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Curcumin Alleviates Streptozotocin-Induced Hyperalgesia in Diabetic Neuropathic Rat via Attenuation of Lipid Peroxidation and Inflammation

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ABSTRACT

Background and Objective: Hyperalgesia is one of the marked clinical signs of patients with diabetes mellitus that affects their lifestyle. This study was conducted to assess the anti-nociceptive effect of chronic administration of curcumin in streptozotocin (STZ)-diabetic rats using formalin, hot tail immersion, and paw pressure tests.

Materials and Methods: This study was conducted on 40 male rats that were divided into control, curcumin-treated control, diabetic, and curcumin-treated diabetic groups. The treatment groups received i.p. administration of curcumin at a dose of 50 mg/kg for 4 weeks. Finally, hyperalgesia were assessed using standard tests. Meanwhile, serum MDA and TNF level was also measured.

Results: Curcumin treatment of diabetic rats significantly reduced pain score in chronic phase of formalin test ($p<0.05$), increased tail flick latency ($p<0.05$) and vocalization score ($p<0.05$) as compared to diabetic group. Curcumin treatment also decreased serum level of MDA ($p<0.05$) and TNF ($p<0.05$).

Conclusion: Chronic administration of curcumin has anti-nociceptive property, partly via attenuation of lipid peroxidation and inflammatory processes.

Keywords: Curcumin, Diabetes mellitus, Anti-nociceptive, Formalin test, Hot tail immersion test, Paw pressure test, Lipid peroxidation, Inflammation

1. Introduction

Painful neuropathy is one of the debilitating complications of diabetes mellitus and is considered a growing healthcare problem (1). Unfortunately, existing treatments strategies are of variable efficacy and do not target underlying pathogenic mechanisms, in part because these mechanisms are not well defined (1). Rat and mouse models of DM are usually used to investigate diabetic neuropathy, with rats in particular being consistently reported to show allodynia and hyperalgesia (1-3). Measures of evoked mechanical, thermal and chemical pain can provide insight into the pathogenesis of the condition (1). Diabetes-induced neuropathy has been well correlated with chronic hyperglycemia (3). Hyperglycemia could lead to increased oxidative stress (enhanced free radical formation and/or a defect in antioxidant defenses), advanced glycation end product formation, impaired nerve growth factor support and augmented inflammatory processes (1).

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Curcumin is a naturally occurring yellow polyphenolic pigment isolated from the rhizome of the plant Curcuma longa. This compound has been known to exert anti-nociceptive effect due to its antioxidant and anti-inflammatory property (4). Evidence on curcumin effectiveness in treating diabetic neuropathy is accumulating (4).

Antioxidant, anti-diabetic, and anti-inflammatory activity of curcumin has been reported (5). This compound shows its cytoprotective effect by scavenging free radicals, reducing pro-inflammatory cytokine TNF-α and attenuating NF-κB (6, 7). We designed this study to investigate the effect of chronic curcumin treatment on hyperalgesia in streptozotocin (STZ)-diabetic neuropathic rat using standard formalin, hot tail immersion, and paw pressure tests and to evaluate the role of oxidative stress and inflammation.

2. Materials and Methods

2.1. Animals

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 190-260 g were housed in an air-conditioned colony room on a 12/12 cycle (21-23°C and 30-40% humidity) and supplied with standard diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with NIH guidelines for the care and use of laboratory animals.

2.2. Experimental protocol

This study was conducted at School of Medicine (Shahed University) in 2014. The rats (n = 40) were randomly allocated and similarly grouped into 4 groups: control, curcumin-treated control, diabetic, and curcumin-treated diabetic. The rats were rendered diabetic by a single intraperitoneal injection of 60 mg kg^{-1} STZ (Sigma-Aldrich, USA) freshly dissolved in cold normal saline. Control animals received equivalent volume of normal saline and vehicle. Diabetes was confirmed by the presence of hyperglycemia, polyphagia, polydipsia, polyuria, and weight loss. One week after STZ injection, blood samples were collected and serum glucose concentrations were measured using glucose oxidation method (Zistshimi, Tehran). Only those animals with serum glucose level higher than 250 mg/dl were selected as diabetic for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. Curcumin (Sigma-Aldrich, USA) was daily administered i.p. at a dose of 50 mg/kg for a period of 4 weeks. Curcumin was dissolved in 10% Cremophor. Changes in body weight, food consumption and water intake were regularly observed during the experimental period.

2.3. Formalin 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. It was then immediately placed in a Plexiglas box. Observations continued for the next 60 min. A nociceptive score was determined for 5-min blocks 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-injection was considered as the early (first) phase and the time interval 15–60 as the late (second) phase.

2.4. Hot tail immersion test

Diabetic thermal hyperalgesia was assessed using tail immersion test. After adaptation, rat tail was immersed in hot water (50°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 with an interval of 2 min and its average was reported. Meanwhile, the cut-off time was 30s.

2.5. Paw pressure test

To test the effect of curcumin on mechanical hyperalgesia, the vocalization thresholds of paw pressure in diabetic rats were measured as described before (2) using an analgesia-meter. Increasing pressure was applied through a plastic

tip onto the dorsal surface between the third and fourth metatarsus of the left hind paw until the rat squeaked. Vocalization thresholds were expressed in grams, and the cutoff was 500 g. Threshold measurements were repeated three times and the average was taken.

2.6. Determination of serum malondialdehyde (MDA) and TNFα concentration

The MDA concentration (thiobarbituric acid reactive substances, TBARS) in the serum was measured. Briefly, 1.0 mL of 20% trichloroacetic acid and 1.0 mL of 1% TBARS reagent were added to 100 µL of serum, then mixed and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at 1000×g for 5 min and the absorbance of the supernatant was read at 532 nm. TBARS results were expressed as MDA equivalents using tetraethoxypropane as standard. TNF□□was determined by rat tumor necrosis factor α ELISA kit (Sigma-Aldrich, USA) and using a microplate reader.

2.7. Statistical analysis

All results are expressed as means ± S.E.M. For multiple comparisons, one-way analysis of variance (ANOVA) was used. When ANOVA showed significant difference, Tukey's post hoc test was applied. Statistical significance was regarded as $p<0.05$.

3. Results

After 6 weeks, the weight of the diabetic rats was found to be significantly decreased as compared to control rats ($p<0.05$) and curcumin treatment caused a lower decrease in diabetic rats as compared to diabetics, so that their weight was significantly higher ($p<0.05$) (Fig. 1). In addition, diabetic rats had also an elevated serum glucose level over those of control rats ($p<0.005$) and treatment of diabetic rats with curcumin caused a significant decrease in the serum glucose ($p<0.01$) relative to vehicle-treated diabetics. Meanwhile, curcumin treatment of control rats did not cause any significant change regarding serum glucose level (Fig. 2).

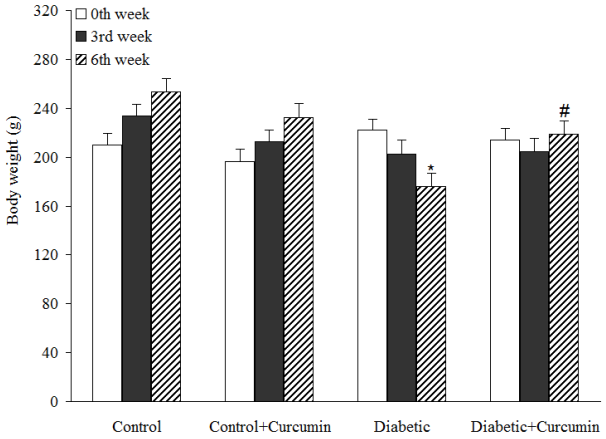

Fig. 1: Body weight in different weeks (means ± S.E.M).
* $p<0.05$ (as compared to week 0 in the same group), # $p<0.05$ (as compared to diabetics in the same week)

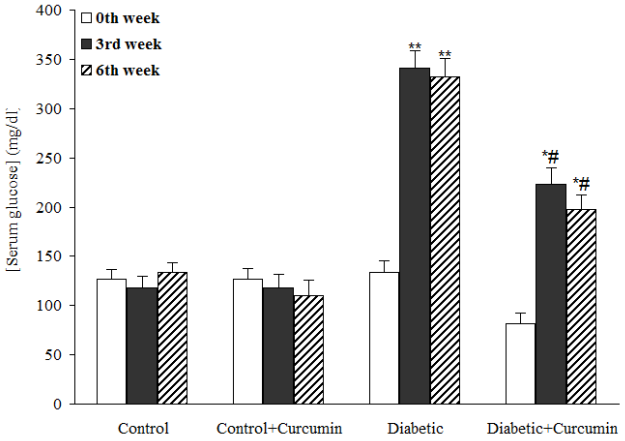

Fig. 2: Serum glucose concentration in different weeks (means ± S.E.M).
* $p<0.01$, ** $p<0.005$ (as compared to week 0 in the same group), # $p<0.01$ (as compared to diabetics in the same week)

3.1. Formalin test

Hind limb formalin injection produced a marked biphasic response in the rats of all groups (Fig. 3). Hyperalgesia was significantly ($p<0.05$) greater in diabetics than in control rats only in late phase of the test. In addition, chronic treatment of diabetic rats with curcumin significantly caused lower nociceptive scores in late phase of the formalin test as compared to diabetic group ($p<0.05$). No such effect was observed for curcumin-treated controls.

3.2. Thermal hyperalgesia

A significant decrease in tail flick latency was observed in diabetic rats after 6 weeks in hot tail immersion test ($p<0.01$) (Fig. 4). This deficit in tail flick response latency was significantly reversed on treatment with curcumin ($p<0.05$).

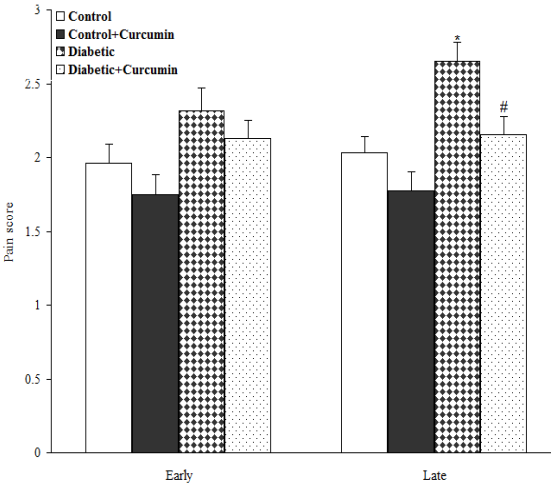

Fig. 3: The effect of curcumin on nociceptive scores in the first (early) and second (late) phases of the formalin test. All data represent mean ± S.E.M.
* $p<0.05$ (as compared to control); # $p<0.05$ (as compared to diabetic)

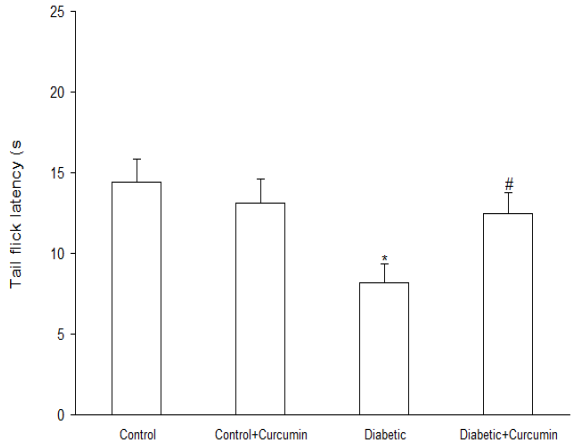

Fig. 4: The effect of curcumin treatment on nociception threshold in hot tail immersion test. All data represent mean ± S.E.M. * $p<0.05$ (as compared to control), # $p<0.05$ (as compared to diabetic)

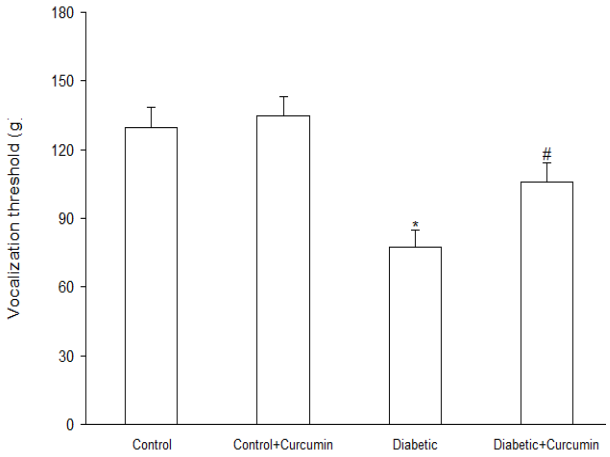

Fig. 5: The effect of curcumin treatment on vacalization threshold in paw pressure test. All data represent mean ± S.E.M. * $p<0.01$ (as compared to control), # $p<0.05$ (as compared to diabetic)

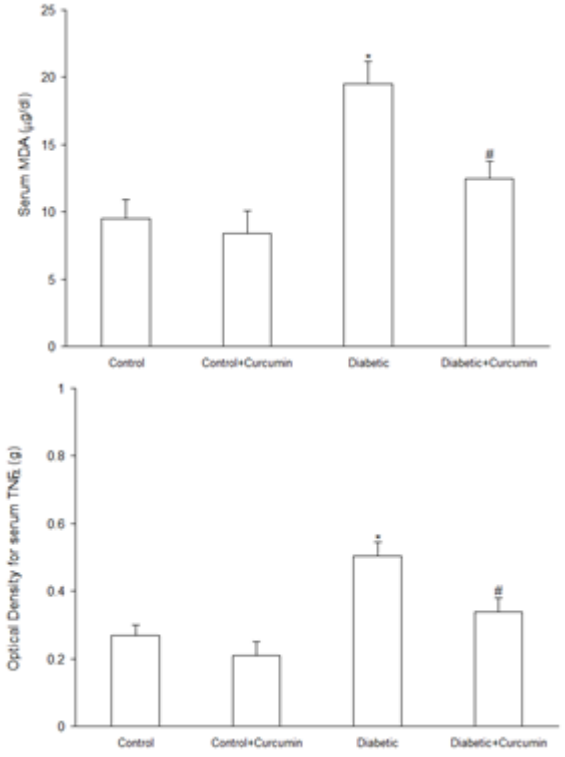

Fig. 6: Serum MDA and TNFα concentration in different groups.
* $P<0.01$ (in comparison with control); # $P<0.05$ (in comparison with diabetic)

3.3. Thermal and mechanical hyperalgesia

A significant decrease in tail flick latency was observed after 6 weeks in diabetic rats in hot tail immersion test ($p < 0.05$). This decrease in tail flick response latency was significantly ($p<0.05$) reversed on treatment with curcumin (Fig. 4). To examine the effect of curcumin on mechanical hyperalgesia, diabetic rats were subjected to the Randall-Selitto test. The vocalization threshold in diabetic rats was lower than that control ($p<0.01$) and curcumin treatment increased the vocalization threshold in diabetic rats ($p<0.05$) (Fig. 5).

3.4. Markers of lipid peroxidation and inflammation

Regarding serum lipid peroxidation (Fig. 6), STZ-induced diabetes resulted in a significant elevation of MDA ($p<0.01$) and treatment of diabetic rats with curcumin significantly attenuated the increased MDA content ($p<0.05$). Meanwhile, serum TNF□ level increased significantly in diabetic group ($p<0.01$) and treatment of diabetic rats with curcumin significantly lowered it ($p<0.05$).

4. Discussion

In this study, development of diabetic neuropathy in STZ-induced diabetic rats was confirmed after 6 weeks, which was consistent with previous reports (2). It has been reported that oxidative stress is responsible for pain impaired processing in diabetic condition (2). Increased free radical mediated-toxicity has been well documented in DM (8). In addition, inflammatory changes are also observed in DM as shown by a higher level of NF-kB and TNF□ (9). NF-kB is a key mediator that regulates immune and inflammatory responses and modulates multiple proinflammatory target genes in endothelial cells, vascular smooth muscle cells, and macrophages (9). The activation of NF-kB leads to the increased production of adhesion molecules, leukocyte-attracting chemokines, various inflammatory cytokines including TNF-alpha and IL-6, and NO through iNOS expression (9).

In our study, administration of curcumin for 4 weeks produced a significant analgesic effect at late phase of the formalin test in diabetic rats in diabetic rats. It has been known that central acting drugs like narcotics inhibit both phases of the formalin test equally, while peripheral acting drugs like aspirin only inhibit the late phase (2). Therefore, the effect of curcumin in this study could be mediated possibly through a peripheral mechanism. One of the possible mechanisms that could partially explain the beneficial analgesic property of curcumin may be attributed to its hypoglycemic and antioxidant effects. Since hyperglycemia in diabetic state could induce some functional alterations in the nervous system (4), curcumin may have attenuated the hyperalgesia in diabetic rats through lowering blood glucose and via attenuation of oxidative stress that was observed in this study. Part of beneficial effect of curcumin in this study could be attributed to its inflammatory effect. As recent evidence shows, many diabetic complications are associated with inflammation and elevated blood and neural tissue inflammatory proteins both in the human and experimental STZ neuropathy. It has also been shown that metabolic changes induced by hyperglycemia lead to deregulation of cytokine control. In this respect, TNFα, IL1β and IL6 content increases in STZ-treated animals (10) that may be reverted by curcumin. The latter compound has shown anti-inflammatory properties (7). In an earlier study, Roghani et al also reported the analgesic effect of curcumin treatment in STZ-diabetic rats and proved the involvement of lipid peroxidation in this respect (11). In our study, more emphasis was on other analgesic tests like paw pressure test and the participation of inflammatory processes in its efficacy.

Taken together, chronic treatment with curcumin showed anti-hyperglycemic and anti-nociceptive effect in diabetic rats and it seems that curcumin may be a good candidate as an anti-diabetic drug to prevent the development of some diabetic complications.

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