RESEARCH ARTICLE

Evaluation of anti-inflammatory activity of *Nigella sativa*: An experimental study

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ABSTRACT

Background: Long-term use of drugs currently used for the treatment of inflammation is associated with serious adverse effects. *Nigella sativa*, an herb exclusively used in traditional medicine, is reported to inhibit both cyclooxygenase (COX) and 5-lipoxygenase pathways of arachidonic acid metabolism. **Aims and Objectives:** To evaluate of antiinflammatory activity *N. sativa* seed fixed oil in different models of inflammation in rats and to compare it with control and aspirin. **Materials and Methods:** Albino Wistar rats of either sex weighing 180-200 g were used in this study. *N. sativa* seed fixed oil was used to evaluate anti-inflammatory by carrageenin-induced hind paw edema, cotton pellet granuloma, and formaldehyde induced arthritis method by oral administration in healthy albino rats. The study was conducted with prior approval of Institutional Animal Ethics Committee. **Results:** In the model of acute inflammation, i.e., carrageenin-induced paw edema in rats, *N. sativa* showed anti-inflammatory activity which was statistically significant as compared to control ($P < 0.001$) but less than aspirin. In cotton pellet induced granuloma method, *N. sativa* significantly decreased the formation of granulomatous tissue, as compared to control ($P < 0.001$). *N. sativa* showed significant anti-inflammatory activity comparable to aspirin in formaldehyde induced arthritis model of chronic inflammation ($P > 0.05$). **Conclusion:** The result of this study suggests that *N. sativa* seed fixed oil possesses significant anti-inflammatory activity in rats.

KEY WORDS: Anti-inflammatory; Fixed Oil; Formaldehyde Induced Arthritis; *Nigella sativa*; Paw Edema

INTRODUCTION

Inflammation is considered as a primary physiological mechanism that helps body to protect itself against various noxious stimuli. It is a defense reaction, the ultimate goal of which is to help the organism get rid of both initial cause of injury (e.g., microbes and toxins) and the consequences of such injury (e.g., necrotic cells and tissues),[1] but many a times it goes unchecked with tissue destruction leading to a spectrum of inflammatory disorders (as in rheumatoid arthritis), and it is under these circumstances a need to resort drug therapy to dampen or abolish the unwanted inflammatory response arises. An uncontrolled and persistent inflammation may act as an etiologic factor for many chronic illnesses. Inflammatory diseases are becoming common in society throughout the world.

At present, both steroidal anti-inflammatory drugs and nonsteroidal anti-inflammatory drugs (NSAIDS) are used in the relief of inflammation. Steroids have an obvious role in the treatment of inflammatory diseases, but due to their toxicity, long-term use of these drugs is associated with serious adverse effects. Prolonged use of NSAID is also associated with side effects.[2] Hence, research for finding a better and safe drug for inflammation has been a continuous, never ending process. Natural

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products, particularly medicinal plants, are believed to be a key source of new chemical substances with potential therapeutic efficacy.

Nigella sativa is a herb extensively used in Unanai, Ayurvedic, Siddhi systems for centuries for various indications including pain, inflammation, and fever.[3,4] Recently, some evidences suggest that *N. sativa* inhibits eicosanoid generation in leukocytes and lipid peroxidation. They are reported to inhibit both cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways of arachidonic acid metabolism.[5] Some studies conducted earlier have reported that it do possess significant analgesic and anti-inflammatory activity.[6,7] Even though substantial work has been carried out to ascertain its anti-nociceptive effects, very little has been done to explore its anti-inflammatory effect on subacute and chronic models of inflammation. Hence, we considered it is worthwhile to know whether it has anti-inflammatory activity in a various model of inflammation.

MATERIALS AND METHODS

This study was conducted in SRTR Government Medical College, Ambajogai, Maharashtra.

Animals

Albino Wistar rats of either sex weighing 180-200 g were used. The rats and mice were grouped in separate cages with six animals in each cage. They were housed in temperature-controlled room (23 ± 1°C) with a 12 h light-12 h dark cycle. They had free access to food and water. The study was conducted after approval from the Institutional Animal Ethics Committee of our institute, which is an approved body by Committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi and animals were cared for in accordance with CPCSEA guidelines.

Chemicals

Aspirin and carboxymethyl cellulose were obtained as kind gift from Medley Pharmaceuticals, Mumbai, India. *N. sativa* seed fixed oil was received as gift sample from Manish Herbals, Mandsaur, Madhya Pradesh. Carrageenan (1% in 0.9% saline) was obtained from commercial sources.

Methods

Animals were divided into following three groups:
1. Control group: Normal saline, dose: 2 ml/kg (p.o.);
2. Standard group (aspirin group): Aspirin, dose: 300 mg/kg (p.o.);
3. Test group (*N. sativa* group): *N. sativa* fixed oil, dose: 10 ml/kg (p.o.).[2,8]

Carrageenan - Induced Hind Paw Edema in Rats[9,10]

Paw edema was induced by an intradermal injection of 0.1 ml of carrageenan (1% in normal saline) into the plantar surface of the right hind paw of the rats.

The acute phase of inflammatory reaction, i.e., edema volume was determined using plethysmometer (modified by Singh and Ghosh) before and at 60 min, 120 min, 180 min after carrageenan injection. All the drugs were administered 1 h before carrageenan.

Percentage inhibition of paw edema, i.e., acute inflammation was calculated using the following formula:

$$\% \text{ Inhibition at given time interval} = \frac{\begin{array}{c}\text{Paw volume in control group}\\ \text{-Paw volume in test group}\end{array}}{\text{Paw volume in control group}}$$

Cotton Pellet Granuloma in Rats

The cotton pellet method as described by Winter CA, Porter CC with slight modification has been used for granuloma formation.[11]

The treatment was started 1 day before the insertion of cotton pellets and continued for 6 days. On the 1st day, the rats were anesthetized by freshly prepared pentobarbitone 40 mg/kg/i.p. Sterilized autoclaved cotton pellet weighing 50 mg ± 1 mg, was implanted in the subcutaneous tissue of right axilla of anesthetized rats. On the 7th day, the cotton pellets together with the granuloma were dissected out carefully, dried overnight at 60°C, and then weighed. The differences in weights before and after were thus obtained for all the animals. The increment in the dry weight was taken as a measure for granuloma formation. The percentage anti-inflammatory effect was calculated using the formula:

$$\text{Persentage anti-inflammatory effect} = \frac{\begin{array}{c}\text{Mean increment in}\\ \text{dry wt in control group}\\ \text{-Mean increment in}\\ \text{dry wt in test group}\end{array}}{\begin{array}{c}\text{Mean increment in}\\ \text{dry wt in control group}\end{array}} \times 100$$

Formaldehyde Induced Arthritis in Rats

Chronic phase of inflammation was induced by subcutaneous injection of 0.1 ml of 2% formaldehyde under the plantar aponeurosis of the right hind paw of albino rats on the 1st and 3rd day of the experiment.[12]

The drug to be tested was given daily for 10 days. The linear cross section (LCS) immediately below the ankle joint of the right hind paw was measured daily with vernier caliper. The difference in LCS on day 1 and day 10 was calculated for all groups. Percentage anti-inflammatory effect of particular drug group was calculated by:

$$\% \text{ Persentage anti-inflammatory effect} = \frac{\begin{array}{l} \text{Mean drfference in} \\ \text{LCS control group} \\ \text{-Mean difference in} \\ \text{LCS test group} \end{array}}{\begin{array}{l} \text{Mean difference in} \\ \text{LCS in control group} \end{array}} \times 100$$

Statistical Analysis

Data were analyzed using Graph pad Prism software version 5.01. Comparison between different groups was done by one-way ANOVA followed by Bonferroni posttest. The $P < 0.05$ was considered statistically significant.

RESULTS

The basal mean paw volume was comparable in all the three groups in carrageenin-induced paw edema model of acute inflammation in rats.

Table 1 shows the mean paw volume increase in all the three groups at a various time interval in carrageenin-induced paw edema model of acute inflammation in rats.

At 2 and 3 h time interval, the mean paw volume increase in aspirin and *N. sativa* treated groups were statistically significantly lower when compared to control group ($P < 0.05$). The mean paw volume increase in aspirin-treated groups was statistically significantly lower when compared to *N. sativa* treated group at 2 and 3 h interval (Table 1).

Table 2 shows the difference in paw volume at 3 h interval and percentage inhibition at 3 h.

At 3 h interval, the paw volume difference in *N. sativa* group and aspirin group was statistically significantly ($P < 0.05$) less as compared to control. Percentage inhibition of acute inflammation was greater in aspirin group than *N. sativa* group at 3 h interval (Table 2).

Mean increment in dry weight was calculated in each group. The increment in dry weight is considered as a measure of inflammation, and a decrease in this increment as compared to control suggests anti-inflammatory activity.

Table 3 shows the mean increment in dry weight and percentage inhibition. As shown in Table 3, the mean increment in the dry weight was 102.70 ± 3.073 mg in the control group, 28.83 ± 0.7923 mg in the aspirin group, and 55.25 ± 1.887 in the *N. sativa* group.

Maximum anti-inflammatory activity was seen in the aspirin group. This difference was statistically significant when compared to other groups.

The anti-inflammatory activity of *N. sativa* group was significantly more than the control group ($P < 0.001$) but less than aspirin group ($P < 0.001$).

The mean difference between the LCS just below the ankle on the 1st and 10th day was calculated for each group. Lower the difference in LCS, higher is the anti-inflammatory action.

Table 4 shows that the least difference in the mean LCS was found in the aspirin group which was statistically significantly ($P < 0.05$) lower than the control group. The difference in the LCS in *N. sativa* group was also significantly less as compared to control group ($P < 0.05$). The mean differences in LCS in aspirin group and *N. sativa* group were comparable.

The percentage anti-inflammatory effect was highest with aspirin group but was comparable with *N. sativa* group ($P > 0.05$).

DISCUSSION

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human disease. Despite the major advances in the modern medicine, the development of new drugs from natural products is still considered important.

N. sativa Linn. is small annual herb distributed throughout India with a long history of medicinal use. Some studies have been conducted to evaluate the anti-inflammatory activity of *N. sativa* and results are encouraging.[6,7,13-15] However, few studies have been conducted in subacute and chronic model of inflammation. Hence, this study was conducted to evaluate its anti-inflammatory activity in an acute, subacute and chronic model of inflammation.

In the model of acute inflammation, i.e., carrageenin-induced paw edema in rats, *N. sativa* showed anti-inflammatory activity which was statistically significant as compared to control.

Similar findings have been reported by Mutabagani and El-Mahdy[14] and Al-Ghamdi[15] wherein, volatile oil of *N. sativa* seeds and aqueous extract of *N. sativa* administered orally to rats significantly reduced the extent of carrageenin-induced foot pad edema but Hajhashemi et al.[13] reported a lack of anti-inflammatory activity of black cumin seed essential oil when administered orally.

Carrageenin-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is devoid of any systemic effect and results are highly reproducible. Carrageenin-induced edema shows a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins whereas, the second phase is known to be influenced by the lipid-derived

Table 1: Effect of different drugs on paw volume in carrageenin-induced paw edema in rats

Groups (*n*=6)	Paw volume increase (in ml)		
	At 1 h	**At 2 h**	**At 3 h**
Control (normal saline 2 ml/kg p.o.)	0.7333±0.06667	1.2670±0.09888	1.9330±0.2108
Aspirin (300 mg/kg p.o.)	0.5333±0.0615	0.2667±0.04216***##	0.3667±0.0333***##
N. sativa (10 ml/kg p.o.)	0.5667±0.04944	0.6333±0.06146***	1.1667±0.1406**

Values are mean±SEM, *n*=6 in each group, ***$P<0.001$ as compared to control, **$P<0.01$ as compared to control, ##$P<0.01$ as compared to *N. sativa*, SEM: Standard error of mean, *N. sativa*: Nigella sativa

Table 2: Effect of different drugs on paw volume in carrageenin-induced paw edema in rats

Groups (*n*=6)	Difference in volume at 3 h (in ml)	% Inhibition at 3 h
Control (normal saline 2 ml/kg p.o.)	1.933±0.2108	
Aspirin (300 mg/kg p.o.)	0.3667±0.0333***##	81.03
N. sativa (10 ml/kg p.o.)	1.1667±0.1406**	39.64

Values are mean±SEM, *n*=6 in each group, **$P<0.01$ as compared to control, ***$P<0.001$ as compared to control, ##$P<0.01$ as compared to *N. sativa,* SEM: Standard error of mean, *N. sativa*: Nigella sativa

Table 3: Effect of different drugs on dry weight of cotton pellet granuloma in rats

Group (*n*=6 in each group)	Mean increment in dry weight with SEM	% inhibition
Control (normal saline 2 ml/kg p.o.)	48.6667±1.0540	
Aspirin (300 mg/kg p.o.)	19.1667±0.7923***###	60.62
N. sativa (10 ml/kg p.o.)	28.5833±0.8983***	41.27

N. sativa: Nigella sativa, SEM: Standard error of the mean, values are mean±SEM, *n*=6 in each group, ***$P<0.001$ as compared to control, ###$P<0.001$ as compared to *N. sativa*

eicosanoids (prostaglandins, leukotrienes, etc.,) with a peak at 180 min.[16] In this study as *N. sativa* had some effect on the carrageenin-induced rat paw edema in late phase of inflammation, it can be assumed that this agent has some inhibitory effect on the peripheral mediators of acute inflammation probably related to arachidonic acid and COX cascade.

In our present investigation, *N. sativa* significantly decreased the formation of granulomatous tissue, in cotton pellet induced granuloma method as compared to control. Dry weight of granuloma projects the effect of test substances on the proliferative phase of inflammation; it was found that *N. sativa* appeared to be effective in inhibition of granuloma formation.

Similar findings were noted by Mutabagani and El-Mahdy.[14] where volatile oil of *N. sativa* seeds and its active principle thymoquinone (TQ) inhibited granuloma formation.

The cotton pellet granuloma method has been widely used to assess the transudative, exudative and a proliferative phase of subacute inflammation. The results of our study indicate that *N. sativa* may have a role in decreasing the granuloma formation and fibrosis associated with inflammation.

N. sativa showed significant anti-inflammatory activity comparable to aspirin in formaldehyde induced arthritis model of chronic inflammation. Although we did not come across any study evaluating the anti-inflammatory activity of *N. sativa* in a similar experimental model, the same has been done in arthritis induced by other methods.

Similarly, Tekeoglu[7] investigated anti-inflammatory effects of TQ on arthritis rat model and concluded that TQ suppressed adjuvant-induced arthritis in rats.

In our study, we have used aspirin as standard comparator and have found that the activity of *N. sativa* was significantly more for chronic inflammation than the control group and was comparable aspirin group. The results of our study supported by other reports from published literature suggest that *N. sativa* may play an adjuvant role which may be beneficial in treatment of diseases characterized by chronic inflammation.

The possible mechanism by which *N. sativa* exerts its anti-inflammatory action may be related to its ability to inhibit eicosanoid generation. TQ and fixed oil of *N. sativa* have been shown to be a potent inhibitor of eicosanoid generation, namely, thromboxane B and leukotrienes B4, by inhibiting both COX and LOX, respectively.[5] TQ an active component in *N. sativa* was also found to inhibit lipid peroxidation. Inflammation is associated with oxidative stress and infiltration of neutrophils and monocytes/macrophages. The release of proinflammatory cytokines from macrophages such as nitric oxide can then, in turn, cause tissue damage. Several lines of evidence suggest that *N. sativa* exhibits an inhibitory effect on nitric oxide production by macrophages,[17] validating traditional use of *N. sativa* seeds for inflammation.

The result of our study supported by other reports suggests that *N. sativa* fixed oil may play a role as an anti-inflammatory activity agent. However, further pharmacological studies are required to assess therapeutic benefits and to elucidate the exact mechanism by which the *N. sativa* inhibits inflammation.

Table 4: Effect of different drugs on LCS below the ankle joint in formaldehyde induced arthritis in rats

Group (*n*=6 in each group)	Mean day 1 LCS (mm)	Mean day 10 LCS (mm)	Mean difference in LCS (mm)	% anti-inflammatory effect
Control (normal saline 2 ml/kg p.o.)	4.117±0.09458	7.517±0.1108	3.400±0.05774	
Aspirin (300 mg/kg p.o.)	3.983±0.06009	4.667±0.1667***	0.6833±0.212***	79.90
N. sativa (10 ml/kg p.o.)	4.017±0.07032	4.717±0.08724***	1.0333±0.049441***	69.60

N. sativa: Nigella sativa, LCS: Linear cross section, values are mean±SEM, *n*=6 in each group, ***P<0.001 as compared to control, SEM: Standard error of the mean

CONCLUSION

Statistically significant activity was seen for *N. sativa* in acute inflammatory model of carrageenin-induced edema as compared to control but less than aspirin. *N. sativa* was also found to possess significant activity in chronic models like cotton pellet granuloma compared to control. In formaldehyde induced arthritis model, *N. sativa* has significant anti-inflammatory activity comparable to aspirin.

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