ORIGINAL ARTICLE

The Effect of *Nigella sativa Linn*. Ground Seed Extract on Cotton Pellet Induced Inflammation in Rats

Saima Parveen[1], Zinnat Ara Begum[2]

Abstract:

The study was done to find out the anti-inflammatory effects of the ethanolic extract of ground seeds of Nigella sativa in inflammed rats. The effect was compared with reference standard drugs aspirin and hydrocortisone. Chronic inflammation was induced by implantation of a sterile cotton pellet in rat's groin region. Treatment with Nigella sativa extract at a dose of 250 mg/kg body weight and at a dose of 500 mg/kg body weight orally daily for 14 days produced anti-inflammatory effect. The percentage of inhibition of granuloma formation were 19.30% and 41.42% respectively. 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.

Introduction:

Inflammation is the result of our body's response to an injury or an infection, in other words, it is a defense mechanism. Unfortunately, this protective response of our immune system can result in damage to our body's organs [1].

Globally, increase in the average lifespan has increased the incidence of communicable and non-communicable diseases. Chronic and degenerative diseases such as cardiovascular diseases, cancer, rheumatic diseases are the most common and significant causes of disability and mortality and carry a high socio-economic cost. Rheumatoid disease is one of the commonest chronic inflammatory conditions and affect people of both sexes, all ethnic groups and all ages.

Rheumatoid arthritis has a world-wide distribution and it affects 0.5-3% of the population and it leads to chronic inflammation, granuloma formation and joint destruction. Diseases of inflammation and immunity can occur when the normal inflammatory response progresses to chronic inflammation either because of long term inappropriate response to a stimulus or because the offending agent is not removed for example, chronic infection, transplantation and autoimmunity[2]. To relieve those conditions various steroidal and non-steroidal drugs are used. Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation. Hence, search for new anti-inflammatory agents that can retain therapeutic efficacy and yet are devoid of

1. Lecturer, Department of Pharmacology and Therapeutics, Holy Family Red Crescent Medical College, Dhaka.
2. Professor of Teaching Methodology, Centre for Medical Education, Dhaka.

these adverse effects is justified. There is much hope of finding active anti-rheumatic compounds from indigenous plants as they are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs. Use of herbal medicine throughout the world is increasing. Plants still remain the primary source of supply of many important drugs used in modern medicine. Therefore, studies are still on in search of more potent, less toxic, cheaper and easily available anti-inflammatory agents. To treat these inflammatory conditions medications are used, but medications can have side effects. However, herbal medications usually are devoid of such problems. For this reason the present study was undertaken to evaluate the anti-inflammatory activity of *Nigella sativa* in experimentally induced inflammation in rats. *Nigella sativa Linn* (Family: Ranuculaceae) is a common spice of South East Asia especially in Bangladesh and it is locally called Kalajira. The plant enjoys vast folklore uses as traditional medicine. The *Nigella sativa* that is kalajira has a healing role for all diseases except death[3]. It has been in use in India and many Middle Eastern communities as natural remedy for many acute and chronic conditions for two thousand years. Traditional use of kalajira as a poultice of ground seeds for inflammatory ailments such as rheumatism, headache and certain skin conditions is supported by modern studies. The seeds are used for anti-inflammatory actions. Thymoquinone, an active constituent of kalajira, is a potent inhibitor of thromboxane B_2 and leukotriene B_2 through inhibition of cyclooxygenase and lipoxygenase respectively[4]. Proper scientific investigation of the properties of ground seed may substitute the presently available anti-inflammatory agents and thereby establish its position in modern medicine as more safe, efficacious and cost-effective drug. The present study was aimed at exploring the possible anti-inflammatory properties of *Nigella sativa Linn*. Therefore, it was designed to evaluate the anti-inflammatory activity of *Nigella sativa* in experimentally induced inflammation. As our knowledge goes, no other work has been done on the chronic anti-inflammatory effect of *Nigella sativa* or kalajira in this country. Considering its medicinal value and availability in this country the study was undertaken on rat models. In this study, anti-inflammatory effects of *Nigella sativa* were compared with both steroidal and non-steroidal anti-inflammatory agents.

The objective of study was to induce chronic inflammation by subcutaneous implantation of cotton pellet, to use two doses of ethanol extract of ground seed of *Nigella sativa* in carrageenin and cotton pellet induced inflammation in rats, to measure dry weight of granuloma, and 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 the Department of Botany, University of Dhaka. After collection, crude ethanol extract was prepared and kept in 4^0C in refrigerator. Long Evans Norweigian rats, collected from Bangabandhu Sheikh Mujib Medical University (3-4 months old, 200-250 gm of weight), had free access to food and water *ad libitum*. Chronic

inflammation was induced by cotton pellet. The anti-inflammatory effect was compared with reference standard drugs aspirin and hydrocortisone.

The rats were divided into five group of six rats each, 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 are anaesthetized with ether[5] and 50 mg of the sterile cotton pellet was inserted one in each axilla and groin of rats by making small subcutaneous incision.

The incisions were sutured by sterile catgut. Group-I received 0.6ml normal saline administered orally for 14 days and served as control. Group-II received ethanol extract of *Nigella sativa* 250 mg/kg body weight administered orally for 14 days. Group-III received ethanol extract of *Nigella sativa* 500 mg/kg body weight administered orally for 14 days. Group-IV received aspirin 100 mg/kg body weight administered orally for 14 days. Group-V received hydrocortisone 2 mg/kg body weight administered subcutaneously for 14 days. The animals were sacrificed by excess anaesthesia on the 14th day and cotton pellets were removed surgically. Pellets were separated from extraneous tissue and dried at 60^{0}C until the weight became 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 was determined and statistically evaluated. The percentage inhibition increases in the weight of the cotton pellet was calculated.

Results:

At the end of the 14 days the pellets were removed from the site of insertion sacrificing the animals[6]. The final weights of the cotton pellets were determined. The mean weights were 207.83±0.69 mg, 177.63±5.31 mg, 142.45±5.58 mg, 164.16±15.86 mg, 146.93±7.12 mg for groups - I, II, III, IV and V respectively. The increment in the weight of cotton pellet in ethanol extract of *N. sativa* 250 mg/kg body weight, ethanol extract of *N.*

Table I: Effects of extracts of *Nigella sativa*, aspirin and hydrocortisone on cotton pellet induced granuloma in rat.

Groups	Initial weight of cotton pellet (mg) (mean±se)	Final weight of cotton pellet (mg) (mean±se)	Increase of weight of cotton pellet (mg) (mean±se)	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 in a test of significance difference from control.
** P<0.001 in a test of significance difference from control

sativa 500 mg/kg body weight, aspirin and hydrocortisone treated groups were 127.63±5.31, 92.45±5.58, 114.16±15.86, and 96.93±7.12 mg respectively. The increment the pellet for the control group on the other hand 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 groups II, III, IV and V respectively. In this chronic study an anti-inflammatory effect was observed at 500 mg/kg body of the ethanolic extract of *N. sativa.*

Discussion:

Treatment with *Nigella sativa* extract at doses of 250 mg/kg body weight orally daily for 14 days produced significant anti-inflammatory effect and at a doses of 500 mg/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 happened in a dose dependent manner. Administration of aspirin and hydrocortisone for 14 days also showed 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 develop during the period of several days. This model demonstrates 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 indicated that the proliferative phase was effectively suppressed by the ethanol extract of *Nigella sativa.*

The anti-inflammatory effect produced by *Nigella sativa* in response to cotton pellet induced granuloma formation is most likely to be mediated through prostaglandins (PGs). This is supported by the fact that aspirin, an inhibitor of PG synthesis [7] produced similar results. Furthermore, *Nigella sativa* produced anti-inflammatory effect comparable to hydrocortisone indicating that it may also act by stabilizing the lysosomal membrane.

Nigella sativa reduces the vascular component of inflammation and impairs 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 reticuloendothelial cells and polymorphonuclear leucocytes resulting a reduction in the cellular exudates [7].

In this study, crude ethanol extracts of ground seed of *Nigella sativa,* and steroidal and non-steroidal anti-inflammatory drugs reduced the weight of granulation tissue. The reduction was statistically significant in comparison to control group and it was more with higher doses of seed extracts.

Further investigations are warranted to reconfirm and identify the anti-inflammatory active principles and elucidate their mechanism of action. Toxicological studies should also be undertaken before any clinical use.

The study was basically a 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 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 conditions. 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 the 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.

References

1. Wilhelm DL. Inflammation and healing. In: Anderson WAD (editor). Pathology, Sixth edition. St. Louis: The C.V. Mosby Company, 1971. pp-14-55.
2. Doherty M, Lanyon, Ralston SH. Musculoskeletal disorders. In: Nicholan AB, Nicki RC, Brian RW, John AAH (editors). Davidson's Principles and Practice of Medicine, Twentieth edition. Edinburgh: Churchill Livingstone, 2006. pp-1066-1142.
3. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother 2003; 17: 299-305.
4. El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5–lipoxygenase products in polymorphonuclear leukocytes in rats. J Ethnopharmacol 2002; 81: 161-164.
5. Chakravarty A, Devi RKB, Rita S, Sharatchandra Kh, Singh ThI. Studies on anti-inflammatory and analgesic activities of aqueous extract of Spilanthes acmella (SPA) in experimental animal models. Indian Journal of Pharmacology 2004; 36: 148-150.
6. Ahmed E, Chowdhury SAR, Khaleque A. Study of the anti-inflammatory effect of garlic in mice. Bangladesh J Physiol Pharmacol 1987; 1: 5-7.
7. Singh GB, Bani S, Singh S, Kaul A. Anti-inflammatory properties of 3-methylpyrazolin-5-(4H)-one-4-[3'-Methoxy-4' (2", 6"- tetra-O-acetyl-β-D-glycopyranosy] benzylidene (compound IIA). Indian Journal of Experimental Biology 1994; 32: 544-547.