

ANTI-INFLAMMATORY ACTIVITY OF *NIGELLA SATIVA* OIL IN RATS

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SUMMARY

Nigella sativa (*N. sativa*), commonly known as black seed, has been a well known herb since ancient times with a wide range of healing properties. The aim of this study was to investigate anti-inflammatory activity of *N. sativa* seed oil at three dosages on carrageenan-induced paw oedema, total white blood cell (TWBC) count and plasma protein in rats. Acute inflammation was induced by subplantar injection of carrageenan (0.1 ml, 1 % w/v) into the rat hind paw. 500 mg/kg, 1000 mg/kg and 1500 mg/kg of *N. sativa* oil were administered orally. Paw oedema, total white blood cell count and plasma protein were assessed. *N. sativa* seed oil exerted significant inhibition of paw oedema at the dosage of 1500 mg/kg at second hour and plasma protein at a dosage of 1000 mg/kg at third hour ($p < 0.05$). No significant inhibition of TWBC count was exerted by *N. sativa* seed oil at third hour after treatment at dosages used in this study. There was also dose-dependent correlation of *N. sativa* seed oil on inhibition of paw oedema. These results support the traditional use of *N. sativa* seed oil for the treatment of inflammatory diseases.

Keywords : anti-inflammatory, *Nigella sativa*, rats

INTRODUCTION

Nigella sativa is an annual herbaceous plant that belongs to the Ranunculaceae family. *N. sativa*, commonly known as black cumin, love in the mist, fitch, and fithches, is a beautiful Middle Eastern herb with many medicinal uses. The seeds of this plant are called black seeds or black cumin. It is a native plant that is widely distributed in Egypt and other parts of the world (Jansen, 1981).

Mature seeds are usually consumed for edible and medical purposes. It has been commonly used as a natural food additive and as a prevention and cure for many ailments in the Middle East and South East Asia for over 2000 years. In Egyptian folk medicine, the seeds are used as carminatives, diuretics, and for delayed menses and lactation, while their oil has protective action against cough and asthma (Soliman, 1978).

The Prophet Muhammad (Peace Be Upon Him) said in his divine wisdom about the black seeds, "Hold onto the use of the black seed for it has a remedy for every illness except death" (reference to the hadith - Sahih Bukhari).

Black seed is a complex substance of more than 100 compounds, some of which have not been identified or studied. The specific seed constituents that have been identified and investigated include fixed oils, saponins, volatile or essential oils, alkaloids and amino acids. It also has traces of calcium, iron, sodium, potassium and crude fiber. The fixed oils constitute 35% of the seed with an unsaturated fatty acid constituent of 73.5% of the fatty acid composition in the seed oil (Atta, 2003). Amino acid analysis of the seed's protein hydrolysate showed the

presence of 15 amino acids, including all nine essential amino acids (Al-Gaby, 1998).

N. sativa has been reported to exhibit many pharmacological effects including anticestode and antinematode (Mahmoud *et al.*, 2002), antifungal (Khan *et al.*, 2003), anti-inflammatory (Al-Ghamdi, 2001), antihistamine activity (Chakravarty, 1993), antitumor (Kumara and Huat, 2001), lactagogue (Agarwal *et al.*, 1979), antidiabetic (Al-Hader *et al.*, 1993), antiulcerogenic (Akhtar *et al.*, 1996), diuretic (Zaoui *et al.*, 2000) and treatment of dyslipidemia and hyperglycaemia (Zaoui *et al.*, 2002).

The present study was therefore undertaken to investigate further the anti-inflammatory effect of *Nigella sativa* oil at different dosages on carrageenan-induced paw oedema, total white blood cell count and plasma protein in rats.

MATERIALS AND METHODS

Animals

Thirty female Sprague-Dawley rats, weighing 150-250 g were used in this study. All animals were kept under standard conditions. They were fed with standard pellets and water *ad libitum*. Experiments were performed between 08:00am. and 3:00pm.

Test materials

Baraka® 450 mg soft gelatin capsule produced by Pharco Pharmaceutical, Alexandria, Egypt was used in this study. Each capsule contains 0.45 ml of *N. sativa* L.

seed oil (El-Baraka seed oil) which contains various constituents including nigellone, fatty acids, glycosides, phenolic components, carotene, minerals, phosphorus and iron and some digestive enzymes.

Ketofen® 5 mg produced by Merial was used in this study as a positive control drug. Each tablet contained 5 mg of ketoprofen.

Lambda-carrageenan® manufactured by Sigma-Aldrich was used to induce acute inflammation of rat paw.

Experiment protocol

Animals were divided into five groups with six rats in each group. The five-animal groups received 500, 1000, 1500 mg/kg of *N. sativa* oil, 1 mg/kg of ketoprofen (positive control) and no treatment (negative control), respectively. All treatments were administered orally.

A volume of 0.1 ml of 1% sterile λ -carrageenan was injected into the subplantar of the right hind paw of the rats, an hour after the drug treatment, while the contralateral paw was injected with 0.1 ml of sterile normal saline.

The paw volume was measured before carrageenan injection and every hour subsequently for a period of 4 hours using water displacement technique (Al-Ghamdi, 2001). A line was marked around the hock joint of both hind limbs before the experiment. The increase in paw volume was measured by dipping the paw until the hock joint in a calibrated syringe filled with water. The displaced water volume was recorded at 0.01 ml accuracy.

Blood was collected via intracardiac puncture by using a 26G needle with the animal under chloroform anaesthesia. One ml of blood was withdrawn each time and collected into EDTA tubes. Blood sampling was done just before and 3 h after carrageenan injection. Blood samples in the EDTA tubes were stored at 4°C and analysed within 24 h of collection. The rats were euthanised after the fourth hour when all necessary procedures had been performed.

Analytical procedures

Carrageenan-induced oedema was measured as follows. The paw volume is the difference between right and left paw volume. Paw oedema volume is the difference of paw volume after and before treatment with carrageenan. Percentage of inhibition was calculated using the following formula:

$$\text{Percentage of inhibition} = 100 \left(1 - \frac{a-x}{b-y} \right)$$

where a is the mean paw volume of treated rats after carrageenan injection

x is the mean paw volume of treated rats before carrageenan injection

b is the mean paw volume of control rats after carrageenan injection

y is the mean paw volume of control rats before carrageenan injection

Total white blood cells were counted manually by using a haemocytometer. Plasma was separated from blood by using microhaematocrit centrifuge. Plasma protein level was determined by using a refractometer.

Statistical analysis

Data were expressed as mean and standard error mean (S.E.M.). Pearson correlation coefficient was used to evaluate correlation between different dosages of *N. sativa* oil on paw oedema. One-way ANOVA test followed by Dunnett multiple comparison tests were used to determine significant differences between different treatment groups for paw oedema. Significant differences between, before and after treatment for total white blood cell count and plasma protein were tested by paired t -test. P -values less than 0.05 ($p < 0.05$) were considered as indicative of significance.

RESULTS

The subplantar injection in the right hind paw with carrageenan induced a progressive oedema in the rats without any treatment (negative control group). Animals treated with *Nigella sativa* oil and ketoprofen showed reduction in paw oedema compared with animals without any treatment (Table 1). The anti-inflammatory effect of *N. sativa* oil was dose-dependent and similar to that of ketoprofen (Table 1). Animals treated with ketoprofen showed significant inhibition of the paw oedema from 2 to 4 h after carrageenan injection ($p < 0.05$), compared to animals without any treatment.

However, *N. sativa* oil only produced significant inhibition ($p < 0.05$) at a higher dosage of 1500 mg/kg at 2 h with an inhibition effect of 58.4%. Correlation between the paw oedema volume and the corresponding *N. sativa* dosage was found to be significant at first hour ($r = -0.470$, $p < 0.05$), highly significant at second hour ($r = -0.545$, $P < 0.01$) and significant at third hour ($r = -0.428$, $p < 0.05$).

Total white blood cell (TWBC) count increased significantly in negative control group ($p < 0.05$) as shown in Table 2. Animals treated with ketoprofen at a dose rate of 1 mg/kg showed a significant reduction in TWBC count three hours after treatment ($p < 0.05$). However, there was no significant difference in TWBC counts in animals treated with *N. sativa* 3 h after and before treatments.

The negative control group showed a significant increase in the plasma protein 3 h after carrageenan injection ($p < 0.05$). Positive control group and *Nigella sativa* oil at a dose rate of 1000 mg/kg showed a significant

Table 1: Effects of *N.sativa* oil (NSO) on carrageenan-induced paw oedema in rats

| Treatment | 1 h ⁺ | | 2 h ⁺⁺ | | 3 h ⁺ | | 4 h | |
|--------------------|------------------------|--------------|------------------------|--------------|------------------------|--------------|------------------------|--------------|
| | Paw oedema volume (ml) | % Inhibition | Paw oedema volume (ml) | % Inhibition | Paw oedema volume (ml) | % Inhibition | Paw oedema volume (ml) | % Inhibition |
| NSO 500 mg/kg | 0.29 ± 0.26 | 26.6 | 0.47 ± 0.18 | 32.8 | 0.59 ± 0.43 | 30.9 | 0.78 ± 0.28 | 17.1 |
| NSO 1000 mg/kg | 0.10 ± 0.05 | 41.6 | 0.29 ± 0.20 | 47.7 | 0.42 ± 0.09 | 38.0 | 0.63 ± 0.12 | 20.6 |
| NSO 1500 mg/kg | 0.06 ± 0.01 | 76.6 | 0.21 ± 0.05* | 58.4 | 0.37 ± 0.10 | 48.4 | 0.63 ± 0.43 | 22.8 |
| Ketoprofen 1 mg/kg | 0.18 ± 0.10 | 30.5 | 0.24 ± 0.08* | 51.0 | 0.21 ± 0.09* | 73.6 | 0.20 ± 0.01* | 76.0 |
| (Positive control) | | | | | | | | |
| No treatment | 0.26 ± 0.08 | — | 0.55 ± 0.25 | — | 0.71 ± 0.17 | — | 0.82 ± 0.27 | — |
| (Negative control) | | | | | | | | |

Values represent the mean ± S.E.M. of 6 animals for each group.

* p < 0.05 compared with -ve control group

+ Correlation is significant at the 0.05 level.

++ Correlation is significant at the 0.01 level.

Table 2: Effects of *N. sativa* oil (NSO) on total white blood cell count and plasma protein in rats

| Treatment | Total white blood cell count (x 10 ⁹ /l) | | Plasma protein (g/l) | |
|---------------------------------------|---|---------------|----------------------|-------------|
| | 0 h | 3 h | 0 h | 3 h |
| NSO 500 mg/kg | 7.17 ± 2.16 | 10.28 ± 3.51 | 76.2 ± 3.4 | 78.3 ± 4.1 |
| NSO 1000 mg/kg | 7.5 ± 1.56 | 9.78 ± 3.34 | 75.7 ± 2.7 | 70.3 ± 3.7* |
| NSO 1500 mg/kg | 9.57 ± 3.56 | 10.78 ± 5.24 | 68.8 ± 6.8 | 62.3 ± 5.4 |
| Ketoprofen 1 mg/kg (positive control) | 9.59 ± 1.25 | 6.29 ± 1.59* | 72.8 ± 4.3 | 65.8 ± 4.2* |
| No treatment (negative control) | 11.18 ± 4.28 | 16.10 ± 5.94* | 57.7 ± 4.7 | 62.0 ± 4.7* |

* p < 0.05 compared with before treatment.

Values represent the mean ± S.E.M. of 6 animals for each group.

p < 0.05 reduction in the plasma protein. However, there was no significant reduction of plasma protein after 3 h of carrageenan injection following administration of the *Nigella sativa* oil at dose rates of 500 mg/kg or 1500 mg/kg. This could probably be due to the high standard error mean (S.E.M.) in plasma protein values before and after treatment. This was mainly contributed by data from two animals which gave high level of plasma protein values before and after treatment.

DISCUSSION

The results showed that *N. sativa* has anti-inflammatory action comparable to that of 1 mg/kg of ketoprofen. This study showed that animals treated with 1500 mg/kg of *N. sativa* oil exhibited significant inhibition of paw oedema. Al-Ghamdi (2001) reported significant inhibition at 500 mg/kg of *N. sativa* seeds crude suspension. The variation of the result may be due to the volume of carrageenan of the same concentration used in both the studies. In the present study, 0.1 ml of carrageenan was used and half of the volume was used to induce acute inflammation in the latter study.

According to Di Rosa *et al.* (1971), histamine and serotonin were mainly released during first 1.5 h after

carrageenan injection. Kinin was released from 1.5 to 2.5 h, followed by prostaglandins until 5 h after carrageenan injection. From the results obtained in this work, oral administration of 1500 mg/kg of *N. sativa* oil produced significant inhibition of the release of kinin. However, this study was unable to establish significant inhibition of the release of histamine, serotonin and prostaglandins in a model of acute inflammation.

Haq *et al.* (1995) in their study of the effect on human lymphocytes by *N. sativa* indicated that *N. sativa* oil activates T-lymphocytes to secrete interleukin-3 (IL-3) and interleukin-1_γ (IL-1_γ). IL-3 is derived from activated T cells: T-helper 1 and T-helper 2, eosinophils and mast cells, while, IL-1_γ is produced mainly by macrophages. Both IL-3 and IL-1_γ are important examples of chemotactic agents that stimulate chemotaxis of leucocytes (Macfarlane *et al.*, 2000). Activation of T-lymphocytes to secrete both IL-3 and IL-1_γ by *N. sativa* oil may contribute to the increase in total white blood cell count shown in the present study.

Chakravarty (1993) reported that nigellone in relatively low concentrations is very active in inhibiting histamine release from rat peritoneal cells by different secretagogues. The researcher suggested that *N. sativa* directly inhibits mast cells through the protein kinase C.

El-Dakhakhny *et al.* (2000) also reported a significant decrease in gastric mucosal histamine in rats treated with 880 mg/kg *N. sativa* oil daily for 2 weeks via oral route. No significant inhibition of paw oedema one hour after carrageenan injection suggested that *N. sativa* at dosages used in this study have little ability to inhibit relatively higher amounts of histamine released from connective tissue mast cells.

There was no significant inhibition and correlation of paw oedema between different dosages of *N. sativa* 4 h after carrageenan injection. This finding supports the postulate suggested by Al-Ghamdi (2001) that *N. sativa* oil may not inhibit the synthesis of prostaglandins. *N. sativa* did not exhibit antipyretic effects in that study, as prostaglandins cause a rise in body temperature during inflammation. Indeed, increased secretion of both IL-3 and IL-1_α are important in eliciting the pain of inflammation and causing the release of prostaglandins (Tizard, 1996).

Inflammation stimulates the synthesis of certain globulins (α₁-globulins and α₂-globulins) by hepatocytes and perhaps immunoglobulins (Ig) by B-lymphocytes. Several cytokines, especially interleukin-6 (IL-6), alter protein synthesis in or protein release from hepatocytes (Stockham and Scott, 2002). Inhibition of plasma protein in this study suggests that there is inhibition of some cytokines or protein from hepatocytes by *N. sativa* seed oil in a model of acute inflammation.

N. sativa showed an anti-oxidant activity by suppressing the chemiluminescence (Haq *et al.*, 1995). The authors also observed no significant difference in killing rates, phagocytic rate and phagocytic activity of polymorphonuclear cells (PMNs) without *N. sativa* or with different concentrations of *N. sativa*, indicating that *N. sativa* did not exert bactericidal activity. In line with this, our study also found no significant effect of different concentrations of *N. sativa* on total white blood cell counts. The suppression of chemiluminescence but not bactericidal activity would suggest that *N. sativa* inhibits PMN myeloperoxidase activity.

No reduction in total white blood cell count in this study indicated no inhibition in the migration of the leucocytes to affected site at treatment dosages. This may be attributed to no inhibition of some chemical mediators that cause emigration of leucocytes (Tizard, 1996).

These results support the traditional use of *N. sativa* seed oil for the treatment of inflammatory diseases. *N. sativa* seed oil exerts its pharmacological activity on acute inflammation induced by 0.1 ml of 1% carrageenan in rat paw by causing a significant reduction in paw oedema volume at a dose rate of 1500 mg/kg at 2 h and the plasma protein at dose rate of 1000 mg/kg at 3 h. However, *N. sativa* seed oil did not cause any reduction in total white blood cell count at dosages used in this investigation.

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