Lycopene ameliorates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rat

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Peripheral neuropathy is one of the common complications of diabetes mellitus. It is frequently associated with debilitating pain. The present study was designed to investigate the effect of lycopene, a carotenoid found in tomatoes, on hyperalgesia and cold allodynia in streptozotocin (STZ) induced diabetic rats. After 4-weeks of STZ injection, diabetic mice exhibited a significant thermal hyperalgesia cold allodynia, hyperglycemia and loss of body weights as compared with control rats. Chronic treatment of lycopene for 4 weeks significantly attenuated the cold allodynia and thermal hyperalgesia. The results emphasize the role of antioxidant such as lycopene as an adjuvant therapy in the treatment of diabetic neuropathy.

Keywords: Diabetic neuropathy, Hyperalgesia, Lycopene, Oxidative stress

Materials and Methods

Animals—Male Wistar rats (200-250 g) procured from Central Animal House facilities of Panjab University, were housed under optimal laboratory conditions maintained on 12:12 hr L:D cycle and had free access to food and water. All animals were acclimatized to laboratory conditions before the experiment. All experiments were carried out between 0900 and 1700 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of animals.

Drugs and reagents—Streptozotocin and lycopene (Sigma, St. Louis, MO, USA), and a glucose oxidase peroxidase diagnostic enzyme kit (Span Diagnostic Chemicals, India), were used in the study.

Induction and assessment of diabetes—A single dose of 65 mg/kg streptozotocin in citrate buffer (pH 4.4, 0.1M) was injected ip to induce diabetes. The age-matched control rats received an equal volume of citrate buffer. Diabetes was confirmed after 48 hr of streptozotocin injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by enzymatic glucose oxidase peroxidase (GOD-PAP) diagnostic kit method. The rats having plasma glucose levels more than 250 mg/dL were selected and used for the present study. Body weight and plasma glucose levels were also
measured before and at the end of the experiment to see the effect of lycopene on these parameters.

**Treatment schedule**—After a basal reading at 4th week of streptozotocin injection, control and diabetic mice were randomly selected and divided into three groups of 6–7 animals each. First group comprised control animals, second group the diabetic control, and the third group consisted of diabetic animals treated with lycopene (4 mg/kg/day) orally. Starting from 4th week till 8th week, groups 1 and 2 received vehicle of lycopene and group 3 received solution of lycopene. Lycopene was first dissolved in 5% Tween 80 and then in double distilled water. Drug solution was freshly prepared and administered in a constant volume of 1 ml/100 g body weight.

**Assessment of thermal hyperalgesia**

Tail-immersion (warm water) test: Tail of mice was immersed in a warm water bath (52.5°C±0.5°C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off time 12 sec). Shortening of the tail withdrawal time indicates hyperalgesia and is attributed to central mechanisms.

Tail-immersion (cold water) test: Tail of mice was immersed in cold water (10°C±0.5°C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off time 15 sec). Shortening of the tail withdrawal time indicates allodynia.

Hot-plate test: The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms. In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55°C±1°C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut-off time was 10 sec in order to avoid damage to the paw.

**Statistical analysis**—Results were expressed as mean ± SE. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Dunnet’s t-test to assess the significance. Statistical significance was considered at \( P<0.05 \). The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

**Results**

**Effect of lycopene on blood glucose and body weights**—After 4 weeks of streptozotocin injection, diabetic rats exhibited significant increase in plasma glucose levels and a significant decrease in the body weights. Lycopene treatment significantly decreased the plasma glucose levels \([P<0.001; 295±10.5 \, \text{mg/dl} \text{ as compared to diabetic rats (} 540±7.48 \, \text{mg/dl})]\] at the end of the 8th week. The body weight was also significantly improved on treatment with lycopene \([P<0.001] \, 257±2.14 \, \text{g as compared to the diabetic mice}\).

**Effect of chronic lycopene treatment on nociceptive threshold in tail-immersion (warm water) test**—At the end of the 4th week, diabetic group exhibited significant decrease in pain threshold from noxious stimuli as compared to control rats \((P<0.001)\). Lycopene administration (4 mg/kg) for 4 weeks starting from 4th week significantly increased the pain threshold from 4th to 8th week compared to control diabetic rats \((P<0.001, \text{Fig. 1})\).

**Effect of chronic lycopene treatment on nociceptive threshold in tail-immersion (cold water) test**—At the end of the 4th week, diabetic animals exhibited significant decrease in pain threshold from non-noxious stimuli as compared to control rats \((P<0.001)\). Lycopene administration (4 mg/kg) for 4 weeks starting from 4th week significantly increased the pain threshold from 4th to 8th week compared to control diabetic rats \((P<0.001, \text{Fig. 2})\).

**Effect of chronic lycopene treatment on nociceptive threshold in hot plate tests**—At the end of the 4th week, diabetic animals exhibited significant decrease in pain threshold from noxious stimuli as compared to control rats \((P<0.001)\). Lycopene administration (4 mg/kg) for 4 weeks starting from 4th week significantly increased the pain threshold from 4th to 8th week compared to control diabetic rats \((P<0.001, \text{Fig. 3})\).

![Fig. 1—Effect of chronic treatment of lycopene on pain threshold in STZ-induced diabetic rats subjected to warm water tail immersion test [Values are mean ± SE. *P<0.001 compared to control (a) and diabetic (b)]](image-url)
Discussion

The markedly decreased nociceptive thresholds in STZ-injected diabetic rats indicated development of significant thermal hyperalgesia and cold-allodynia. The hyperalgesic response in the tail withdrawal test is generally attributed to central mechanisms, whereas the hyperalgesic response on the hot plate is considered to result from a combination of both central and peripheral mechanisms.

Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy. Present study on the tail-immersion and hot-plate methods indicates that lycopene prevents both spinal and supraspinal neuropathy in diabetic rats. Dimethylthiourea, a hydroxyl radical scavenger, also prevented the development of mechanical hyperalgesia in the diabetic rats. Lycopene is a powerful antioxidant with a singlet-oxygen-quenching capacity greater than that of β-carotene and vitamin E by 47 and 100 times, respectively. Lycopene is also a potent antiproliferative, anti-inflammatory, hypocholesterolemic, TNF-α, and peroxynitrite inhibitory agent. Lycopene modulates cyclooxygenase synthesis pathway. Lycopene can trap singlet oxygen and reduce mutagenesis in the Ames test. The antioxidant activity of carotenoids in multilamellar liposomes has been assayed by inhibition of formation of thiobarbituric acid reactive substances. Thus the antinociceptive effect of lycopene may be attributed to its powerful antioxidant activity. However, lycopene may have improved neuronal blood flow by its direct vasorelaxant properties.

In conclusion, lycopene may constitute a potential therapeutic adjuvant in antidiabetic regimen for diabetic neuropathic pain.

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References


