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Antinociceptive activity of *Tribulus terrestris* oral feeding in diabetic rats: Involvement of lipid peroxidation

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ABSTRACT

Background and Objective: Due to the presence of some evidence for anti-diabetic and antioxidant activity of *Tribulus terrestris* (TT), this study was designed to investigate the anti-nociceptive effect of *TT* in streptozotocin-diabetic rats using formalin test and hot tail immersion tests.

Materials and Methods: Rats were divided into control, TT-treated control, diabetic, sodium salicylate (SS)-treated diabetic (as positive control), and TT-treated diabetic groups. The treatment groups received oral administration of TT-mixed pelleted food (3%) for 5 weeks. Serum level of malondialdehyde (MDA) as a reliable marker of lipid peroxidation was also measured.

Results: TT treatment of diabetic rats reduced pain score only in chronic phase of the formalin test ($p<0.05$). Meanwhile, SS administration significantly reduced pain score only at chronic phase of the test ($p<0.05$). Regarding hot tail immersion test, diabetic rats showed a significant reduction in tail flick latency as compared to control ones ($p<0.05$). Although TT treatment of diabetic rats increased this latency relative to untreated diabetics but the existing difference was not statistically significant. TT treatment of diabetic rats also significantly reduced MDA level versus untreated diabetics ($p<0.01$).

Conclusion: Taken together, 5-week administration of TT could attenuate nociceptive score in chronic phase of formalin test in streptozotocin-induced experimental model of diabetes mellitus and has no significant effect on thermal pain threshold and part of its beneficial effect is exerted via attenuation of lipid peroxidation and possibly reduction of oxidative stress.

1. Introduction

Diabetes mellitus (DM) is regarded as one of the debilitating problems around the world and number of diabetic pateints is estimated to profoundly increase by the year 2030 (1). Uncontrolled continued chronic hyperglycemia in DM leads to severe complications including neuropathy, retinopathy, and autonomic dysfunctions. Diabetic neuropathy as observed with some deranged conditions of nociception (i.e. hyperalgesia) is the most common complication with an incidence of more than 50% (2). Diabetes-induced deficits in motor and sensory nerve conduction velocities and other manifestations of peripheral diabetic neuropathy have been well correlated with chronic hyperglycemia (3). Hyperglycemia could also lead to increased oxidative stress (enhanced free radical formation and/or a defect in antioxidant defenses), advanced glycation end product (AGE) production, and impaired nerve growth factor support (3, 4). Oxidative stress may be one of the major responsible mechanisms in the development of diabetic neuropathy (4).

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Some interests exist on the use of medicinal plant in reducing the devastating complications of DM (5, 6). 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 (7). 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 (7). This enhanced nociception has been observed in the early stages of diabetic neuropathy in STZ-diabetic rats (7). 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 (8, 9). On this bais, *Tribulus terrestris* (TT) has been known as a medicinal foodstuff with traditional use in diabetes (10). *Tribulus terrestris* L. (Zygophyllaceae) is a perennial creeping herb with a widespread distribution in Mediterranean, subtropical and desert climates worldwide. In traditional folk medicine, it has been used since ancient times as an aphrodisiac as well as to treat urinary infections, inflammation, oedema and other ailments (11, 12). The protective effect of TT in diabetes has already been established (10). It has also been shown that standardized aqueous extract of TT could attenuate hyperalgesia in experimentally induced diabetic neuropathic pain model via attenuation of oxidative stress and inflammation (13).

Therefore, this study was carried out to evaluate the antinociceptive effect of TT feeding in STZ-induced diabetic rats using standard formalin and tail immersion tests.

2. Materials and Methods

TT was obtained from the local grocery (Tehran, Iran) in June and was systemically identified by the botanists at Department of Biology (Shaheed Beheshti University, Tehran, Iran). Then, its powder was mixed with standard rat chow at a weight ratio of 3%.

2.1. Animals

Male albino Wistar rats (n=45) (Pasteur's institute, Tehran, Iran) weighing 180-240 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, TT-treated control, diabetic, sodium salicylate-treated diabetic used as positive control and TT-treated diabetic. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 60 mg/kg) 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 at 3rd and 6th weeks 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

In this test, each animal was acclimatized to the observation box before any testing began. Then, it was given a subcutaneous injection of 50 µl 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. Hot tail immersion test

Diabetic thermal hyperalgesia was assessed using tail immersion test. After adaptation, rat tail was immersed in warm water (51°C) and the tail flick response latency (withdrawal response of tail) was observed as the end point response. Each experiment was repeated 4 times for each animal and its average was reported. Meanwhile, a cut-off time of 30 s was also considered.

2.4. Assessment of serum lipid peroxidation

The animals were anesthetised at the end of 6th week post-STZ injection and after collection of blood samples via cardiac puncture and sera were collected to estimate biochemical parameters as follows:

The MDA concentration (thiobarbituric acid reactive substances, TBARS) was measured as dsecribed before (14). For measurement of MDA concentration as thiobarbituric acid reactive substances or 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 final values were calculated from the tetraethoxypropane standard curve.

2.5. Data and statistical analysis

All values were given as mean ± S.E.M. Statistical analysis was carried out using repeated measure one-way ANOVA and one-way ANOVA followed by Tukey's *post-hoc* test. A statistical p value less than 0.05 was considered significant.

3. Results

Body weight and serum glucose level were measured before and at 3rd and 6th week after the experiment (Figures 1-2). There were no significant differences between the groups before the experiment. At the end of 6 weeks, the body weight of the untreated (P<0.05) was found to be significantly lower as compared to control rats. It was of interest that weight of TT-treated diabetic rats was greater than untreated-diabetic ones and the existing difference was significant (p<0.05). In addition, untreated- and TT-treated diabetic rats also had elevated serum glucose level over those of control rats (P<0.005-0.001). In this respect, treatment of diabetic rats with TT caused a significant reduction in the latter parameter in comparison with untreated-diabetic ones (P<0.05). On the other hand, the weight and serum glucose level of TT-treated control rats was not significantly different versus untreated-control animals.

Formalin produced a marked biphasic response in the rats of all groups. Formalin-induced hyperalgesia was significantly (P<0.05-0.01) more marked in untreated-diabetic than in control rats in both phases of the formalin test (Fig. 3). Treatment of diabetic 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 diabetic rats. In contrast, treatment of non-diabetic rats with TT caused lower nociceptive scores in second phase of the formalin test (P<0.05) in comparison with untreated diabetics (Fig. 3).

A significant decrease in tail flick latency was observed after 6 weeks of diabetes in hot tail immersion test (p<0.05) (Fig. 4) and this deficit in tail flick response latency was not significantly reversed on treatment with TT.

Measurement of serum MDA level in different groups showed that diabetic rats had a significantly higher serum level of MDA as a reliable marker of lipid peroxidation relative to control (p<0.01) and this elevation was significantly attenuated following TT treatment (p<0.05).

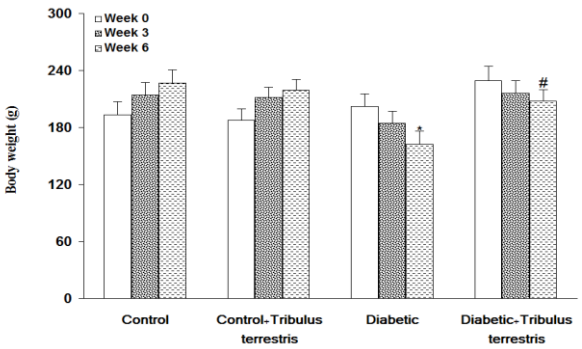

Fig. 1. Body weight in different weeks (means ± S.E.M).
* p<0.05 (as compared to week 0 in the same group);
# p<0.05 (relative to diabetic group in the same week)

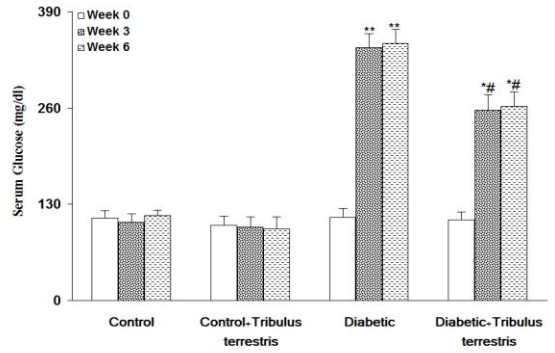

Fig. 2. Serum glucose concentration in different weeks (means ± S.E.M).
* p<0.005, ** p<0.001 (as compared to week 0 in the same group); # p<0.05 (relative to diabetic group in the same week)

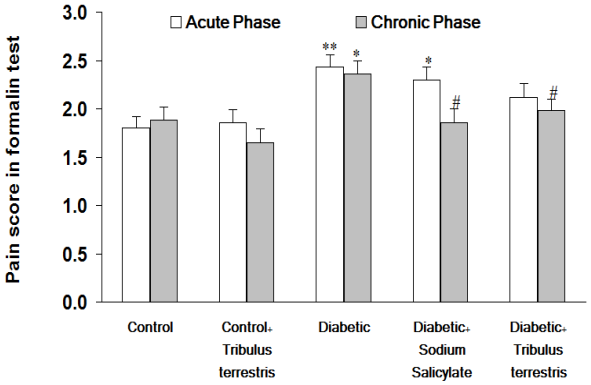

Fig. 3. The effect of TT and sodium salicylate (SS) on nociceptive scores in the first (early) and second (late) phases of the formalin test. All data represent mean ± S.E.M.
* p<0.05, ** p<0.01 (as compared to control); # p<0.05 (as compared to diabetic)

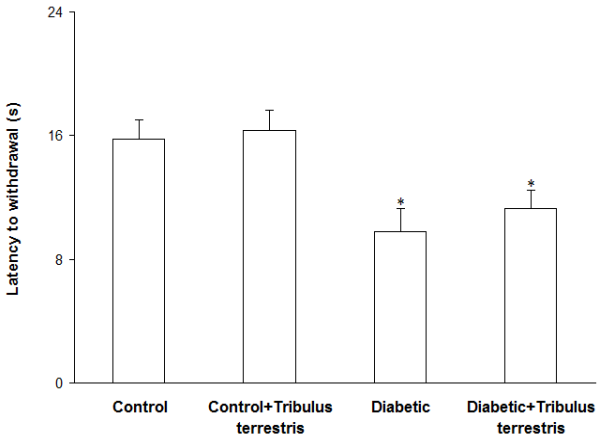

Fig. 4. Effect of TT on hyperalgesia in hot tail immersion test. All data represent mean ± S.E.M.
* p<0.05 (as compared to control)

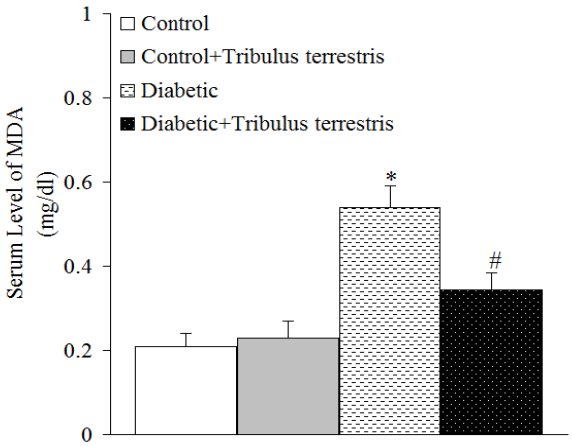

Fig. 5. Effect of TT on serum MDA levelin different groups. All data represent mean ± S.E.M.
* p<0.01 (as compared to control), # p<0.05 (as compared to diabetic)

4. Discussion

In this study, the possible antinociceptive effect of TT feeding in STZ-induced diabetic rats using formalin test and hot tail immersion test was investigated. The obtained results showed that TT feeding could attenuate nociceptive score in chronic phase of formalin test in streptozotocin-induced experimental model of diabetes mellitus and has no significant effect on thermal pain threshold and part of its beneficial effect is exerted via attenuation of lipid peroxidation and possibly through reduction of oxidative stress.

Our results clearly showed that there is an enhanced nociceptive reactivity in both phases of the formalin test in diabetic rats. It has been reported taht diabetic rats exhibited an elevated hyperalgesic response to painful stimuli of different natures that may mimic parts of painful diabetic neuropathy (15) and on this basis STZ-induced diabetic rats have been routinely applied as a valid model of painful diabetic neuropathy to test possible analgesic or hypoalgesic agents (16). Although exact mechanisms responsible for these problems have not been fullu elucidated, hyperglycemia-induced toxicity in the nervous system (17), an elevated activity of afferent fibres terminating in the spinal cord, enhanced level of glutamate and overactivation of NMDA receptors, a lower activity of opioidergic and GABAergic inhibitory systems, altered reactivity of dopaminergic receptors and changed reactivity of dopaminergic system, changes in activity of calcium channels and alterations in endogenous

opiate levels and decreased activity of nNOS-cGMP system in neurons of dorsal root ganglion may be involved in the nociceptive modulation in diabetic state (15, 16, 18, 19).

In our study, TT feeding for a period of 5 weeks produced a significant antinociceptive effect in chronic phase of the formalin test ion STZ-diabetic rats that was the same as sodium salicylate-treated group. It has been shown that centrally-acting drugs like narcotics could inhibit both phases of the formalin test (20) and peripherally-acting agents including aspirin could suppress the chronic phase of the formalin test (21, 22). Thus, it is logical that analgesic effect of sodium salicylate in our study has been via a peripheral mechanism and TT has exerted its benbeficial effect in the same manner. Possible mechanisms involved in analgesic effect of TT in present study may be due to its hypoglycemic and anti-hyperglycemic and antioxidative effect that has been reported in earlier studies (13, 23). In this respect, a previous study showed that standardized aqueous extract of Tribulus terristris (known also as nerunjil) is able to increase the superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, and decrease the lipid peroxide levels in a dose-dependent manner and to increase pain threshold response (13). It is possible that part of beneficial effect of TT in our study has been mediated through potentiating antioxidant defensive system.

In summary, our finding showed that administration of TT could lower nociceptive score in chronic phase of the formalin test in STZ-induced experimental model of diabetes mellitus and has no significant effect on thermal pain threshold and part of its beneficial effect is exerted via attenuation of lipid peroxidation and possibly reduction of oxidative stress.

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