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Original article

Diosgenin ameliorates development of neuropathic pain in diabetic rats: Involvement of oxidative stress and inflammation

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ABSTRACT

Neuropathic pain is one of the prevalent complications of diabetes mellitus (DM). Oxidative stress and inflammation are the principal determinants for its development. Pharmacological interventions targeted at alleviating or suppressing these pathways are clinically promising. Diosgenin is a natural steroidal saponin with anti-diabetic and multiple protective properties. This study was designed to study the efficacy of chronic diosgenin administration on alleviation of hyperalgesia in streptozotocin (STZ)-diabetic rats. Rats were allocated to control, diosgenin-treated control, diabetic, and diosgenin-treated-diabetic groups. Diosgenin was daily administered at a dose of 40 mg/kg for 5 weeks. Nociceptive behavior was assessed using paw pressure, hot tail immersion, and formalin tests. In addition, some oxidative stress and inflammation markers were measured. Diosgenin treatment of diabetic group increased mechanical and thermal nociceptive thresholds and lowered pain score at late phase of the formalin test, but not at its early phase. Biochemical analysis of serum samples and sciatic nerve and dorsal root ganglion (DRG) lysates showed restoration or improvement of nuclear factor-<kappa>B (NF-κB), malondialdehyde (MDA) level, activity of superoxide dismutase (SOD), catalase, tumor necrosis factor α (TNFα), and interleukin 1β (IL-1β) upon diosgenin treatment of diabetic rats. The obtained results exhibited antinociceptive potential of diosgenin in diabetic rats through lowering oxidative stress and inflammation and improving antioxidant defense system. This suggests possible therapeutic potential of diosgenin for alleviation and management of diabetic neuropathic pain.

1. Introduction

Diabetes mellitus (DM) is a major public health concern and a prevalent endocrine disease that is associated with development of severe complications in numerous tissues of the body. It is estimated that 415 million of adults have diabetes in the world and there are 318 million of undiagnosed adults with impaired glucose tolerance which puts them at high risk [1]. Increased sensitivity to noxious stimuli (known as hyperalgesia) is one of the prevalent complications of some diabetic patients [2]. Since as many as 39% of patients with chronic pain may be untreated, thus, managing diabetic neuropathic pain in clinical setting is an emerging therapeutic challenge [3]. Streptozotocin (STZ)-diabetic rats exhibit a hyperalgesic behavior following application of noxious stimuli and this is usually used as a reliable model for studying painful diabetic neuropathy [4–6] and this has also been used to test the efficacy of promising analgesic compounds [7]. Design of novel compounds for relief of painful diabetic neuropathy is strongly warranted. In this respect, management of DM and its complications using medicinal plants and their active ingredients have been increasingly employed in the society as reviewed before [8,9].

Diosgenin is a steroidal sapogenin present in fenugreek and *Dioscorea* spp. in the form of glycosides [10]. Until now, it has shown many advantageous effects like cardiovascular protective effect [11,12], anti-hyperglycemic and hypoglycemic activity [12,13], and hypolipidemic effect [14]. Diosgenin could also ameliorate oxidative stress and potentiate antioxidant defense

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system, lower inflammation, and is capable to exert an anti-apoptotic effect [11]. Diosgenin also attenuates cognitive deterioration and oxidative damage induced by D-galactose in senescent mice [15]. These properties mentioned for diosgenin strongly indicate that it may be an appropriate candidate to prevent or retard diabetic neuropathic hyperalgesia. To the best of our knowledge, there has not been any reports regarding its effect on nociceptive behavior in diabetic animals. Therefore, this study was done to study this issue and to explore possible involvement of oxidative stress and inflammation.

2. Materials and methods

2.1. Experimental design

In this study, we used male albino Wistar rats (procured from Pasteur's Institute, Tehran, Iran, 9–10 weeks old with a weight range of 210–250 g). Animals were held in an animal house (temperature at 21–23 °C and humidity at 40–60% with 12:12 h lighting period) with free access to food and water. Applied maneuvers were in line with NIH guidelines for the care and use of laboratory animals and were approved by Ethics Committee of Shahed University (Tehran, Iran) in 2014. The rats (n = 40) were randomly allocated to 4 equal-sized groups, i.e. control, diosgenin-treated control, diabetic, and diosgenin-treated diabetic. Diabetes was induced by a single *i.p.* injection of streptozotocin (STZ; 60 mg/kg; SigmaAldrich, USA) freshly dissolved in cold normal saline [12,16]. Seven days after STZ injection, overnight fasting blood samples were collected and serum glucose concentration was determined using glucose oxidase kit from ParsAzmun (Tehran, Iran). Animals with a serum glucose level above 250 mg/dl were nominated for further evaluation. Diosgenin (SigmaAldrich, USA) was administered *p.o.* using rat gavage needle at a dose of 40 mg/kg/day (dissolved in Kolliphor), started 8 days after STZ injection for 5 weeks. Dose of diosgenin was taken from our earlier study on its anti-diabetic and antioxidant activity [12]. Body weight was measured every week and serum glucose level was determined two days before STZ injection and 21 and 42 days after STZ injection. All behavioral tests were conducted from 10:00 a.m. till 04:00 p.m.) by a trained experimenter that was blind to treatments. Tests for assessment of pain including formalin, hot tail immersion, and paw pressure tests were conducted at 6th week after STZ injection with 2-day intervals between them to prevent and/or minimize animal sufferings.

2.2. Paw pressure test

For evaluation of mechanical hyperalgesia, paw pressure test was applied using rodent pincher analgesia meter (IITC Life Science, USA). This device allows measurement of mechanical nociceptive threshold in an accurate and simple method [17]. Increasing pressure was applied through a plastic tip attached to its pincher onto the dorsal surface between the third and fourth metatarsus of the left hind paw until the rat withdrew the paw, squeaked, or struggled. The cutoff force was set at 500 g to prevent tissue damage. In this test, three trials separated by at least 10 min were performed for each rat and mean value was the final data for each animal. The obtained thresholds were reported in grams.

2.3. Hot tail immersion test

Assessment of thermal hyperalgesic threshold was done using hot tail immersion test as described before [17]. After being adapted, animal tail was immersed in hot water (51 °C) and tail withdrawal latency was recorded with a cut-off time of 30 s to prevent tissue injury. This experiment was repeated four times for each rat with an inert-trial interval of 5 min and its average was obtained.

2.4. Formalin test

A previously used method was chosen for this test [5]. Briefly, each animal was adapted to the observation box for 10 min. Then, 50 μl of 2.5% formalin solution was injected into the plantar surface of one hind paw and the rat was placed in a Plexiglas box and observations lasted for 60 min. Nociceptive behavior was scored at 5-min blocks by measuring the amount of time spent in each of the four behavioral scores, i.e. "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 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 period was regarded as the early phase and time interval 15–60 was the late phase.

2.5. Biochemical assessment

2.5.1. Assay of serum and sciatic nerve and DRG level of MDA and SOD and catalase activity and serum TNFα and IL-1β

The animals were sacrificed at the end of 6th week post-STZ injection and after collection of blood samples via cardiac puncture, sciatic nerves (proximal to the trifurcation) and DRG at lumbar levels L4–L5 were quickly dissected out on an ice-chilled glass plate and tissue homogenates were prepared in lysis buffer (pH 7.4) containing protease inhibitor cocktail (SigmaAldrich, USA) and sera or supernatants were employed to estimate biochemical parameters as follows:

The MDA concentration (thiobarbituric acid reactive substances, TBARS) was measured as dsecribed before [18]. For measurement of MDA concentration as thiobarbituric acid reactive substances (TBARS), trichloroacetic acid (TCA) and TBARS reagent were added to supernatant sample, mixed and incubated at boiling water for 90 min. Upon rapid cooling on ice, samples were centrifuged at 3000 × *g* for 10 min and the absorbance was read at 532 nm and its final value was obtained from tetraethoxypropane standard curve.

Superoxide dismutase (SOD) activity was assayed in accordance to an earlier report [19]. For this purpose, supernatant sample was mixed with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min, and nitroblue tetrazolium was added. Thereafter, blue formazan was monitored at 550 nm using a spectrophotometer. "The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit (NU) of SOD activity".

For catalase activity assay, Claiborne's method was applied [20]. Briefly, H_2O_2 was added to a combination of potassium phosphate buffer (50 mM, pH 7.0) and supernatant and rate of H_2O_2 decomposition was determined for 2 min at 1 min intervals at 240 nm.

Serum level of TNFα and IL-1β and tissue level of NF-kB was determined using sandwich enzyme-linked immunosorbent assay and commercial kits according to manufacturer's instructions (Cloud-Clone Corp. (Houston, USA)). Absorbance of samples was determined at 450 nm by Synergy HT microplate reader (BioTek, USA) and values were reported as their final concentration according to plotted standard curves.