ORIGINAL ARTICLE

COMPARISON OF ANTI-INFLAMMATORY ACTIVITY OF *NIGELLA SATIVA* AND DICLOFENAC SODIUM IN ALBINO RATS

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Background: *Nigella sativa* or "*Kalonji*" is a naturally occurring plant in Pakistan and other countries which possesses a wide range of medicinal properties, the anti-inflammatory property being one of these. Diclofenac sodium is a commonly used anti-inflammatory drug. The purpose of this study was to compare the anti-inflammatory effect of ethanolic extract of *Nigella sativa* seeds with that of diclofenac sodium in albino rats. **Methods:** This laboratory randomized controlled trial (RCT) was conducted in the Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore. The study was carried out on 90 male albino rats. Five percent formalin in a dose of 50 µl was injected into sub-plantar surface of right hind paw of each rat to produce inflammation. The rats were randomly divided into three groups of thirty each. Group A was given normal saline (control); group B was given *Nigella sativa* seed extract; and group C received diclofenac sodium, as a reference drug. Increase in paw diameter, and total and differential leukocyte counts were measured as markers of inflammation. **Results:** *Nigella sativa* seeds extract caused significant ($p<0.05$) reduction in the paw inflammatory response in albino rats. The effect was longer in duration than the effect caused by diclofenac sodium; however, the extract was comparatively less potent than diclofenac sodium. The extract had no significant effect ($p>0.05$) on the total or differential leukocyte counts. **Conclusion:** Our results suggest that ethanolic extract of *Nigella sativa* seeds possesses potent anti-inflammatory effect in albino rats however, this effect is comparatively less but prolonged than that produced by diclofenac sodium.

Keywords: *Nigella sativa,* formalin test, inflammation, diclofenac sodium

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INTRODUCTION

Nigella sativa or "*Kalonji*" is a traditionally used medicinal plant. It is widely grown in different parts of the world including Pakistan. Its seeds have been used as medicines for the treatment of obesity, dyspepsia, diarrhoea, indigestion, puerperal disorders, and various skin diseases. *Kalonji* seeds contain a volatile oil, a fixed oil, proteins, amino acids, reducing sugars, alkaloids, organic acids, saponins, fats, vitamins and minerals.[1]

The results of using *Nigella sativa* oil[2] and various extracts[3,4] for relieving inflammation have been encouraging. Thymoquinone is the major active principle of *Nigella sativa* and most of its pharmacodynamics effects are due to thymoquinone. Al-Ali *et al*[5] carried out a study to determine LD_{50} of thymoquinone both in mice and rats, orally as well as intraperitoneally. Autopsy and histopathology of liver, kidney, heart and lungs were also determined. The study showed that the LD_{50} in albino rats after intraperitoneal injection was 57.5 mg/kg and after oral ingestion was 794.3 mg/kg.

Different medicinal plants and their active ingredients are being evaluated these days because of their potential anti-inflammatory effects. We, in our study, used the ethanolic extract of *Nigella sativa* to determine its effect on an artificial model of inflammation in albino rats. Commonly used anti-inflammatory drug diclofenac sodium was used for comparison of effects.

Analgesic and anti-inflammatory drug abuse has become a major problem in our country due to over-the-counter sale of such drugs and these have their adverse effects too. Research on medicinal plants like *Kalonji* may provide opportunity of use of safe, cheap and effective natural anti-inflammatory agent in our country.

The objective of this study was to compare the anti-inflammatory effect of ethanolic extract of *Nigella sativa* seeds with that of diclofenac sodium in albino rats.

MATERIAL AND METHODS

This laboratory based randomized controlled trial (RCT) was conducted in the Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore. Ninety adult, healthy male albino rats, each weighing 250–300 grams, were obtained from National Institute of Health, Islamabad. Animals were housed in groups of 30 per cage for at least one week before the start of experiments. Housing conditions were thermostatically maintained at 26±2

°C and with a light/dark cycle (lights on: 0900–2100). Animals were given food and water *ad libitum*.

The following drugs/chemicals were used: Ethanol (Merck, USA), Diclofenac sodium (Novartis, Pakistan), Sterilized distilled water (Otsuka, Pakistan), Disposable syringes (BD, Pakistan), and Formalin (Merck, USA).

Ethanolic extract of *Nigella sativa* seeds was made and standardized using facilities available at Applied Chemistry Research Centre, PCSIR labs, Lahore. *Nigella sativa* seeds obtained from local market were dried and then crushed into a coarse powder using an electric grinder. This powder was then extracted with ethanol using Soxhlet extractor. The extract was filtered and the solvent (ethanol) evaporated in vacuum with a rotatory evaporator. This yielded a blackish-brown concentrate. This concentrate was kept at 4 °C prior to use. The crude extract was dissolved in sterilized distilled water and then diluted to the desired concentration.[6]

Ninety rats were randomly divided into 3 groups of 30 each. Group A: (Control, n=30): was given normal saline, 10 ml/Kg of body weight, intra-peritoneally[4], Group B: (Experimental, n=30): was given ethanolic extract of *Nigella sativa* seeds in a dose of 50 mg/Kg of body weight intra-peritoneally[4], and Group C: (Reference, n=30): was given diclofenac sodium, 25 mg/Kg of body weight, intra-peritoneally[4].

A standard and internationally accepted model of experimental inflammation, "formalin test" was used for the production of artificial inflammation.[7] Five percent (5%) formalin in a dose of 50 micro-litres was injected into sub-plantar surface of right hind paw of each rat to produce inflammation.[4,7] Increase in the hind paw diameter (oedema) was used as a measure of inflammation. Paw diameter was measured immediately before (0 hour) and at 1, 3, 10 and 25 hours after formalin injection using Vernier's caliper.[4] Percentage reduction of inflammation was calculated using the formula:

$$\text{Reduction (\%)} = (1 - Dt/Dc) \times 100$$

Where Dt and Dc represent the mean paw diameter in treated (Nigella/diclofenac) and control groups, respectively. Leucocytosis occurs within a few hours after the onset of inflammation.[8]

After 25 hours of formalin injection, blood sample was drawn from each rat. The blood was added immediately with anti-coagulant ethylene diamine tetra acetic acid (EDTA) for determination of white blood cell counts. Total and differential leukocyte counts were determined according to the method of Dacie and Lewis.[9]

Data was entered into SPSS version 15.0. Mean±standard error of mean (SEM) were calculated for continuous variables. One way ANOVA followed by post hoc LSD test (multiple comparisons) was applied to find out the statistical significance among the three groups. The difference was considered significant if the p value was less than 0.05; and, highly significant if the *p* value was less than 0.001.

RESULTS

The oedema (increase in paw diameter) produced in each of three groups is shown in table-1. At 1 hour, 3 hour and 25 hour, the oedema produced in the *Nigella sativa* and diclofenac groups, was significantly ($p<0.05$) less than that of the control group. At 10 hour, the oedema produced in diclofenac group was significantly ($p<0.05$) less than that of control group and *Nigella sativa* group; but, the oedema produced in the *Nigella sativa* group was not significantly ($p=0.127$) less than that of control group. At 1 hour, 3 hour and 10 hour, the oedema produced in the diclofenac group was significantly ($p<0.05$) less than that of *Nigella sativa* group. While at 25 hour, the oedema produced in the diclofenac group was not significantly ($p=0.733$) different from that of *Nigella sativa* group. The percentage reduction in inflammation (paw diameter) caused by the *Nigella sativa* (Group-B) and diclofenac sodium (Group-C) at different time intervals is shown in figure-1. At 1 hour, 3 hour and 10 hour, reduction of the inflammation (i.e., reduction in paw diameter) caused by the diclofenac sodium was more than that caused by *Nigella sativa*. However, at 25 hour, reduction of the inflammation caused by *Nigella sativa* was more than that caused by diclofenac sodium. The total leukocyte counts (TLC) of group B and group C was not significantly ($p>0.05$) different from the TLC of the control group. TLC of the *Nigella sativa* group was also not significantly ($p=0.819$) different from that of the diclofenac group (Table-2). The differential leukocyte counts (DLC) of the three groups are given in the table-3. The percentages of the neutrophils of *Nigella sativa* and diclofenac groups were not significantly ($p>0.05$) different from that of the control group. The percentage of the neutrophils of diclofenac group was also not significantly ($p=0.61$) different from that of the *Nigella sativa* group.

Table-1: Oedema (increase in paw diameter) produced in groups A, B and C.

Groups	Mean±SEM oedema at different time intervals (mm)			
	1 hour	3 hour	10 hour	25 hour
A (Control)	0.80±0.04	0.98±0.04	0.51±0.08	0.30±0.02
B (*Nigella Sativa*)	0.49±0.03*	0.66±0.03*	0.39±0.05**	0.22±0.03*
C (Diclofenac)	0.30±0.02*	0.36±0.03*	0.21±0.03*	0.23±0.02*

*$p<0.05$ as compared to control (significant), **$p=0.127$ as compared to control (non-significant)

Table-2: Total leukocyte count (TLC) in groups A, B and C.

Groups	Mean±SEM TLC (cells/mm^3)
A (Control)	10996.67±279.078
B (*Nigella Sativa*)	10531.67±303.906*
C (Diclofenac)	10618.33±209.577**

*p=0.222 as compared to control (non-significant), **p=0.319 as compared to control (non-significant)

Table-3.Differential leukocyte counts (DLC) of rats in groups A, B and C.

Groups	Mean±SEM white blood cells (%)				
	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
A (Control)	51.03±2.78	44.5±2.81	1.97±0.22	2.23±0.36	0.30±0.12
B (Nigella Sativa)	45.77±1.71*	47.83±1.64*	3.37±0.29**	2.27±0.24*	0.77±0.17**
C (Diclofenac)	47.70±3.23*	47.90±3.27*	2.37±0.35*	1.77±0.28*	0.27±0.08*

*p>0.05 as compared to control (non-significant), **p<0.05 as compared to control and diclofenac groups (significant)

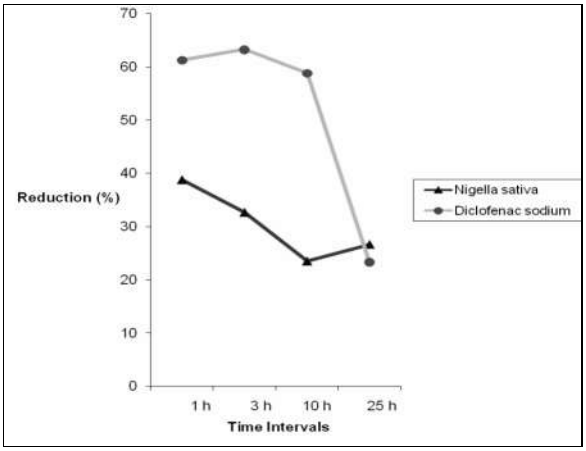

Figure-1: The percentage reduction of the inflammation (paw diameter) in group B (*Nigella sativa*) and group C (Diclofenac), at different time intervals.

DISCUSSION

The present study compared the anti-inflammatory effects of ethanolic extract of *Nigella sativa* seeds and diclofenac sodium. Our results showed that the ethanolic extract of Nigella sativa produced reduction in the oedema at all the time intervals. Maximum reduction was observed at 1 hour (38.75%). The reference drug diclofenac sodium also produced reduction of inflammation at all time intervals but its maximum effect was seen at 3 hour (63.26%). At all the time intervals, reduction in the oedema caused by diclofenac sodium was comparatively greater than that produced by *Nigella sativa*; except at 25 hour, when *Nigella sativa* caused 26.66% reduction of inflammation as compared to 23.33% caused by diclofenac. This showed that the duration of the anti-inflammatory activity of *Nigella sativa* was longer than that of the reference drug, i.e., diclofenac sodium.

Ghamdi[3] measured anti-inflammatory activity of aqueous extract of *Nigella sativa* seeds 3 hours after inducing inflammation with carrageenan. He reported that *Nigella sativa* produced a significant (p=0.018) reduction of carrageenan induced paw oedema when compared to the control group and the anti-inflammatory effect was comparable to that produced by aspirin. In our study, TLC of the groups A, B and C were within the normal ranges (normal TLC of albino rats=6000–18000 cells/ml)[10], and TLC of the three groups were also not significantly different from each other. The DLC showed that neutrophilia was present in all the three groups (normal neutrophil count in albino rats=10–30%).[10] The percentage of the neutrophils in *Nigella sativa* treated group was the least of all groups, but it was not significantly different from that of the control or diclofenac group (p>0.05). The percentage of neutrophils in diclofenac treated group was also not significantly different from that of the control group and *Nigella sativa* treated group (p>0.05). This shows that the acute anti-inflammatory activity of *Nigella sativa* is not by significant reduction of the white cell counts; however, as suggested by Hajhashemi *et al*[11], inhibition/reduction of release of prostaglandins, leukotrienes and some other compounds from the leukocytes by thymoquinone may be responsible for anti-inflammatory activity.

CONCLUSION

It is concluded that *Nigella sativa* seeds have potent anti-inflammatory effect. This effect is comparatively less but prolonged than that of diclofenac sodium.

AUTHOR'S CONTRIBUTION

MUB: Conducted this study under supervision of HJQ. TS: Helped in the study design and data analysis.

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