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Original Research Article

# A Study of Anti-inflammatory Activity of Nigella Sativa in Experimental Models of Acute & Subacute Inflammation

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**Conflict of interest: Nil**

**Abstract**

**Aims and objectives:** To evaluate antiinflammatory activity of Nigella sativa seeds in acute & sub-acute models of inflammation in rats & to compare it with control & indomethacin

**Materials & Methods**: Male wistar rats of weight 100-150 gram were used in the study. Ethanolic extract of N.sativa was used to evaluate antiinflammatory activity by carrageenan induced hind paw edema, kaolin induced paw edema & cotton pellet granuloma by oral gavage method. Institutional Ethical Committee approval was taken before start of study.

**Results:** In model of acute inflammation i.e. carrageenan induced paw edema in rats, N sativa in graded doses & indomethacin exhibited antiinflammatory activity which was statistically significant as compared to control ($p<0.05$). Anti-inflammatory activity with 500 mg/kg & 1000 mg/kg Nigella sativa were not significantly different ($p>0.05$) to those of Indomethacin at all-time intervals tested. In kaolin induced paw edema, similar results were obtained as that of carrageenan induced paw edema. In cotton pellet granuloma method, N. sativa significantly decreased the formation of granuloma tissue as compared to control. There was a significant ($P< 0.05$) decrease in weight of cotton pellet at day 14 in all the groups treated with graded doses of N. sativa and Indomethacin in comparison to control group. Weight reduction of granuloma in N. sativa 1000mg was not significantly different ($p>0.05$) from Indomethacin group.

**Conclusion:** The results suggest that N. sativa has significant anti-inflammatory activity that is comparable to that of indomethacin at 500mg/kg & 1000mg/kg.

**Keywords:** Inflammation, Nigella sativa, carrageenan, kaolin, paw edema, cotton pellet granuloma, indomethacin.

## Introduction

Inflammation is an essential immune response that enables survival during infection or injury and maintains tissue homeostasis under a variety of noxious conditions[1,3]. Acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis & resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to variety of chronic inflammatory diseases[2]. Several studies have demonstrated that inflammatory response represents the "common soil" of the multifactorial diseases, encompassing both chronic inflammatory rheumatic disorders and a wide variety of conditions including type 2 diabetes, cardiovascular and neurodegenerative diseases, obesity, cancer, asthma, and ageing[3]. Three main pathways, NF-kB, MAPK, and JAK-STAT, play major roles in inflammation, and dysregulation of one or more of these pathways may lead to inflammation – associated disease[4-6].

Presently, non-steroidal anti-inflammatory drugs (NSAIDs) & steroidal inflammatory drugs are used to suppress inflammation but long term use of these drugs in chronic inflammation is associated with various side effects & toxicities[7]. Among several medicinal plants, Nigella sativa ((Ranunculaceae) has been considered one of the most treasured nutrient-rich herb in history around the world and numerous scientific studies are in progress to validate the traditionally claimed uses of small seed of this species[8,9]. These traditional uses of *N. sativa* seeds are largely attributed to their wide array of medicinal properties, including antioxidant, anti-inflammatory, immunomodulatory, anticancer, neuroprotective, antimicrobial, antihypertensive, cardioprotective, antidiabetic, gastroprotective, and nephroprotective and hepatoprotective properties[10]. Mechanism by which N.sativa shows its anti-inflammatory action has been explored, they are reported to inhibit eicosanoids generation & lipid peroxidation[11].

### Material and method

**Study design:** Experimental study

**Sample size:** 30 Male Wistar rats (weighing 100-150gm) were obtained from CDRI (The Central Drug Research Institute) were used. The animals were housed in polycarbonate cages in a room with a 12h day – night cycle, temperature of 22º c ± 2ºc and humidity of 45%–64%. Animals were fed with a standard pellet diet (Hindustan Lever Ltd, Mumbai, India) and water ad libitum. The study was conducted after approval from Institutional Animal Ethical Committee which is, in turn , approved by Committee for the Purpose of Control and supervision of experiments on Animals ( CPCSEA), New Delhi.

**Study Place:** This experimental study was done in research laboratory of Deptt. of Pharmacology of a premier Institute of Uttar Pradesh (U.P.), INDIA.

### Drugs and chemicals

Indomethacin: Jagsonpal Pharma; Carrageenan: Loba Chemie, Kaolin : Thomas Baker

Sterilized Cotton: Doctor’s

### Plants

*Nigella sativa* seeds were procured from IITC Organic India Pvt. Ltd.-Lucknow, Uttar Pradesh, INDIA. and authenticated

by a botanist at NBRI, Lucknow. Seeds of *Nigella sativa* were thoroughly washed in distilled water to remove impurities and dried in shade. The seeds were grounded to powder with the help of mortar and pestle, 150 g of powder was soaked in 250 ml of 99% ethanol (analytical grade) in a closed container at room temperature for 7 days with periodic stirring with a sterile glass rod. After 7 days it was filtered with the help of Whatman's filter paper no.1 and the filtrate transferred in a petri dish and left in shade for 3 days to allow evaporation of ethanol. The extract so obtained was greenish black in colour and had a typical smell which was different to that of *Nigella sativa* it was then weighed in electronic weighing balance and was 50 g in weight. Equal amount of distilled water that is 50 ml had been added as vehicle. The extract was transferred in aliquots of 1ml each and was stored at $4^0$C for further use.

**Methods**

**Animals are divided into following groups:**

1. Control group: Distilled water
2. Standard group: Indomethacin group
3. Test group (N. sativa)- 3 groups of different doses

**Carrageenan-induced hind paw edema in rats[12]**

This experiment was carried out for evaluation of the inhibitory effects of anti-inflammatory drugs on the edema formation induced by carrageenan.

The animals were divided randomly in five groups of six animals each.

Group 1: Control were given Distilled water orally 30 minutes before carrageenan injection
Group 2: Test group were given *Nigella Sativa* (250mg/kg) orally 30 minutes before carrageenan injection
Group 3: Test group were given *Nigella Sativa* (500mg/kg) orally 30 minutes before carrageenan injection
Group 4: Test group were given *Nigella Sativa* (1000mg/kg) orally 30 minutes before carrageenan injection
Group 5: Standard group were given Indomethacin (15mg/kg) orally 30 minutes before carrageenan injection.
Dose of indomethacin extrapolated from human dose (50mg tds) to rats.

Carrageenan was prepared as 1% solution in sterile normal saline solution (NSS). The animal was then restrained in a plastic cage and its right hind paw was sterilized with 70% ethanol.0.05 ml of 1% carrageenan solution was injected subplantarly to the right hind paw by using a sterile syringe with 26 guage needle. The determination of paw volume was done by the mercury displacement method by using plethysmometer. The reading of each paw volume was made three times in order to get more precise result.

Results were expressed as percentage of inhibition of edema, calculated according to the formula,

**Percent Inhibition = $[(V_t–V_0)_{control} – (V_t–V_0)_{treated}]/(V_t–V_0)_{control} \times 100$.**

$V_0$—initial volume, $V_t$- volume after certain time of carrageenan injection

An increased volume of the paw was determined 0, 15, 30, 60, 120, 180, 240, 300 minutes and after carrageenan injection and compared with the normal paw volume which was determined prior to carrageenan injection.

**Kaolin-induced hind paw edema in rats[13]**

0.10 ml of 10% kaolin suspension was injected sub plantarly into the right hind paw by using a sterile syringe with 26 gauge needle. The paw volume was plethysmometrically determined prior to and at fixed time intervals of 0, 2, 4, 8, 12, 24, 48 hours after kaolin injection. The animals were divided randomly in five groups of six animals each

Group 1: Control were given Distilled water orally 30 minutes before kaolin injection.

Group 2: Test group were given *Nigella Sativa* (250mg/kg) orally 30 minutes before kaolin injection.

Group 3: Test group were given *Nigella Sativa* (500mg/kg) orally 30 minutes before kaolin injection.

Group 4: Test group were given *Nigella Sativa* (1000mg/kg) orally 30 minutes before kaolin injection.

Group 5: Positive control group were given Indomethacin (15mg/kg) orally 30 minutes before kaolin injection.

Results were expressed as percentage of inhibition of edema, calculated according to the formula,

**Percent Inhibition = $[(V_t - V_0)_{control} - (V_t - V_0)_{treated}]/(V_t - V_0)_{control} \times 100$**

Dose of indomethacin extrapolated from human dose (50mg tds) to rats.

**Cotton pellet induced granuloma formation in rats[14]:**

This model is adopted to be a measure of the ability of the drug to interfere with the proliferative components of inflammatory process. Absorbent cotton wool was cut into small pieces, weighing 20±1 mg and made up to pellets. The cotton pellets were dried and sterilized in a hot air oven at $120^0$Cfor 2 hrs. Animals were anesthetized with ether and sterile cotton pellet was implanted subcutaneously and placed in scapular region. After closing the wound with suture, the animals were allowed to recover. Indomethacin and Nigella sativa or control vehicle were administered orally in once a daily doses regimen throughout the experimental period (14 days). On the last day of experiment the implanted pellet was dissected out and carefully removed from the surrounding tissues. After drying overnight at $70^0$C, each granuloma pellets were weighed and the granuloma formation as well as granuloma inhibition of the tested agent were calculated:

$$\textbf{GF (mg/mg cotton)} = \frac{W_{14} - W_0}{W_0}$$

$$\textbf{\% G I} = \frac{\textbf{GF of control group} - \textbf{GF of tested group}}{\textbf{GF of control group}}$$

**$W_{14}$ =** mg dry weight of granuloma pellet at $14^{th}$ day
**$W_0$ =** mg dry weight of cotton pellet determined before implantation
**GF =** granuloma formation **GI =** granuloma inhibition

**Results**

After administration of carrageenan in right paw there was a progressive increase in paw volume at 0, 15, 30, 60, 120, 180, 240, 300 minutes (Table 1). The Mean value of displacement of Hg (ml) in *Nigella sativa* (250mg/kg) group at same time interval, shows that there was an increase in displacement values till 30 minutes and decrease thereafter. The values are significantly ($p < 0.05$) different from control and Indomethacin group at 180, 240, 300 minutes. (Table 1)

The Mean value of displacement of Hg (ml) in *Nigella sativa* (500mg/kg) group exhibits an increase in displacement values till 30 minutes and decrease thereafter. The values are significantly ($p < 0.05$) different from control group at 120,180, 240, 300 minutes. The Mean value of displacement of Hg (ml) in *Nigella sativa* (1000mg/kg) concludes similar results.(Table 1)

The Mean value of displacement of Hg (ml) in Indomethacin (15mg/kg) group reveals similar pattern of increase in displacements. The values are significantly ($p < 0.05$) different from control group at 30, 60,120,180, 240, 300 minutes. Results

show that Indomethacin starts its action in <30 minutes as compared to *Nigella sativa*. Values of displacement with 500 mg/kg, 1000 mg/kg Nigella sativa were similar (p>0.05) to those of Indomethacin at all-time intervals tested. (Table1)

**Table 1: Effect of Nigella sativa ethanolic extract on Carrageenan Induced Paw Edema in Rats. Values indicate displacement of Hg (ml) (Mean±SD)**

| Time (mins.) | 0 | 15 | 30 | 60 | 120 | 180 | 240 | 300 |
|---|---|---|---|---|---|---|---|---|
| Control | 1.92±0.08 | 2.13±0.16 | 2.5±0.21$^{\#}$ | 2.6±0.13$^{\#}$ | 2.85±0.22$^{\#}$ | 2.93±0.20$^{\#}$ | 2.9±0.11$^{\#}$ | 2.9±0.11$^{\#}$ |
| *N. sativa* (250 mg/Kg) | 2.03±0.15 | 2.18±0.13 | 2.48±0.27 | 2.45±0.27 | 2.43±0.29 | 2.5±0.17$^{\#,*}$ | 2.37±0.15$^{\#,*}$ | 2.37±0.15$^{\#,*}$ |
| *N. sativa* (500 mg/kg) | 1.95±0.11 | 1.93±0.09 | 2.17±0.24 | 2.2±0.23 | 2.1±0.1$^{*}$ | 2.08±0.07$^{*}$ | 2.05±0.08$^{*}$ | 2.05±0.08$^{*}$ |
| *N. sativa* (1000 mg/kg) | 2±0.15 | 2.03±0.12 | 2.12±0.17 | 2.18±0.24 | 2.07±0.11$^{*}$ | 2.08±0.09$^{*}$ | 2.08±0.09$^{*}$ | 2.08±0.09$^{*}$ |
| Indomethacin | 2±0.12 | 2.1±0.15 | 2.12±0.15$^{*}$ | 2.07±0.09$^{*}$ | 2.03±0.07$^{*}$ | 2.03±0.07$^{*}$ | 2.03±0.07$^{*}$ | 2.03±0.07$^{*}$ |
| ANOVA<br>F<br>P | <br>0.735<br>0.577 | <br>2.770<br>0.049 | <br>4.721<br>0.006 | <br>6.178<br>0.001 | <br>22.352<br>0.000 | <br>50.968<br>0.000 | <br>70.359<br>0.000 | <br>70.359<br>0.000 |

* P < 0.05 in comparison to control; # P < 0.05 in comparison to Indomethacin

After administration of kaolin in right paw, a progressive increase in paw volume has been seen at 0, 2, 4, 8, 12, 24, 48 hrs. (Table 2). The mean value of displacement of Hg (ml) in *Nigella sativa* (250mg/kg) group at same time interval shows that there was an increase in displacement values till 24 hours and decrease thereafter. The values are significantly (p < 0.05) different from control group at 48 hrs. (Table 2). The mean value of displacement of Hg (ml) in *Nigella sativa* (500mg/kg) group indicates an increase in displacement values till 8 hours and decrease thereafter. The values are significantly (p < 0.05) different from control group at 24 & 48 hrs. (Table 2)

The mean value of displacement of Hg (ml) in *Nigella sativa* (1000mg/kg) group exhibits an increase in displacement values till 2 hours and decrease thereafter. The values are significantly (p < 0.05) different from control group at 12, 24, 48 hrs. (Table 2)

The mean value of displacement of Hg (ml) in Indomethacin (15mg/kg) group were at 0, 2, 4, 8, 12, 24, 48 hrs. shows that there was an increase in displacement values till 8 hrs. and decrease thereafter. The values are significantly (p < 0.05) different from control group at 12, 24, 48 hrs. (Table 2)

Values of displacement with 500 mg/kg, 1000 mg/kg *Nigella sativa* were similar (p>0.05) to those of Indomethacin at all-time intervals tested. ((Table 2).

**Table 2: Effect of Nigella sativa ethanolic extract on Kaolin Induced Paw Edema in Rats values indicate displacement of Hg (ml) (Mean±SD)**

| Time (in hrs.) | 0 | 2 | 4 | 8 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|---|
| Control | 1.78±0.23 | 1.98±0.25 | 2.12±0.27 | 2.3±0.3 | 2.55±0.25# | 2.77±0.23# | 2.87±0.21#,* |
| *N. sativa* (250 mg/kg) | 1.88±0.24 | 2.03±0.08 | 2.2±0.33 | 2.2±0.4 | 2.27±0.37 | 2.28±0.27 | 2.27±0.27* |
| *N. sativa* (500 mg/kg) | 1.92±0.24 | 2.1±0.11 | 2.13±0.20 | 2.18±0.22 | 2.13±0.25 | 2.1±0.13* | 2.1±0.13* |
| *N. sativa* (1000 mg/kg) | 1.97±0.08 | 2.21±0.27 | 2.17±0.10 | 2.17±0.10 | 2.08±0.10* | 2.05±0.08* | 2.03±0.05* |
| Indomethacin(15 mg/kg) | 1.98±0.10 | 2.02±0.04 | 2.02±0.04 | 2.05±0.15 | 2.02±0.13* | 1.98±0.10* | 2.02±0.12* |
| ANOVA<br>F<br>P | <br>1.025<br>0.414 | <br>1.643<br>0.195 | <br>0.599<br>0.667 | <br>0.713<br>0.591 | <br>4.580<br>0.007 | <br>18.670<br>0.000 | <br>25.267<br>0.000 |

* P < 0.05 in comparison to control; # P < 0.05 in comparison to Indomethacin

Mean weight of cotton Pellet in control group was 5.13±0.63 mg at day 0 and 67.45±16.33 mg at day 14. There was a significant (P< 0.05) decrease in weight of cotton Pellet at day 14 in all the groups treated with graded doses of *Nigella Sativa* and Indomethacin in comparison to control group.(Table 3)

*Nigella Sativa* in lower doses (250mg/kg, 500mg/kg) decreased the weight of cotton pellet which was significantly different from Indomethacin group. Weight reduction in *Nigella sativa* 1000mg was similar (p>0.05) to Indomethacin group.(Table 3)

The percentage change in dry weight of granuloma was highest in control group (1257.03±462.97) followed by Nigella sativa group and lowest in Indomethacin group. The percentage change was significantly lower than control group in all Nigella sativa groups and Indomethacin group. Percentage change in dry weight of granuloma with 1000mg/kg Nigella sativa was comparable to Indomethacin group (p>0.05) (Table 3)

**Table 3: Effect of Nigella sativa ethanolic extract on Cotton Pellet Induced Granuloma Formation in Rats values indicate weight of Cotton Pellet in granuloma formation in rats (Mean±SD)**

| | Day 0 (mg) | Day14 (mg) | Percentage change (%) |
|---|---|---|---|
| Control | 5.13±0.63 | 67.45±16.33# | 1257.03±462.97# |
| *N. sativa* (250 mg/kg) | 5.02±0.13 | 23.8±4.51*,# | 376.02±99.65*,# |
| *N. sativa* (500 mg/kg) | 5.4±0.65 | 17.21±3.8*# | 223.57±85.78*,# |
| *N. sativa* (1000 mg/kg) | 4.92±0.18 | 9.87±1.45* | 100.86±30.85* |
| Indomethacin (15 mg/kg) | 5±0.56 | 9.82±1.33* | 96.91±24.51* |
| ANOVA<br>F<br>P | <br>0.901<br>0.479 | <br>57.022<br>0.000 | <br>30.458<br>0.000 |

* P < 0.05 in comparison to control; # P < 0.05 in comparison to Indomethacin

## Discussion

After administration of 0.05 ml of 1% Carrageenan a significant increase in paw volume was seen in control group. After

administration of 0.10 ml of 10% Kaolin a significant increase in paw volume was seen in control group.

Administration of *Nigella sativa* ethanolic extract (250 mg/kg, 500 mg/kg, 1000 mg/kg) significantly ameliorated the increase in paw volume by carrageenan in dose dependent manner. These ameliorated values of paw volume were significantly ($P < 0.05$) different from control group. Our results are in conformity with those of Thabrew M et al., 2002[15] who reported that *Nigella sativa* ethanolic extract corrected changes in paw volume caused by injection of carrageenan in rat paw. Several studies have reported anti-inflammatory effect of different extract and ethanolic extract of *Nigella sativa* in carrageenan induced paw edema model. Pise HN & Padwal SL, 2017[16], also reported decrease in carageenan induced paw edema in rats by aqueous extract of N. sativa.

Ethanolic extract of N. sativa decreased kaolin induced rat paw edema in all doses as compared to control & its effects at 500mg/kg & at 1000mg/kg were similar to that of indomethacin. The results are in line with Kamal A, 2010[17].

We also tested anti-inflammatory activity in sub-acute inflammation by observing the reduction of weight of cotton pellet granuloma. The results showed significant decrease in weight of cotton pellet granuloma by N. sativa as compared to control. Reduction in granuloma formation was not statistically significantly different from that of indomethacin, standard anti-inflammatory drug. Our results are in accordance with Mutabagani and El-Mahdy, 1997[18] who concluded that the volatile oil of N. sativa and thymoquinone injected i.p. exhibited a dose-dependent anti-inflammatory effect against carrageenan-induced rat hind paw edema and cotton seed pellet granuloma comparable to the reference drug indomethacin (3 mg/kg, i. p.). Pise HN and Padwal SL, 2017[16] also inferred similar results. The proposed mechanism by which N. sativa exhibits its anti-inflammatory activity is inhibition of eicosanoid generation & lipid peroxidation. TQ & fixed oil present acts as principle constituents. Inflammation is associated with recruitment of neutrophils, monocytes &/or macrophages. Macrophages release nitric oxide, proinflammatory cytokine, which may cause tissue damage. There are few studies which confirm inhibitory role of N. sativa on nitric oxide production by macrophages[19].

In recent years, ethnobotanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. They obviously deserve scrutiny more vigorously such as phytochemical investigation, biological evaluation on experimental animal models, toxicity studies and investigation of molecular mechanism of actions of isolated phytoconstituents

## Conclusion:

N. sativa (in all doses) possesses significant anti-inflammatory activity in carrageenan induced hind paw edema & kaolin induced paw edema, acute models of inflammation as compared to control. At high dose i.e., 1000mg/kg, its anti-inflammatory activity is comparable to that of indomethacin. Similarly, N. sativa exhibited anti-inflammatory activity in sub-acute model of inflammation i.e. cotton pellet granuloma formation significantly as compared to control & at dose of 1000mg/kg its anti-inflammatory activity is comparable to that of indomethacin.

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