Original Article

"EFFECT OF NIGELLA SATIVA SEEDS EXTRACT ON SERUM C-REACTIVE PROTEIN IN ALBINO RATS"

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ABSTRACT:

Background:

C-reactive protein (CRP) is an acute phase protein. It predicts future risk of cardiovascular diseases. Different medicinal plants and their active ingredients possess the ability to reduce serum CRP levels and hence inflammatory disorders and cardiovascular diseases. In our study, ethanolic extract of *Nigella sativa* seeds was evaluated in albino rats for its possible effect on serum CRP levels.

OBJECTIVE:

The objective of this study was to determine the effect of ethanolic extract of *Nigella sativa* seeds on an acute inflammatory biomarker/mediator, C-reactive protein (CRP) in albino rats.

STUDY DESIGN:

Randomized controlled trial (RCT).

PLACE AND DURATION OF STUDY:

Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore; from September to November, 2009.

SUBJECTS AND METHODS:

The study was carried out on 90 male albino rats. Five percent (5%) 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. CRP levels in each group were measured from blood samples taken 25 hours after giving formalin.

RESULTS:

The ethanolic extract of *Nigella sativa* seeds, given intraperitoneally, caused highly significant ($p<0.001$) reduction in serum CRP levels as compared to control group. The reduction in CRP levels by ethanolic extract of *Nigella sativa* was also significantly ($p<0.05$) more than that produced by diclofenac sodium.

CONCLUSION:

Our results suggest that *Nigella sativa* possesses ability to reduce serum CRP levels significantly, after production of artificial inflammation, in albino rats

KEY WORDS:

Nigella sativa, CRP, inflammation.

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INTRODUCTION:

C-reactive protein (CRP) is an acute phase protein produced by liver, and its concentration is increased during infections and inflammation. An

acute-phase protein has been defined as one whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 25 percent during inflammatory disorders. The changes in the concentrations of acute-phase proteins are due largely to changes in their production by hepatocytes [1].

CRP is a pentraxin protein (five non-covalently associated protomers arranged symmetrically around a central core). It is classified as first-line defense molecule against pathogenic organisms as it binds to phosphocholine of bacterial and fungal membranes and activates the complement system. It also stimulates phagocytic cells that remove apoptotic and necrotic cells thus contributing to healing of injured tissue. Production of CRP from the liver is stimulated by cytokines associated with non-specific tissue injury such as interleukin-1β, interleukin-6, and tumor necrotic factor [2]. It has been shown that CRP predicts future risk for cardiovascular disease in apparently healthy persons, independent of established risk factors. Serum CRP has been shown to predict myocardial infarction, coronary artery disease (CAD), stroke, peripheral arterial disease, sudden death etc [3]. It has also been documented that CRP is not only a marker of inflammation and cardiovascular events but also a mediator of these conditions [4].

Nigella sativa or "Kalonji" is a traditionally used medicinal plant. It is widely grown in different parts of the world including Pakistan. Its seeds are commonly used in different Pakistani foods, spices and pickles. Traditionally, these have been used as medicine for the treatment of diarrhea, indigestion, dyspepsia, puerperal disorders, obesity and skin disorders. *Kalonji* seeds contain a volatile oil, a fixed oil, proteins, amino acids, reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, saponins, fats, vitamins and minerals [5].

The results of using *Nigella sativa* oil [6] and various extracts [7,8] for relieving inflammation have been encouraging. Thymoquinone is the major active principle of *Nigella sativa* and most of its pharmacodynamic effects are due to thymoquinone. Al-Ali *et al* [9] 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 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 ability to reduce serum CRP levels and hence inflammatory disorders and cardiovascular diseases [10]. We, in our study, used the ethanolic extract of *Nigella sativa* to determine its effect on this marker/mediator of inflammation. A standard anti-inflammatory drug diclofenac sodium was used for comparison of effect of *Nigella sativa* and diclofenac sodium on the CRP levels.

MATERIALS AND METHODS:

ANIMALS:

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 ^{0}C and a light/dark cycle (lights on: 0900-2100). Animals were given food and water ad libitum.

CHEMICALS AND DRUGS:

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

PREPARATION OF EXTRACT:

Ethanol 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 ^{0}C prior to use. The crude extract was dissolved in sterilized distilled water and then diluted to the desired concentration [11].

PRODUCTION OF INFLAMMATION:

A standard and internationally accepted model of experimental inflammation, "formalin test" was used for the production of artificial inflammation

[8,12]. The 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.

Group B (Experimental, n=30): was given ethanol extract of *Nigella sativa* seeds in a dose of 50 mg/Kg of body weight intra-peritoneally [8].

Group C (Reference, n=30): was given diclofenac sodium, 25 mg/Kg of body weight, intra-peritoneally.

Five percent (5%) formalin in a dose of 50 micro-liters was injected into sub-plantar surface of right hind paw of each rat to produce inflammation (Fig. 1).

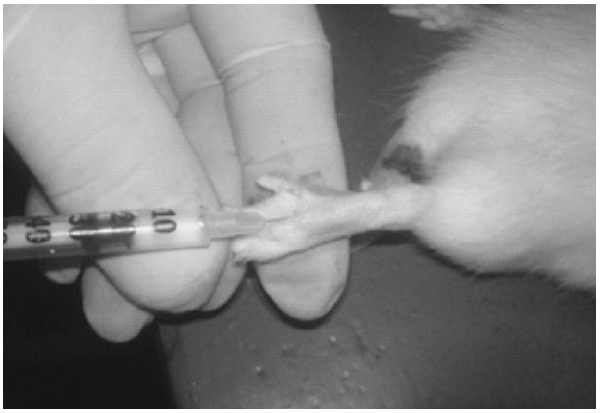

Fig. 1: Formalin being injected into sub-plantar surface of right hind paw of the rat.

CRP concentration peaks in blood about 25 hours after the onset of inflammation [13]. After 25 hours of formalin injection, each rat was anesthetized using ether. For this purpose, the rat was placed inside a close, transparent glass chamber containing ether-soaked cotton. The rat, thus anesthetized, was placed on a dissection board at its back and after palpation of lower rib and sternal margin, the needle of 3 ml disposable syringe was inserted directly into heart, taking care that it may not pierce its posterior wall. Two milliliter blood sample was obtained in this way.

After 15-20 minutes, blood samples were placed in centrifuge machine and centrifuged at 5000 rpm for 15 minutes. The serum was separated and stored at -20 ^{0}C for determination of CRP at later stage. The Labor Diagnostika Nord GmbH & Co. CRP ELISA kit (Ref. No. DM E-4600, Lot No. 8297) was used for the determination of serum C-reactive protein levels.

STATISTICAL ANALYSIS:

Data was entered into SPSS version 15.0. Descriptive analysis was carried out to find out mean ± SEM values of data obtained.

One way ANOVA followed by post hoc LSD test (multiple comparisons) was applied to find out the statistically significant different values among the three groups.

The values were considered significant if the p value was less than 0.05; and, highly significant if the p value was less than 0.001.

RESULTS:

Table 1 and Fig. 2 show the serum C-reactive protein (CRP) levels of the three groups. One way ANOVA showed that the CRP levels of group B (*Nigella sativa* group) and group C (diclofenac sodium group) were highly significantly less than the CRP levels of the group A i.e. control group (p=0.000).

Post hoc LSD test showed that the CRP level of the *Nigella sativa* group was highly significantly lower than that of the diclofenac group (p=0.000).

Table 1: Mean ± SEM serum C-reactive protein (CRP) levels of the three groups

Groups	**Mean ± SEM serum CRP** (µg/ml)
A	422.90±5.69
B	217.65±3.31*
C	277.73±4.18*

* p=0.000 as compared to control (highly significant)

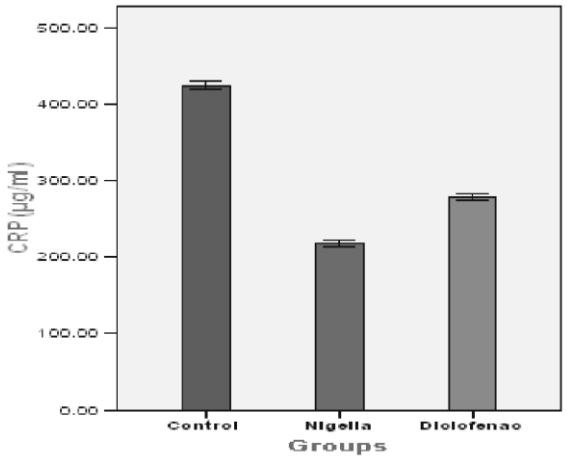

Fig. 2: Mean ± SEM serum C-reactive protein (CRP) levels of the three groups.

DISCUSSION:

Our study assessed the effect of ethanolic extract of *Nigella sativa* seeds on acute inflammatory biomarker/mediator, CRP. Formalin was used to produce artificial inflammation. The analysis of C-reactive protein levels of the three groups revealed that CRP level of *Nigella sativa* treated group was highly significantly less than those of the control group and diclofenac treated group (p=0.000).

Hajhashemi *et al.*[6] measured anti-inflammatory activity of essential oil of *Nigella sativa* seeds 4 hours after inducing inflammation with carrageenan. Maximum inhibition of the inflammatory response was caused by the essential oil at an intraperitoneal dose of 400 µl/Kg (87% inhibition of the inflammation). They evaluated anti-inflammatory activity by measuring some physical parameters but no chemical mediator or marker of inflammation was measured. Hajhashemi suggested that the inhibition of prostaglandins, leukotrienes and oxygen radicals by thymoquinone may be responsible for anti-inflammatory activity of essential oil.

Tanko [8] used ethanolic extract of *Nigella sativa* to check its efficacy against formalin induced artificial inflammation in albino rats. The extract was successful in reducing various physical parameters of inflammation like paw edema, but the researchers didn't explore its effect on chemical mediators of inflammation. They suggested that anti-inflammatory effect of the extract observed might be due to the presence of flavonoid which inhibit phosphodiesterases which are involved in cell activation, and their effect depend upon the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the sites of injuries.

Takeoglu *et al.*[14] evaluated the efficacy of volatile oil of *Nigella sativa* against experimentally induced inflammation (rheumatoid arthritis) in rats and measured various inflammatory cytokines such as TNF-α and IL-1β. Arthritis was induced in rats by Freund's incomplete adjuvant. They compared the anti-inflammatory properties of *Nigella sativa* oil with methotrexate. The pro-inflammatory cytokines, TNF-α and IL-1β, levels were significantly lower in the *Nigella sativa* treated group than those in the control group and methotrexate treated group. This effect of *Nigella sativa* is consistent with the results of the present study. In our study too, the ethanolic extract of *Nigella sativa* was able to lower the serum CRP levels highly significantly more than by diclofenac sodium.

In conclusion, our results show that the ethanolic extract of *Nigella sativa* possesses ability to reduce the serum levels of acute inflammatory biomarker CRP highly significantly in albino rats. The potential of *Nigella sativa* to reduce CRP should be further investigated involving chronic inflammatory models. It may prove beneficial regarding the regression of development of various chronic inflammatory disorders such as atherosclerosis and ischemic heart diseases.

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