JALALABAD MEDICAL JOURNAL

Original Article

Effect of Nigella Sativa Oil on Inflammation in Rats

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ABSTRACT

The use of Nigella sativa (Kalozira) has been used for a long, in various countries as a spice, food preservative and remedy for the treatment of many disorders. The objective of this study was to assess the effects of Nigella sativa oil (NSO) against inflammation after single administration with 3 increasing doses in rats. The anti-inflammatory effect was evaluated by paw oedema test. This experimental study was conducted in the department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2016 to February 2017. For this purpose, 20 Long Evans rats of both sexes were divided into control (10 ml/kg of 1% solution of Tween 20) and experimental groups (NSO 0.5, with 0.5 ml/kg NSO; NSO 1, with 1 ml/kg NSO; NSO 1.5, with 1.5 ml/kg NSO) with 5 rats in each group. All the agents were given intraperitoneally (i.p) two and half an hour before the paw oedema test. The inflammation was lowered by all three doses (0.5, 1, 1.5 ml/kg; i.p) but the result was only significant at doses of 1ml/kg ($p<0.05$) and 1.5 ml/kg ($p<0.01$) groups, in comparison to that of control group. Therefore, it may be concluded that, increasing the dose of NSO was progressively more effective in lowering inflammation.

Keywords: *Inflammation, Nigella sativa, Paw oedema test.*

[Jalalabad Med J 2019; 16(2): 47-50]

INTRODUCTION

Inflammation is a complex set of interactions among the many chemical factors and cells in response to various kinds of injuries, like: traumatic, infectious, post-ischemic, toxic or autoimmune injury[1]. It is an essential immune response that enables self protection during infection, injury and maintains tissue homeostasis in different noxious condition[2]. If tissue damage is inevitable the focus shifts from protection of tissue to promotion of healing of the damaged tissue through inflammation[3]. Among the different methods for measurement of inflammation in test animals, the paw oedema test is simple, accurate and reliable. In this test, subcutaneous administration of formalin in any paw

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causes a local tissue injury and subsequently paws oedema[4]. Analgesic and anti-inflammatory drugs are causing a lot of unwanted effects like gastritis, gastric ulcers and renal damage[5]. In recent years, many studies have been conducted throughout the world to find alternatives to the traditional anti-inflammatory drugs to replace them so that their adverse effect can be minimized.

Nigella sativa is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean Sea. This widely distributed plant is native to Arab countries[6]. In Islamic literature, it is considered as one of the greatest form of healing medicine. It was mentioned by prophet Mohammad (PBUH) as remedy for all diseases except death[7]. The Nigella sativa, have shown anti-inflammatory effects in various animal models[8,9]. The volatile oil (0.66 ml and 1.55 ml/kg, i.p) of Nigella sativa and thymoquinone (0.5, 1.0, 5 mg/kg, i.p) exhibited a dose-dependent anti-inflammatory effect against carrageenan-induced rat hind paw oedema[10]. Similarly, the aqueous extract of Nigella sativa (500 mg/kg, p.o) possesses anti-inflammatory action in carrageenan-induced paw oedema similar to 100 mg/kg aspirin[8]. Moreover, oral administration of essential oil of Nigella sativa at a dose of 100, 200 and 400 l/kg did not exert a significant anti-inflammatory effect in the carrageenan test, but intraperitoneal injection of the same doses significantly inhibited carrageenan induced rat paw oedema[9]. However to the best of our knowledge no published data were available in assessing the anti-inflammatory effects of single supplementations of NSO in increasing doses like 0.5 ml/kg, 1 ml/kg and 1.5 ml/kg body weight in rats. So this study was done to assess the effects of Nigella sativa oil (NSO) against inflammation after single administration with 3 increasing doses in rats.

MATERIALS AND METHODS

This experimental study was conducted in the pain laboratory, department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2016 to February 2017. All experiments and animal care were performed according to the guidelines set in the 'the ethical guidelines for experimentation in laboratory animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh[11]. Twenty healthy adult Long Evans rats weighing 180 to 220 gm of either sexes were obtained from animal house of Bangladesh University of Health Sciences (BUHS), Mirpur 1, Dhaka[8,12]. They were housed in specially built plastic cages with 5 rats per cage under a 12/12 hour light/dark cycle[13,14]. The ambient room temperature was maintained at around 27-28 °C, corresponding to the thermoneutral zone for rodents[15]. The rats had free access to standard laboratory food and cooled boiled water ad libitum. They were kept there for a period of 7 consecutive days for acclimatization prior to the experiment. To avoid circadian influences all the experiments were performed at daytime between 08:00 and 15:00 hours[16]. The Nigella sativa oil (Drug International Pharmaceuticals, Bangladesh) was dissolved in 1% solution of Tween 20. On the basis of doses of supplementation all the rats were divided into 4 groups (5 rats/group). The control group received only 1% solution of Tween 20 (10 ml/kg body weight)[9], experimental Group NSO 0.5, NSO 1 and NSO 1.5, received 0.5 ml/kg, 1 ml/kg and 1.5 ml/kg body weight of NSO respectively, in equal volume to that of 1% solution of Tween 20[17]. The rat was intraperitoneally injected with 1% solution of Tween 20 or NSO 0.5 ml/kg or 1 ml/kg or 1.5 ml/kg body weight. One hour later, the rat was restrained manually by a thick towel and the right hind paw was exposed. Fifty (50) l of dilute (2.5%) formalin was injected subcutaneously into the plantar aspect of the right hind paw with an insulin syringe. After 90 minutes the animal was sacrificed. Each rat was anesthetized by chloral hydrate (400 mg/kg body weight, i.p) and after cessation of the respiratory chest movement; decapitation was done to confirm death[18]. Both the hind paws of the sacrificed rat were cut at their knee joints by sharp scissors[15]. Then the volumes of both paws were measured using a water plethysmometer[4]. Paw volume was measured by substracting amount of water column before paw immersion from amount of water column after paw immersion. Net oedema volume was calculated by subtracting the left paw volume from the right paw volume[15]. The data were expressed as mean±SEM (standard error of mean) and were statistically analyzed by a computer with statistical package for social science (SPSS) using ANOVA (among groups), followed by Bonferroni's post hoc test (between groups). In the interpretation of results, $p \leq 0.05$ was considered as the level of significance.

RESULTS

In this study, mean±SEM of paw oedema volumes in paw oedema test were 0.32±0.04, 0.22±0.04, 0.18±0.02 and 0.14±0.02 ml of water in group control, NSO 0.5, NSO 1 and NSO 1.5 respectively (Table-I). The

differences of these mean values of oedema volumes among the groups were statistically significant ($p \leq 0.01$). The mean value of both group NSO 1 and NSO 1.5 was significantly ($p \leq 0.05$, p 0.01) lower than that of control though the mean value of NSO 0.5 was not significantly lower than that of control. Moreover, the mean value of group NSO 0.5 was higher than that of group NSO 1 and NSO 1.5 though the differences were not statistically significant. Also, the mean value of group NSO 1 was not significantly higher than group NSO 1.5 (Figure-1). Table-II showed the statistical analyses between different groups of rats.

Table-I: *Paw oedema volume in different groups of rats (n=20).*

Groups	Number	Paw Oedema Volume (ml of water)
Control	5	0.32±0.04 (0.2 to 0.4)
NSO 0.5	5	0.22±0.04 (0.1 to 0.3)
NSO 1	5	0.18±0.02 (0.1 to 0.2)
NSO 1.5	5	0.14±0.02 (0.1 to 0.2)

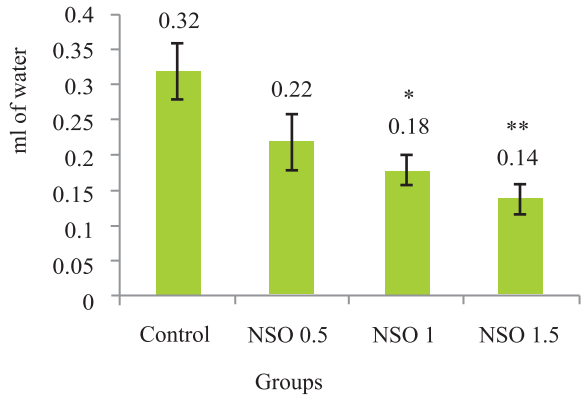

Figure-I: *Bar diagram showing paw oedema volume in different groups of rats. Each bar symbolizes for mean±SEM for 5 rats (n=5).*
***= p≤0.01, *= p≤0.05 Compared to control.*

Table-II: *Statistical analysis between different groups of rats (n=5).*

Groups	p value
Control vs NSO 0.5 vs NSO 1 vs NSO 1.5	0.005**
Control vs NSO 0.5	0.214ns
Control vs NSO 1	0.033*
Control vs NSO 1.5	0.005**
NSO 0.5 vs NSO 1	1.000ns
NSO 0.5 vs NSO 1.5	0.511ns
NSO 1 vs NSO 1.5	1.000ns

ns= Not significant,**= p≤0.01, *= p≤0.05 Compared to control.

DISCUSSION

In the present study, the inflammation was lowered by all three doses (0.5, 1, 1.5 ml/kg; i.p) of NSO but the result was only significant at doses of 1 ml/kg (i.p) and 1.5 ml/kg (i.p) groups, in comparison to that of control group. However, the NSO supplementation at 1.5 ml/kg (i.p) group was shown to be significantly more effective than the other two dose groups. Similar observations were also reported by various investigators, though the doses and extract of Nigella sativa were different[9,19]. Numerous molecular targets are involved in the anti-inflammatory activity of Nigella sativa. NSO might act by inhibiting histamine release from mast cells[20]. It might inhibit thromboxane in cycloxygenase pathway and leucotriene in 5-lipoxygenase pathway of aracidonic acid metabolism. Both these substances also might inhibit the membrane lipid peroxidation[21]. It has been suggested that, NSO might inhibit inducible nitric oxide synthase (iNOS) enzyme and therefore decrease the production of free radical nitric oxide (NO)[22].This might in turn decreased the vascular smooth muscle relaxation and reduced the inflammation[23].

It has also been suggested that, Nigella sativa might exert anti-inflammatory effect by inhibiting production of interleukin (IL) - 1β, tumour necrosis factor - α (TNF-α), IL-4, IL-5, IL-13 and eosinophils[24,25,26]. In addition, NSO might abolish proinflammatory mediators metalloproteinase-13, cycloxygenase-2 (COX-2) and prostaglandin E2 (PGE2) production. It might also exert anti-inflammatory effect by blocking LPS-induced phosphorylation of p38 mitogen-activated protein kinase, nuclear factor-kappa B-p65, extracellular-regulated kinase[25].

All these inflammatory molecules mediates inflammation by causing vasodilataion (histamine, prostaglandin), endothelial activation (histamine, TNF, IL-1), increased vascular permeability (histamine, serotonin, leucotrienes), pain (prostaglandin, cytokines), vascular smooth muscle relaxation (NO) and fever (prostaglandin, IL-1, TNF)[23].

CONCLUSION

In the present study, intraperitoneal administration of Nigella sativa oil at three different doses has shown anti-inflammatory effects and the higher dose group was more effective than the lower dose groups. So, it is concluded that increasing the dose of Nigella sativa oil may be progressively more effective in lowering inflammation.

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