171.59 ± 1.23 respectively (Table- 1). All units were expressed in mm^3 . The percentage inhibition of oedema formation in group - II, III, IV and V were 28.75%, 43.79%, 40.52%, 47.71% at a dose of *N. sativa* 250mg/kg, *N. sativa*

Table-1: Effects of administration of ethanol extract of *N. sativa*, aspirin and hydrocortisone on carrageenan-induced paw oedema after 1 hour of carrageenan injection.

Groups	Initial (0 hr) Paw volume (MEAN \pm SEM) mm^3	Paw volume after 1 hr of Carrageenan injection (MEAN \pm SEM) mm^3	Increased Paw volume (MEAN \pm SEM) mm^3	Inhibition of edema formation after 1 hr %
Group I	117.25 ± 1.28	193.75 ± 2.14	76.50 ± 2.11	—
Group II	121.05 ± 3.32	175.55 ± 2.1	$54.50 \pm 1.24^*$	28.75%
Group III	133.69 ± 2.48	176.69 ± 1.17	$43.00 \pm 4.65^*$	43.79%
Group IV	128.63 ± 5.16	174.13 ± 1.68	$45.50 \pm 3.82^*$	40.52%
Group V	131.59 ± 4.63	171.59 ± 1.23	$40.00 \pm 2.11^*$	47.71%

* $P < 0.05$

500mg/kg, aspirin 100mg/kg and hydrocortisone 2mg/kg body weight respectively in comparison to control (Table-1). From the result it was found that a significant anti-inflammatory effect was exhibited by the ethanolic extract of *N. sativa* at 500mg/kg body weight with 43.79% inhibition.

Discussion

The frequency of intake of NSAIDs and their reported common side effects is increasing day by day, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. To give a scientific validation to the plant *N. sativa*, an attempt was made to study the anti-inflammatory activity⁴ of the ethanolic extract of its seeds. Administration of ethanol extract of ground seed of *N. sativa* at a dose of 250 mg/kg and 500mg/kg body weight orally exerted anti-inflammatory effect, where the percentage of inhibition of oedema formation was 28.75% and 43.79% respectively. And the effect was dose dependent. Following the administration of aspirin and hydrocortisone the percentage of inhibition of oedema were 40.52% in aspirin and 47.71% in hydrocortisone. The effect of *N. sativa* extract at a dose of 500mg/kg body weight was better than that of non-steroidal reference standard aspirin, and was little bit less than that of steroidal hydrocortisone.

N. sativa might have inhibited the release of histamine and serotonin (5-HT) and the formation of TNF- α , IL-1 β , and IL-6 and enhanced the production of IL-10, thus resulting in an overall attenuation of the pro-inflammatory/inflammatory mediators and cytokine ratio in Carrageenan-injected paws.⁵ *N. sativa* might have effect on vascular component of inflammation by affecting the release or formation of inflammatory mediators such as PGs, histamine, leucotrienes etc. It may also inhibit the amoeboid activity of the reticuloendothelial cells and polymorphonuclear leucocytes resulting a reduction in the cellular exudates.⁶

Thus it can be concluded that ground seed of the plant *N. sativa* possess significant anti-inflammatory activity in rats. Further studies involving the purification of the chemical constituents of the plant and the investigations to elucidate mechanism of action and safety profile may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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