The Effect of Oral Administration of *Withania Somnifera* Root on Formalin-Induced Pain in Diabetic Rats

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ABSTRACT

Introduction: Hyperalgesia is considered as one the marked signs of subchronic diabetes mellitus that could affect the life style of the patients. With considering the potential anti-diabetic effect of the medicinal plant Withania somnifera (WS)(ashwagandha), this study was designed to investigate the analgesic effect of WS on formalin-induced nociceptive responses (standard formalin test) in diabetic rats.

Methods: Rats were divided into control, WS-treated control, diabetic, sodium salicylate (SS)-treated control and diabetic and WS-treated diabetic groups. For induction of diabetes, streptozotocin (STZ) was used at a single dose. The treatment groups received oral administration of ashwagandha -mixed rat pellet (6.25%) for two months.

Results: The results showed that diabetic rats exhibited a higher score of pain at both phases of the formalin test and WS-treated diabetic rats exhibited a lower nociceptive score at both phases of the test ($p<0.05$). Meanwhile, SS administration significantly reduced pain score only at chronic phase of the test in the diabetic group ($p<0.01$).

Discussion: Taken together, these results indicate that two-month administration of ashwagandha could attenuate nociceptive score in an experimental model of diabetes mellitus and this may be considered as a potential treatment for 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 (1). 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 (2). This enhanced nociception has been observed in the early stages of diabetic neuropathy in STZ-diabetic rats (3). 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 (4). Although the use of herbal and natural supplements in some countries has increased dramatically in recent years (5), but claims of their therapeutic effects abound, medical research has not always supported their effectiveness (6). Therefore, this study was carried out to evaluate the antinociceptive effect of Withania somnifera (WS) in STZ-induced diabetic rats using standard formalin test.

2. Methods

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 265-335 g were housed in an air-con-

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ditioned colony room at 21 ± 1 °C and supplied with standard pellet 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 (n =54) were randomly divided into 5 experimental groups; i.e. control, WS-treated control, sodium salicylate (Sigma Chemical, St. Louis, Mo., USA)-treated diabetic rats used as positive control, diabetic, and WS-treated diabetic. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 60 mg Kg/l; Upjohn, St. Quentin en Yvelines, France) dissolved in cold 0.9% saline immediately before use. Powder of WS was mixed with standard rat chow at a weight ratio of 6.25% and administered for a period of two months starting on day +3. 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).

2.1. 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.

2.2. 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 8th week after the experiment. There were no significant differences between the groups before the experiment. At the end of 8 weeks, the body weight of the untreated ($P<0.005$) and WS-treated diabetic ($P<0.01$) rats was found to be significantly lower as compared to control rats.

In addition, untreated- and WS-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 WS caused a significant reduction in the latter parameter in comparison with untreated-diabetic ones ($P<0.05$).

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. 1). 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 WS caused lower nociceptive

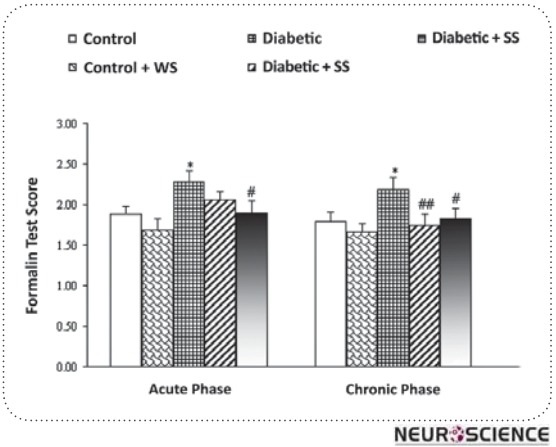

Figure 1. The effect of Withania somnifera feeding (6.25%) and sodium salicylate (SS, 200 mg/Kg) on nociceptive scores in first (early) and second (late) phases of the formalin test. All data represent mean ± S.E.M.

* $P<0.05$ (as compared to control)

# $P<0.05$, ## $P<0.01$ (as compared to control)

scores in both phases of the formalin test ($P<0.05$) in comparison with untreated control ones (Fig. 1). Furthermore, diabetic animals receiving WS 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 antinociceptive effect of Withania somnifera in STZ-induced diabetic rats using formalin test was investigated. The results 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 (2) 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 (3).

It was also demonstrated that administration of WS for two months 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 (7), while peripheral acting drugs like aspirin only inhibit the late phase (8). Therefore, the effect of sodium salicylate in this study has been mediated through a peripheral mechanism, while the effect of WS could be mediated through a central and possibly, via a peripheral mechanism.

Taken together, these results indicate that two-month administration of ashwagandha (withania somnifera) could attenuate nociceptive score in an experimental model of diabetes mellitus and this may be considered as a potential treatment for painful diabetic neuropathy.

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