Antinociceptive Effect of Black Seed Feeding in Streptozotocin-Diabetic Rats

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ABSTRACT

Introduction: Diabetic rats display exaggerated hyperalgesic behavior in response to noxious stimuli that may resemble and model aspects of painful diabetic neuropathy in humans. This study was designed to investigate the effect of Nigella sativum (NS) on formalin-induced nociceptive responses (standard formalin test) in streptozotocin (STZ)-induced diabetic rats.

Methods: For this purpose, STZ-diabetic rats received Nigella sativum mixed with standard rat chow at a weight ratio of 6.25% orally for a period of one month.

Results: It was found out that NS treatment did cause a significant reduction in blood glucose in diabetic rats and NS-treated diabetic rats exhibited a lower nociceptive score as compared to untreated-diabetic ones. Meanwhile, NS treatment reduced the nociceptive score in both phases of the formalin test. In contrast, sodium salicylate as positive control only reduced this score in the second phase of the test.

Discussion: The results suggest therapeutic potential of NS feeding for treating painful diabetic neuropathy.

1. Introduction

Streptozotocin-induced diabetes in the rat has been increasingly used as a model of painful diabetic neuropathy to assess the efficacies of potential analgesic agents. It has been well known that diabetic rats display exaggerated hyperalgesic behavior in response to noxious stimuli like paw formalin injection that may resemble and model aspects of painful diabetic neuropathy. This enhanced nociception has been observed in the early stages of diabetic neuropathy in STZ-diabetic rats. Despite great achievements in analgesic drugs development, there is still a need for new analgesics devoid of the side effects presented by opioids or non-steroidal anti-inflammatory drugs for the treatment of some acute and chronic pain conditions (1-4). On this foundation, Nigella sativum (NS) has been known as a medicinal foodstuff with traditional use in diabetes. Beneficial effects of this medicinal plant have been demonstrated in diabetic animals and both insulin-dependent and non-insulin-dependent diabetic subjects (5-6). Although the use of herbal and natural supplements in some countries has increased dramatically in recent years, but claims of their therapeutic effects abound, medical research has not always supported their effectiveness. Therefore, this study was carried out to evaluate the antinociceptive effect of NS feeding in STZ-induced diabetic rats using standard formalin test.

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2. Methods

NS was obtained from the local grocery (Tehran, Iran) in april and was systemically identified by the botanists in Department of Biology (Shaheed Beheshti University, Tehran, Iran). Then, its powder was mixed with standard rat chow at a weight ratio of 6.25%.

2.1. Animals

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 205-255 g (7-9 weeks old) were housed in an air-conditioned colony room (3-4/cage) on a light/dark cycle at 21 ± 2°C and supplied with pelleted diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of Shahed University (Tehran, Iran) and in accordance with the NIH guidelines for the care and use of laboratory animals.

The animals were randomly divided into five experimental groups; i.e. control (n =18) receiving 0.9% saline, NS-treated control (n =10), sodium salicylate (Sigma Chemical, St. Louis, Mo., USA) -treated rats (n = 5) used as positive control, vehicle-treated diabetic (n =10), and NS-treated diabetic (n =11). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 60 mg Kg/l; Upjohn) dissolved in cold 0.9% saline immediately before use. Sodium salicylate (200 mg/Kg, i.p.) was administered 1 h before conducting the formalin test. Serum glucose level and body weight were monitored at the start and end of the experiment. Diabetes was verified by a serum glucose level higher than 250 mg/dl using glucose oxidation method (glucose oxidase kit, Zistchimie, Tehran).

2.2. Formalin Test

The applied method for formalin test was according to the previously described method. Briefly, each animal was acclimatized to the observation box before any testing began. Then, it was given a subcutaneous injection of 50 µ l of 2.5% formalin into the plantar surface of one hind paw using a 25-gauge syringe needle. Each rat was then immediately placed in a Plexiglas box (40 x 40 x 40 cm) positioned over a mirror angled at 45 ° to allow an unobstructed view of the paws by the observer.

Observations to determine nociceptive responses began upon placing the rat into the box and continued for the next 60 min. A nociceptive score was determined for each 5 min block during that period by measuring the amount of time spent in each of the four behavioral categories: 0, the position and posture of the injected hind paw is indistinguishable from the contralateral paw; 1, the injected paw has little or no weight placed on it; 2, the injected paw is elevated and is not in contact with any surface; 3, the injected paw is licked, bitten, or shaken. Then, a weighted nociceptive score, ranging from 0 to 3 was calculated by multiplying the time spent in each category by the category weight, summing these products and dividing by the total time for each 5 min block of time. The first 10 min post-formalin was considered as the early phase, and the time interval 15-60 as the late phase.

2.3. Data and Statistical Analysis

All values were given as mean ± S.E.M. Statistical analysis was carried out using student's paired t-test and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical P value less than 0.05 was considered significant.

3. Results

Body weight and serum glucose level were measured before and at 4th week after the experiment (Table 1). There were no significant differences between the groups before the experiment. At the end of 4 weeks, the body weight of the untreated ($P<0.005$) and NS-treated diabetic ($P<0.01$) rats was found to be significantly lower as compared to control rats. It was of interest that although weight of NS-treated diabetic rats was 6.7% greater than

Table 1. Body weight and serum glucose level of control, diabetic, and Nigella sativum (NS) extract - treated control and diabetic rats

	Body weight (g)		Serum glucose (mg/dl)	
	Week +0	Week +4	Week +0	Week +4
Control	238.1 ± 4.2	267.5 ± 5.9	102.7 ± 4.1	96.8 ± 3.5
Control + NS	237.7 ± 5.8	245.4 ± 4.3	99.8 ± 5.6*	91.7 ± 4.2
Diabetic	241.7 ± 4.3	190.7 ± 6.7 **	104.7 ± 4.7	379.8 ± 15.7 ***
Diabetic + NS	234.3 ± 3.7	211.4 ± 7.1*	96.5 ± 5.8	281.7 ± 12.9**

* P<0.01, ** P<0.005, *** P<0.001 (Compared to control)

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untreated-diabetic ones, but the existing difference was not significant. In addition, untreated- and NS-treated diabetic rats also had elevated serum glucose level over those of control rats (P<0.001). In this respect, treatment of diabetic rats with NS caused a significant reduction in the latter parameter in comparison with untreated-diabetic ones (P<0.05). On the other hand, although the weight and serum glucose level of NS-treated control rats was 8.2% and 5.2% lower than untreated-control animals, but the existing difference was not significant.

Formalin produced a marked biphasic response in the rats of all groups. Formalin-induced hyperalgesia was significantly (P<0.05) more marked in untreated-diabetic than in control rats in both phases of the formalin test (Fig. 2). Treatment of rats with sodium salicylate (200 mg/Kg, i.p.) caused a significant reduction (P<0.05) in nociceptive score only in the second phase of the formalin test as compared to control rats (Fig. 1). In contrast, treatment of non-diabetic rats with NS caused lower nociceptive scores in both phases of the formalin test (P<0.05) in comparison with untreated control ones (Fig. 1). Furthermore, diabetic animals receiving NS showed a less intensive nociceptive behavior, especially for the first phase of the test, ac compared to untreated diabetic rats.

4. Discussion

In this study, the possible antinociceptive effect of NS feeding in STZ-induced diabetic rats using formalin test was investigated. There are two main conclusions to be drawn from the obtained results as follows:

First, the results clearly demonstrated that there is an intensified nociceptive response in both phases of the formalin test in diabetic rats. It is a well-established fact that diabetic rats display exaggerated hyperalgesic behavior in response to noxious stimuli that may model aspects of painful diabetic neuropathy (7) and for this reason STZ-induced diabetic rats have been increasingly used as a model of painful diabetic neuropathy to assess the efficacies of potential analgesic agents (8). Although evaluation of mechanisms causing these symptoms is complicated because of the overlap between the systemic effects of hyperglycemia and its toxic effects within the peripheral nervous system, but direct functional toxicity of hyperglycemia in the peripheral nervous system (9), an increased activity of primary afferent fibers leading to an increased excitatory tone within the spinal cord, increased release of glutamate and activation of the NMDA receptor, reduced activity of both opioidergic and GABAergic inhibitory systems, decreased activity of nNOS-cGMP system in neurons of dorsal root ganglion, altered sensitivity of the dopaminergic receptors and altered responsiveness of the dopaminergic system, possibly through the enhancement and/or deactivation of the endogenous Met-enkephalinergic system, alterations in L-type Ca2+ channels and some changes in central and peripheral endogenous opiate levels could be involved in the modulation of nociception in diabetic rats (10-12).

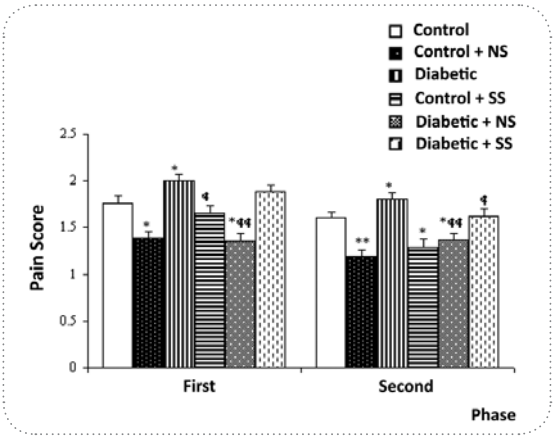

Figure 1. The effect of Nigella sativum feeding and sodium salicylate (SS, 200 mg/Kg) on nociceptive scores evoked by one injection of 2.5% formalin into the plantar surface of one hindpaw on nociceptive scores in first (early) and second (late) phases of the formalin test. All data represent mean ± S.E.M.

* P<0.05, ** P<0.01 (Compared to control);

□P<0.05, □□ P<0.01 (Diabetic compared to control)

Secondly, administration of NS could produce a significant antinociceptive effect in both phases of the formalin test in control and diabetic rats. On the other hand, sodium salicylate significantly reduced the nociceptive score only in the second phase of the formalin test. It has been known that centrally-acting drugs like narcotics inhibit both phases of the formalin test equally, while peripheral acting drugs like aspirin only inhibit the late phase (13). The effect of sodium salicylate in this study has been mediated through a peripheral mechanism, while the effect of NS could be mediated through a central and possibly, via a peripheral mechanism. One of the possible mechanisms which could partially explain the beneficial analgesic effect of NS in this study may be attributed to its hypoglycemic and antioxidant effect. Since hyperglycemia in diabetic state could induce some functional alterations in the nervous system, NS through lowering blood glucose could attenuate the hyperalgesia, as has been observed in the present study. On the other hand, since oxidative stress play a key role in the complications of diabetes, therefore, NS administration to diabetic animals could cause partial reversal of the disturbed antioxidant levels and peroxidative damage.

The data reported herein confirm that diabetes-induced hyperalgesia is attenuated following NS feeding as determined by formalin test and this may be of potential benefit in painful diabetic neuropathy.

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