ORIGINAL ARTICLE

Anti-Inflammatory Activity of *Nigella sativa*: A Study on Animal Model

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Abstract:

A prospective study was carried out to find out the anti-inflammatory effects of the ethanolic extract of ground seeds of Nigella sativa in inflammed rats at the Department of Pharmacology and Therapeutics, Dhaka Medical College, during the period from January to December 2008. The effect was compared among sixty Long Evan Norwegian rats with reference standard aspirin and hydrocortisone. Acute inflammation was induced by Carrageenan injection at the sub-plantar surface of the hind paw of rat. Ethanol extract of ground seed of Nigella sativa, aspirin, hydrocortisone and normal saline (as control) were administered to evaluate anti-inflammatory effects. The anti-inflammatory effects (acute) measured by 'inhibited oedema formation' were 43.79% by Nigella sativa, 40.52% by aspirin, 47.71% by hydrocortisone. Again chronic inflammation was induced by implantation of a sterile cotton pellet in rat's groin region for 14 days and treated with Nigella sativa extract, aspirin, hydrocortisone and normal saline. The anti-inflammatory effects (chronic) were measured by weighing cotton pellet to evaluate 'inhibited granuloma formation' and were 41.42%, 27.67% and 38.58% respectively. Moreover, Nigella sativa extract was administered in two different doses (250mg/kg and 500 mg/kg body weight) and significant anti-inflammatory effect was observed by the higher dose.

Introduction:

The occurrence of inflammatory disorder is seen world wide with no racial predilection. However the poor and developing countries are lacking proper management of inflammatory diseases. As a result the prevalence of inflammatory conditions are considerably high in developing countries including Bangladesh. 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 evolve more acceptable agents which will be devoid of potential adverse effect. Use of herbal medicine throughout the world is increasing. Plants still remaining the primary source of supply of many important drugs used in modern medicine. The *Nigella sativa Linn* (Family: Ranuculaceae) is a common spice of south east Asia.*Nigella sativa* (locally called Kalajira) has been in use in Bangladesh, India and many Middle Eastern communities as natural remedy of many acute conditions for two thousand years[1]. Various research works stated that Thymoquinone- an active component of

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Nigella sativa is a potent inhibitor of PGs, histamine, 5HT, leucotrienes and polymorphnuclear leucocytes. Plants still remain the primary source of supply of many important drugs used in modern medicine. Therefore, studies are still going on in search of more potent, less toxic, cheaper and easily available anti-inflammatory agents. To treat inflammatory conditions medications are used, but medications can have side effects. However herbal medications usually are devoid of such problems. *Nigella sativa Linn* (Family: Ranuculaceae) is a common spice of south East Asia especially in Bangladesh and locally called Kalajira. . The plant enjoys vast folklore uses as traditional medicine. The Nigella sativa that is kalajira is a healing for all diseases except death [1]. Considering its medicinal value and availability in Bangladesh, the study was done to evaluate the anti-inflammatory effect of the ground seed of Nigella sativa in rat model and acute and chronic inflammation were compared with both steroidal and non steroidal anti-inflammatory agents. Objective of the study was to induce acute and chronic inflammation by 1% Carrageenan injection and by subcutaneous implantation of cotton pellet to compare the effects of ethanol extract of *Nigella sativa* with Aspirin and Hydrocortisone.

Materials and method:

The ground seed of Nigella sativa was collected from National Herborium and was taxonomically identified by Department of Botany, University of Dhaka. After collection, the seeds were sun-dried and finally ground to coarse powder. The powdered plant material was extracted with distilled ethanol at room temperature for 10 days. The filtrate was concentrated in vacuum (50°C) yielding the crude ethanol extract. The crude extract obtained as such was kept and dried in refrigerator at 4°C for three days. The extract was diluted with normal saline prior to any pharmacological use. After collection of crude ethanol extract was formed and kept in 4^0C in refrigerator. The extract was oil in nature. Tween-80 (suspending agent) and water was added with oil to make a suspension. This suspension was arbitrarily divided into two doses as low dose (250mg/kg) and high dose (500mg/kg) for dose dependent response.

Long Evans Norweigian rats, collected from, BSMMU (3-4 months old, 200-250gm of weight), had free access to food and water *ad libitum.* Acute inflammation was induced by Carrageenan a chemical agent. The animals were kept in the laboratory environment for seven days and fasted overnight and weighed before the experiment.

The animals were randomly divided into different groups consisted of six rats in each group. Group I: Received 0.6ml normal saline administered orally and served as control. Group-II: Received ethanol extract of *Nigella 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 one hour of drug administration, 0.1 ml of 1% Carrageenan in sterile saline solution was injected into the sub-plantar surface of the right hind paw for the production of acute inflammation. Paw volumes were measured by volume displacement method using plethysmometer[2] after 1hour of carrageenan injection.

For chronic inflammation the rats were divided into five groups (n=6), fasted overnight and allowed free access to water. The rats were administered with vehicle, standard drug and test drugs. One hour after the first dosing, the rats were anesthetized with ether[3] and 50mg of the sterile cotton pellet was inserted one in each axilla and groin of rats by making small incisions were sutured by sterile catgut. Group-I: received subcutaneous incision. The 0.6ml normal saline administered orally for 14 days and served as control. Group-II: received ethanol extract of *Nigella sativa* 250mg/kg body weight administered orally for 14 days. Group-III: received ethanol extract of *Nigella sativa* 500mg/kg body weight administered orally for 14 days. Group-IV: received aspirin 100mg/kg body weight administered orally for 14 days. Group-V: received hydrocortisone 2mg/kg body weight administered subcutaneously for 14 days. The animals were sacrificed by excess anesthesia on the 14th day and cotton pellets were removed surgically. Pellets were separated from extraneous tissue and dried at 60^{0}C unit weight become constant. The net dry weight i.e. after subtracting the initial weight of the cotton pellet was determined. The average weight of the pellet of the control group as well as of the test groups was calculated. The percent change of the granuloma weight relatively with vehicle control is determined and statistically evaluated. The percentage inhibition increases in the weight of the cotton pellet is calculated. Significance of difference between groups was assessed by using ANOVA Test.

Results:

Acute inflammation

The mean initial (0 hour) 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 one 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-I) All units were expressed in cub.mm. The percentage inhibition of oedema formation in group - II, III, IV and V were 28.75%, 43.79%, 40.52%, 47.71% respectively (Table-I) in comparison to control. 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.

Table-I: Effects of ethanol extract of *Nigella sativa*, aspirin and hydrocortisone on Carrageenan-induced paw oedema after 1 hour

Groups	Initial (0hr) Paw volume	Paw volume after 1 hour of Carrageenan injection	Increased paw volume (cub.mm.)	Inhibition of oedema formationa
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±1.24*	28.75%
Group III	133.69±2.48	176.69±1.17	43.00±4.65*	43.79%
Group IV	128.63±5.16	174.13±1.68	45.50±3.82*	40.52%
Group V	131.59±4.63	171.59±1.23	40.00±2.11*	47.71%

* P<0.05 in a test of significance difference from control.

Chronic inflammation

At the end of the chronic anti-inflammatory study after 14 days the pellets were removed from the site of insertion sacrificing the animals[4]. The final weight of the cotton pellets was determined. The weights were 207.83±0.69mg, 177.63±5.31mg, 142.45±5.58mg, 164.16±15.86mg, 146.93±7.12mg for group - I, II, III, IV and V respectively. The increment in the weight of cotton pellet in ethanol extract of *N. sativa* 250mg/kg body weight, ethanol extract of *N. sativa* 500mg/kg body weight, aspirin and hydrocortisone treated *groups* were 127.63±5.31, 92.45±5.58, 114.16±15.86, 96.93±7.12mg respectively (Table-II). Where as, the increment the pellet for the control group was 157.83±8.69 mg. The percentage of inhibition of granuloma formation were 19.13, 41.42, 27.67, 38.58 as compared to the control for ethanol extract of *N. sativa* 250mg/kg, ethanol extract of *N. sativa* 500mg/kg, aspirin 100mg/kg and hydrocortisone 2mg/kg body weight respectively (Table - II). In this chronic study an anti-inflammatory effect was observed at 500mg/kg body of the ethanolic extract of *N. sativa.*

Table - II: Effects of extracts of Nigella sativa, Aspirin and Hydrocortisone on cotton pellet induced granuloma in rat.

Groups	Initial weight of cotton pellet	Final weight of cotton pellet	Increase of weight of cotton pellet (mg)	Inhibition of granuloma formation %
Group I	50±0.22	207.83±0.69	157.83±8.69	--
Group II	50±0.22	177.63±5.31	127.63±5.31*	19.13
Group III	50±0.22	142.45±5.58	92.45±5.58**	41.42
Group IV	50±0.22	164.16±15.86	114.16±15.86**	27.67
Group V	50±0.22	146.93±7.12	96.93±7.12**	38.58

* P<0.05= significant difference from control.
** P<0.001=highly significant difference from control

Discussion:

The above mentioned models have given a broad spectrum for the evaluation of the anti-inflammatory activity.In different models,the inflammation was produced by different inducers by releasing anti-inflammatory mediators.Each having different mechanism of action for producing inflammation either by increasing the vascular permeability,the infiltrations of leukocytes from the blood into the tissue or granuloma formation and tissue repair.

To give a scientific validation to the plant *N. sativa*, an attempt was made to study the anti-inflammatory activity of the ethanolic extract of its seeds[5]. From the result it was observed that the effect of at dose of 500mg/kg body weight was better than that of non-steroidal reference standard aspirin, and little bit less that the steroidal reference standard hydrocortisone.

In this study, *Nigella sativa* may inhibit release of histamine and serotonin (5-HT) and the formation of TNF-α, IL-1ß, and IL-6 and enhanced the production of IL-10, thus resulting in an overall attenuation of the pro-inflammatory / anti-inflammatory mediators and cytokine ratio in Carrageenan-injected

paws, which may contribute to its anti-inflammatory effect [6].

Nigella sativa reduces the vascular component of inflammation and impair the release or formation of inflammatory mediators such as PGs, histamine, leucotrines etc. responsible for increasing vascular permeability and inflammation. It may also inhibit the amoeboid activity of the reticulo-endothelial cells and polymorph-nuclear leucocytes resulting a reduction in the cellular exudates[7].

Treatment with *Nigella sativa* extract at doses of 250mg/kg body weight orally daily for 14 days produced significant anti-inflammatory effect and at a doses of 500mg/kg body weight orally daily for 14 days produced significant anti-inflammatory effect and the percentage of inhibition of granuloma formation were 19.30% and 41.42% respectively. This was also in a dose dependent manner. Following administration of aspirin and hydrocortisone for 14 days showed also anti-inflammatory effect and the percentage of inhibition of granuloma formation were 27.67% and 38.58% respectively.

In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the ethanol extract of *Nigella sativa*.

In this study, crude ethanol extracts of ground seed of *Nigella sativa* steroidal and non-steroidal anti-inflammatory drugs daily for 14 days reduced weight of granulation tissue. The reduction was statistically significant in comparison to control group which was observed at the higher does (500mg/kg body weight). But the reductions of weight of granulation tissue in case of steroidal and non-steroidal anti-inflammatory drugs were highly significant in comparison to ethanol extract of ground seed of *Nigella sativa*.

The study was basically pharmacological one and both the modern drugs and herbal products were used to influence the biological system. It was evident that the biological systems have certain limitations, like individual variations, interference in the response with the system, variability in methods and other factors, which might have interfered with primary findings. However, the results obtained in this experiment may not represent the exact effect. Despite all these limitations, interpretation of the results obtained in this study was made carefully and cautiously.

The study provides an initial step in demonstrating the anti-inflammatory effect of ethanol extract of ground seed of *Nigella sativa*. The obtained data support the basis for future use of *Nigella sativa* in traditional system of medicine. Thus, it could be a new agent in reducing morbidity and mortality resulting from inflammatory disease condition. The findings presented here provide a baseline for future studies designed to quantify the effects of ethanol extract of ground seed of *Nigella sativa*. The experimental results suggest that the possible mechanism of anti-inflammatory activity of polyamines may be due to their impairment of the release or formation of inflammatory mediators such as histamine, 5-HT, PGs, and lysosomal membrane stabilization as supported by present experimental findings.

Studies on polyamines may be helpful in developing a new approach for better understanding of the inflammatory process and the generation of new anti-inflammatory drugs.

Further investigations are warranted to reconfirm and identify the anti-inflammatory active principles and elucidate their mechanism of action. Toxicological studies should also be under taken before any clinical use. The experimental results suggest that the possible mechanism of anti-inflammatory activity of polyamines may be due to their impairment of the release or formation of inflammatory mediators such as histamine, 5-HT, PGs, and lysosomal membrane stabilization as supported by present experimental findings. Studies on polyamines may be helpful in developing a new approach for better understanding of the inflammatory process and the generation of new anti-inflammatory drugs.

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