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Study of Anti-Inflammatory Effects of Black Cumin (Nigella Sativa) and Honey on Mice (Mus Musculus)

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Abstract

The study was conducted to determine the effect of *Nigella sativa* (Kalonji) and Honey asan anti-inflammatory agent for humans and animals. The study was carried out on 20 Albino Mice of almost equal size and weight. All the mice were given 5% solution of formalin in a dose of 0.5ml injection in their right hind paw to produce artificial inflammation. The mice were divided into four groups of five animals in each and were randomly allotted to four treatments as Group A (Control) where no *Nigella sativa* extract and honey were given, Group B where the mice were given only the ethanolic extract of *Nigella sativa* in the dose of 0.05ml injection as a remedy of inflammation, Group C where the mice were given only the honey orally in a dose of 0.05mg and Group D where mice were given 50% (0.025ml*)* intraperitoneally *of Nigella sativa* extract and 50% (0.75mg) of honey as an anti-inflammatory agents. The data was statistically analyzed by the Analysis of Variance (ANOVA) and the results showed that the inflammation was significantly ($p<0.05$) reduced in mice given treatments compared to untreated control groupand among treated groups. The mice given the extract of *Nigella sativa* (Group B) showed better results ($p<0.05$) in reducing the inflammation compared to other groups (C and D), Group D where the mice were given 50% (0.025ml) *Nigella sativa* extract and 50% (0.75mg) honey showed better results ($p<0.05$) than mice given only honey. Overall, both the extract of *Nigella sativa* and the honey were almost equally successful in reducing the inflammation in mice which showed that these two agents can successfully be used as anti-inflammatory drugs in humans andanimals.

Introduction

Inflammation is a basic physiological mechanism that aids the body in protecting itself from harmful stimuli. It is a defensive mechanism whose primary purpose is to assist theorganism to get rid of both the original harm and its aftereffects. However, occasionally it might lead to inflammatory illnesses if left unchecked. Today, a variety of therapeutic herbs, including *Nigella sativa*, are utilized to treat these conditions due to their anti-inflammatory qualities. These days, a variety of painkillers and anti-inflammatory medications are also utilized, in spite of adverse effects. Therefore, using a naturally occurring plant (*Nigella sativa*) to treat inflammation has several advantages. (10) Inflammation is treated with both

steroidal anti-inflammatory medications (SAIs) andnonsteroidal anti-inflammatory drugs (NSAIDS). Although steroids perform a clear role in the treatment of inflammatory disorders, but prolonged use of these medications is linked to substantial side effects due to their toxicity. Nonsteroidal anti-inflammatory medication usage foran extended period is also linked to negative side effects.(15).

Black cumin(Nigella sativa):

Nigella Sativa, often known as "Black Cumin" or "Kalonji," is a dicotyledonous plant that grows in a variety of places, including Pakistan. India, Iran, and Pakistan. It has so many therapeutic qualities, as it is used to treat a variety of diseases such as skin conditions, diarrhea, obesity, anxiety, hypertension, diabetes, inflammation, and cough. (3) The composition of *N. sativa* seeds varies, but they have moisture, oils, proteins, carbs, vitamins, and minerals. The fixed and essential oil in black cumin seed is the source of many health benefits associated to *N. sativa*. Both saturated and unsaturated fatty acids are present in fixed oil in small but significant levels for their action. Dihomo-linoleic acid, which is found in the fixed oil of seeds, is a potent antioxidant. (2) Black cumin (*Nigella sativa*), a highly nutraceutical plant with numerous health benefits, is gaining interest from the scientific community, the medical community, and pharmaceutical firms. Black cumin's primary bioactive ingredient, thymoquinone (TQ), has shown the pleiotropic pharmacological actions that can improve immunity, cell survival, and energy metabolism. TQ also reduces oxidative stress and inflammation. (8) In addition to being utilized in food processing, seeds are also used in linen and wool clothing as insect repellents, and essential oils are used in a variety of cosmetics. (9)

Thymoquinone and other bioactive chemicals discovered in Nigella sativa have been designated as valuable therapeutic herbs, and there is strong evidence in the literature to support this claim. *Nigella sativa* has received recognition as a remarkable medicinal plant with regard to pathogenic microorganisms, such as human-infected harmful viral species. Recent animal studies have revealed that *Nigella sativa* seed extract is effective against a number of viruses, including Newcastle disease virus (NDV), Peste des Petits Ruminants (PPR) virus, Papaya ringspot virus, and avian influenza (H9N2). (9) According to reports, *Nigella sativa* seeds significantly reduce symptoms of amenorrhea, internal haemorrhage, paralysis, bronchitis, cough, pain, fever, influenza, and dermatitis. (7). Protection from illness-induced nephrotoxicity and hepatotoxicity is one of the known pharmacological activities of the crude extracts of the *Nigella sativa* seeds. Anti-inflammatory, analgesic, antipyretic, antibacterial, and anti-cancer properties are present in the seeds and oil of this plant. (1) The defense against liver and kidney damage brought on by disease or toxins is one of the pharmacological effects of the crude *Nigella sativa* seed extracts. Anti-inflammatory, analgesic, antipyretic, antibacterial, and anti-cancer properties are also present in the seeds and oil of *Nigella sativa*. (1)

Due to the anti-inflammatory and antioxidant properties of flavonoids and phenolic acids found in honey, honey may have a significant impact on human health. There seems to be some variation in how honey affects immunity and wound healing. Honey has been shown to block the effects of pro-inflammatory substances including TNF- (Tumor Necrosis Factor) and IL-6 in cancer patients (Interleukin). (13) Depending on a number of circumstances, honey includes macro-and micronutrients. Bee species, floral supply, and environmental and processing variables round out the list. In addition to sugar, protein, enzymes, minerals, vitamins, amino acids, and a variety of polyphenols, honey contains 200 distinct compounds. Each honey has a unique colour, flavour, viscosity, and medicinal properties due to the various ratio of these chemicals. (17) The content, sensory perception, and appearance of

honey vary according to the plant from which it is collected. The main nutritional and health-relevant components are carbohydrates, predominantly fructose and glucose but also over 25 different oligosaccharides. (5) A naturally occurring food that is ready to eat and high in flavonoids is recognised for its ability to heal wounds by acting as both an antioxidant and an antibacterial. Studies on the anti-inflammatory properties of honeys might now be supported by the fact that flavonoids reduce inflammatory processes. (19)

Honey has traditionally been used to cure a variety of illnesses, including eczema, piles, gastrointestinal disorders, respiratory disease, sore throats, TB, thirst, coughing, weariness, and hepatitis. It is additionally utilised as a dietary supplement. The use of honey in the treatment and prevention of injuries, diabetic, cancers, breathing problems, cardiovascular, neurologic, and gastrointestinal disorders is supported by a wealth of scientific data. (18) Apart from that honey being consumed and used to treat injuries, Clinical illnesses ranging from gastrointestinal system (GIT) disorders to ocular symptoms are treated effectively. It's been used as a dietary supplement for a very long time and medical treatment. Additionally, it has been used to successfully treat periodontitis, burns, and wounds. (12).

MATERIAL AND METHODS

Apparatus and Reagents

As per the plan of study, the apparatus and chemicals were used such as 70% Ethanol, sterilized distilled water, disposable syringes, and 5% formalin.

Plan of work

The study was conducted in School of Zoology, Minhaj University Lahore using 20 healthy albino mice. Twenty adult, healthy albino mice (males and females), weighing between 34 and 35 grams a piece, were procured from The University of Veterinary and Animal Sciences Lahore. They were practically all the same age. Before the tests began, the animals were kept in cages for a week in groups of five mice each to counteract the behavioral alterations brought on by travel anxiety. Mice were provided with prepared regular food and clean water, and housing conditions were thermostatically controlled at room temperature with constant humidity and light/dark cycles. Their cages were cleaned every day and they were given fresh food on daily basis.

Experimental design

20 albino mice were included in the study and were divided into four groups of five in each. The mice of all groups were induced inflammation artificially with the 5% formalin solution. Each mice was injected with a 0.05ml dose of this solution on the platter surface of the right hind paw with the help of disposable syringe. Paws size of each mice was measured at 0 hour before injecting the 5% solution of formalin and after 24 hours of injection with the help of vernier caliper.

Experimental Procedure

Each mice in all the groups was labeled with a number from one to five with the help of marker to identify them. The mice were kept in groups of five in separate cages/boxes and mice diet and free access of clean water was made available. Before and after creating the inflammation artificially with the help of 5% formalin injections, paws sizes were measured with the help of vernier calipers. The inflamed paws were treated by injecting 0.05 ml ethanolic extract of *Nigella sativa* intraperitoneally to each mice and oral feeding of honey mixed with mice diet was given at the rate of 1.5 mg per mice after every four days as one dose for the experiment period of 21 days as per detail given in Table-1.

Table 1.Details showing the experiment groups of mice and the treatments given to them

Groups	**Extract of *Nigella sativa* and Honey**
Group A (Control)	None, only 0.0 5ml 0f distilled water wasinjected per dose per mice.
Group B	0.05 ml injection of *Nigella sativa* extractper mice per dose
Group C	1.5 mg of honey per mice per dose orally.
Group D	0.025ml injection of *Nigella sativa* and 0.75 mg of Honey (orally) per mice perdose.

Results

The paw sizes of all mice in each group measured with the help of vernier calipers from day 0 to experiment period of 21 days with a gap of 4 days in each successive measurement.

The findings of this experiment at the end of 21st day showed that the inflammation was significantly reduced ($p<0.05$) in mice of group C (0.45vs 0.40cm) and in mice of group D (0.45 vs 0.235cm). Among other treated groups, the mice treated with 50% honey and 50% *Nigella sativa* extract (Group D) showed better results in reducing the inflammation compared to group canimals where the mice were given pure honey (0.45 vs 0.40cm). Overall, the results of this study indicated that the extract of *Nigella sativa* was

Table 2. showing the average paw size of different experiment groups after every 4 days

Groups **Days**	**Group A (Control)**	**Group B (Ethanolic extract of *Nigella sativa 0.05ml*)**	**Group C (Honey 1.5mg)**	**Group D (*Nigella sativa* +Honey) (0.025ml+0.75mg)**
Day 0	0.30 cm	0.30cm	0.25cm	0.25 cm
After 24 hoursof Injecting Formalin	0.40 cm	0.45 cm	0.50 cm	0.45 cm
Day 1	0.50 cm	0.45 cm	0.50 cm	0.50 cm
Day 4	0.50 cm	0.45 cm	0.50 cm	0.55 cm
Day 8	0.50 cm	0.45 cm	0.50 cm	0.55 cm
Day 12	0.50 cm	0.40 cm	0.50 cm	0.50 cm
Day 16	0.45 cm	0.35 cm	0.45 cm	0.45 cm
Day 21	0.45 cm	0.30 cm	0.40 cm	0.35 cm

much better in reducing inflammation compared to pure honey. The findings of the current study or those of several studies already undertaken which elaborated that the *Nigella sativa* is an effective agent to reduce the inflammation and can be safely used for the treatment of inflammatory cases both in animals andhumans.

Statistical Analysis

The paw sizes were measured after every 4 days interval to see the effectiveness of treatments agents used in the experiment. The software Minitab's one-way Analysis of Variance (ANOVA) method was used for the statistical analysis. There was no effect ($p>0.05$) of the reagents (ethanolic extract of *Nigella*

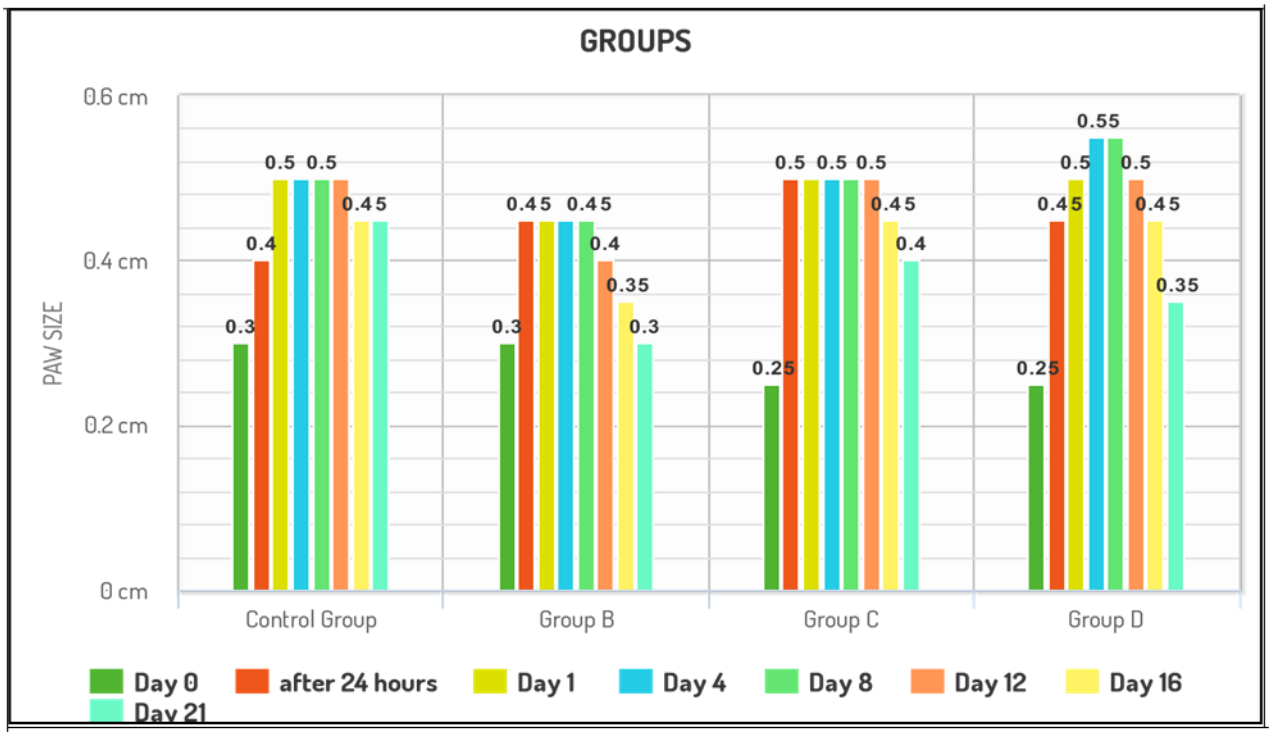

Figure 1. Graphical representation of paw size of all the Groups at Day 0, after 24 hours of formalin injection, Day 1,4,8,12, 16 and 21

sativa and honey) seen on the animals at the start 10 days. The reagents showed their effectiveness slowly and on the day 16 and 21, Group B mice given *Nigella sativa* showed positive results ($p<0.05$) which reduced the inflammation completely in mice. The details of statistical analysis is given at table 3.

Discussion

In many previous studies, *Nigella sativa* (N. sativa) has been proved to have several pharmacological actions, including analgesic, antibacterial, anti-inflammatory, spasmolytic, and bronchodilator. It also has anticancer, antitussive, anticancer, anticancer, anti-diabetic, anti-tussive, immunomodulator, and antioxidant properties. On peritoneal Wistar rat mast cells,

N. sativa ethanol extract was shown to have anti-inflammatory effects. (11) Rats were utilised as models for subacute (complete Freund's adjuvant-induced) as well as acute (carrageenan-induced) inflammation. The objective was to evaluate *Nigella sativa* oil's (NSO) anti-inflammatory, antinociceptive, and antioxidant properties in both models. NSO's ability to reduce inflammation in cases of acute inflammation was studied. NSO has similar anti-inflammatory effects to those produced by diclofenac.

Table 3. Showed the paw sizes of mice of all the groups at day 0, 24 hours after injecting formalin, day 1, 4,8,12, 16, 21. Control Group (No treatment), Group B (0.05ml Nigella sativa), Group C (1.5mg honey), Group D (0.025ml Nigella sativa+0.75mg honey). Significant differences ($p \leq 0.05$) between groups on different days are denoted with distinct letters (A, B, C, and D). The P value dropped to zero at day 21, indicating a high level of significance ($p < 0.05$).

Days	Group A	Group B	Group C	Group D	Significance level	P-Value	F-Value
Day 0	0.28 ± 0.02^{A}	0.28 ± 0.02^{AB}	0.23 ± 0.02	0.27 ± 0.01^{B}	0.05	0.211	1.67
After 24 Hours ofinjecting formalin	0.42 ± 0.02^{A}	0.42 ± 0.02^{AB}	0.46 ± 0.02^{B}	0.44 ± 0.03^{B}	0.05	0.74	0.41
Day 1	0.45 ± 0.04^{B}	0.43 ± 0.03^{A}	0.48 ± 0.02^{A}	0.47 ± 0.02^{A}	0.05	0.66	0.53
Day 4	0.48 ± 0.03^{A}	0.46 ± 0.06^{AB}	0.45 ± 0.03^{B}	0.51 ± 0.04^{A}	0.05	0.78	0.35
Day 8	0.50 ± 0.03^{B}	0.44 ± 0.04^{A}	0.45 ± 0.02	0.49 ± 0.05^{AB}	0.05	0.68	0.49
Day 12	0.48 ± 0.04^{B}	0.41 ± 0.04^{A}	0.42 ± 0.03^{AB}	0.45 ± 0.04^{A}	0.05	0.64	0.55
Day 16	0.46 ± 0.04^{B}	0.35 ± 0.02^{A}	0.38 ± 0.02^{A}	0.34 ± 0.02^{A}	0.05	0.47	3.28
Day 21	0.44 ± 0.03	0.31 ± 0.01	0.34 ± 0.02	0.33 ± 0.02	0.05	0.008	5.49

When the mechanical analgesia was assessed, the analgesic effect of NSO was only seen in the sub-acute inflammatory condition. Additionally, diclofenac's analgesic and anti-inflammatory effects in sub-acute inflammation tended to be enhanced when NSO was added to it. The findings of this study demonstrated the significance of NSO as a source of bioactive chemicals, particularly p-cymene, TQ (Thymoquinone), and -thujene, which are very efficient in the treatment of inflammation. (16)

Eight stingless bee honey varietals from southern Brazil were examined for their phenolic content, reducing capacity, free antioxidant activity, and anti-inflammatory impact against lipopolysaccharides that activated RAW264.7 macrophages. At the studied concentrations, stingless bee honey did not appear to be hazardous and it stopped inflamed macrophages from releasing nitric oxide and a pro-inflammatory cytokine. All of these findings imply that stingless bee honey may be a substantial source of naturally occurring compounds with antioxidant and anti-inflammatory characteristics. (4)

In the present study, the objective was to evaluate *Nigella sativa*'s anti-inflammatory abilities and compare them to those of honey. In this study, albino mice were used. In order to treat them, artificial inflammation in the right hind paws was first induced with the help of 5% formalin. *Nigella sativa*, honey, *Nigella sativa* and honey, and no treatment was used to control the inflammation. Paw size data were statistically analyzed using ANOVA using the Minitab software's instructions. Comparative analysis between the control and treatment groups was evident in several outcomes. The size of the paws varied between treatment groups. According to this study, the experiment was conducted for up to 21 days. After causing the artificial inflammation with 5% formalin solution the mice were treated with the treatment dose after every 4 days and no dose to control. The paw sizes were measured from the day 0 before causing the inflammation. There was no difference ($p>0.05$) between control and the other groups. After inducing the inflammation, paw sizes were measured from all the groups on the day 4, 8,12,16 and 21. There was a gradual decrease in the inflammation in *Nigella sativa* treatment group. The reduction in inflammation was also seen in mice of group C (honey), but it was way less than the *Nigella sativa* group. At day 16, *Nigella sativa* treated the inflammation significantly($p>0.05$). On the day 21st, inflammation was completely treated. It was observed that the group treated with honey showed the reduction in inflammation but slower than *Nigella sativa* treatment.

Nigella sativa extract was more helpful to treat the artificial inflammation in mice as compared to honey. At day 0, paw size of mice of all the groups were measured and it was between 2.5-3.0 cm. At day 0, no significant ($p>0.05$) change was seen in the paw sizes of mice of all the groups. These mice of all the groups were injected with a 5% formalin solution in the dose of 0.05ml to induce inflammation. This 5% formalin solution remains successful to cause inflammation in all the mice`s paws of all the groups. These mice were then treated with *Nigella sativa* and honey and no dose to control group. The treatment dose was given after every four days. At day 4, there was no significant ($p>0.05$) change in paw size of mice of all the groups. At day 8, Paw size of the control group was significantly ($p<0.05$) inflamed than the group treated with *Nigella sativa* (Group B) and other treated groups. At the day 12, Group B (*Nigella sativa* treatment) showed significant($p<0.05$) decrease in the inflammation compared to control group and other treatment groups. At the day 12, mice of Group D (*Nigella sativa* +honey) also showed a significant ($p<0.05$) decrease in paw inflammation compared to the control group. At the day 16, there was further significant ($p<0.05$) decrease in inflammation in the mice of *Nigella sativa* treatment group (Group B) and mice of Group D (*Nigella sativa*+honey) also showed decrease in inflammation. At the day 16, Mice of Group C (honey treatment) also showed some decrease in paw inflammation. At day 21 (last day) of the experiment, inflammation in mice of Group B (*Nigella sativa* treatment) were complete-

ly reduced, which indicated that *Nigella sativa* was the most effective to treat the inflammation. Group D (*Nigella sativa* +honey) showed significant ($p<0.05$) reduction in inflammation but less than *Nigella sativa*. Group C (honey treatment) treated the inflammation to some extent, but it was less effective than *Nigella sativa*.

The results of this study showed that *Nigella sativa* can be effectively used to treat the inflammation. It showed the gradual and consistent decrease in inflammation. The further studies needed to find out how we can increase the effectiveness of honey in this regard.

Conclusion

This study showed that *Nigella sativa* can effectively be utilized to treat inflammation asa natural ingredient. There is undoubtedly still much to learn about the effectiveness of these natural edible reagents. The results of the present investigation demonstrated that *Nigella sativa* extract can be used to treat the inflammation and is very easy to make at room temperature. In this study, the artificial inflammation of albino mice was treated with *Nigella sativa* extract, honey, *Nigella sativa* +honey and no treatment as control. On all the treatment dose, control group as described was the same as the day 1st of inflammation. But there was a gradual decreasein the inflammation of other groups. Group B (*Nigella sativa* treatment) showed significant ($p<0.05$) decrease in inflammation day by day which showed that *Nigella sativa* is rich in anti-inflammatory properties. It is demonstrated that honey also possesses anti-inflammatory properties, but it is less effective than *Nigella sativa*.

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