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# Quercetin attenuates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rats

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Neuropathic pain is one of the important microvascular complications of diabetes. Oxidative stress and superoxide play a critical role in the development of neurovascular complications in diabetes. Aim of the present study was to evaluate the effect of quercetin, a bioflavonoid on thermal nociceptive responses in streptozotocin (STZ)-induced diabetic rats assessed by tail-immersion and hot plate methods. After 4-weeks of a single intravenous STZ injection (45 mg/kg body weight), diabetic rats exhibited a significant thermal hyperalgesia and cold allodynia along with increased plasma glucose and decreased body weights as compared with control rats. Chronic treatment with quercetin (10 mg/kg body weight; p.o) for 4-weeks starting from the 4th week of STZ-injection significantly attenuated the cold allodynia as well as hyperalgesia. Results indicate that quercetin, a natural antioxidant, may be helpful in diabetic neuropathy.

**Keywords:** Diabetic neuropathy, Hyperalgesia, Quercetin, Cold allodynia, Tail-immersion, Hot plate.

Neuropathic pain is one of the most common complications in diabetes mellitus. It is mostly characterized by hyperalgesia and allodynia. Clinical and experimental studies have revealed that reactive oxygen species (ROS) play a significant role in pathophysiology of neuropathic pain in diabetes. Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone) is a phenolic compound widely distributed in the plant kingdom. It is found in many frequently consumed foods, including apples, onion, tea, berries and brassica vegetables. Renewed interest has been observed in recent years on multiple activities of novel bioflavonoids. They are reported to have many beneficial effects on human health, including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergic activity, cataract prevention, antiviral activity and anti-inflammatory effects[1, 2]. There are sporadic reports on the analgesic activity of certain flavonoids such as hydroxy ethyl rutoside[3] and gossypin[4]. Recently it has been reported that quercetin has analgesic activity[5] in naive animals tested in few test systems[6]. Moreover, quercetin has been reported to inhibit oxidative damage in various tissues of STZ-induced diabetic rats[7].

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Based on analgesic and antioxidant properties of quercetin, the present study has been aimed to evaluate the effect of quercetin on thermal hyperalgesia and cold allodynia, the indices of diabetic neuropathic pain in rats.

## Materials and Methods

*Animals*—Male Sprague-Dawley rats (200-250 g) bred in Central Animal House facilities of Panjab University were housed under optimal laboratory conditions. Animals were acclimatized to laboratory conditions before the experiments that were carried out between 0900 and 1700 hr. Approval of Institutional Animal Ethics Committee was obtained.

*Treatment schedule*—After a basal reading at 4th week of STZ injection, control and diabetic rats were randomly selected and divided in three groups of 6-7 animals each. i.e. control, diabetic control and diabetic group treated with quercetin. Starting from the 4th week till 8th week, the control and diabetic control groups received vehicle of quercetin and another diabetic treated group received suspension of quercetin (10 mg/kg body weight/day) orally. Quercetin (Sigma Chemical, St.Louis, MO, USA) suspension was prepared in 0.5 % carboxy methyl cellulose solution. All the drugs were administered in a constant volume of 0.5 ml/ 100g body weight of rat.

*Induction and assessment of diabetes*—A single dose of 45-mg/kg body weight STZ (Sigma Chemical,

St.Louis, MO, USA) prepared in citrate buffer (*p*H 4.4, 0.1 M) was injected through tail vein[8]. The age matched control rats received citrate buffer and used along with diabetic animals. Diabetes was confirmed after 48 hr of STZ injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by enzymatic GOD-PAP (glucose oxidage peroxidase) diagnostic kit method (Span Diagnostic Chemicals, India)[9]. The rats having plasma glucose levels more than 250 mg/dl[10] were selected and used for the present study. Body weight and plasma glucose level were also measured before and at the end of experiment to see the effect of quercetin on these parameters.

*Assessment of thermal hyperalgesia and cold allodynia—Tail-immersion (warm water) test:* Tail of rat was immersed in a warm water (47° ± 1°C) bath until tail withdrawal (flicking response) or signs of struggle were observed (cut-off 15 sec). Shortening of the tail-withdrawal time indicates hyperalgesia.

*Tail-immersion (cold water) test*—The procedure was same as warm water test but the temperature of water was set at 10° ± 0.5°C, a temperature that is normally innocuous[11]. The shortened duration of tail immersion indicates allodynia. The cut-off time was 15 sec.

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

*Statistical analysis*—The nociceptive threshold (latency in seconds) to thermal noxious and non-noxious stimuli was measured and expressed as mean ± S.E. The hyperalgesic and allodynia responses were analysed by ANOVA followed by Tukey's *t* test. $P < 0.05$ was considered as significant. Student's *t* test was used to compare the values from two groups.

## Results

*Blood glucose and body weights*—Diabetic rats exhibited significantly increased plasma glucose levels compared to control rats ($P<0.001$). There was a marked decrease in the body weight of STZ-injected rats as compared to control rats ($P<0.001$). These parameters were unaffected by quercetin treatment.

*Effect of chronic quercetin treatment on diabetic pain threshold in tail-immersion (warm water) tests*—At the end of 4th week, diabetic animals exhibited significant decrease in pain threshold from noxious stimuli as compared to control rats ($P<0.001$). Quercetin administration from the 4th week to 8th week significantly increased the tail withdrawal latencies from 4th to 8th week compared to control diabetic rats ($P<0.001$, Fig. 1A).

*Effect of chronic quercetin treatment on diabetic pain threshold in tail-immersion (cold water) tests*—At the end of 4th week, diabetic animals exhibited significant decrease in pain threshold from non-noxious stimuli as compared to control rats ($P<0.001$). Quercetin administration from the 4th week to 8th week significantly increased the tail withdrawal latencies from 4th to 8th week compared to control diabetic rats ($P<0.001$, Fig. 1B).

*Effect of chronic quercetin treatment on diabetic pain threshold in hot plate tests*—At the end of 4th week, diabetic animals exhibited significant decrease in pain threshold as compared to control rats ($P<0.05$). Quercetin administration from the 4th week to 8th week significantly increased the tail withdrawal latencies from 4th to 8th week compared to control diabetic rats ($P<0.001$, Fig. 1C).

## Discussion

The markedly decreased nociceptive thresholds in STZ-injected diabetic rats as compared with control rats indicated development of significant thermal hyperalgesia and cold-allodynia. Mechanical hyperalgesia, thermal allodynia and formalin-evoked flinching in STZ-rats have been demonstrated previously[12, 13]. The hyperalgesic response in the tail-withdrawal test is generally attributed to central mechanisms[14, 15], whereas the hyperalgesic response on the hot plate is considered to result from a combination of both central and peripheral mechanisms[15].

Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy[16]. Present study on the tail-immersion and hot plate methods indicates that quercetin prevents the both spinal and supraspinal neuropathy in diabetic mice. This is in agreement with the reports where glutathione (GSH) and alpha-lipoic acid, well known, antioxidants significantly prevented thermal and mechanical hyperalgesia[17,18]. It is also reported that dimethylthiourea, a hydroxyl scavenger, also prevented the development of mechanical hyperalgesia in the diabetic rats[19]. The mechanism by

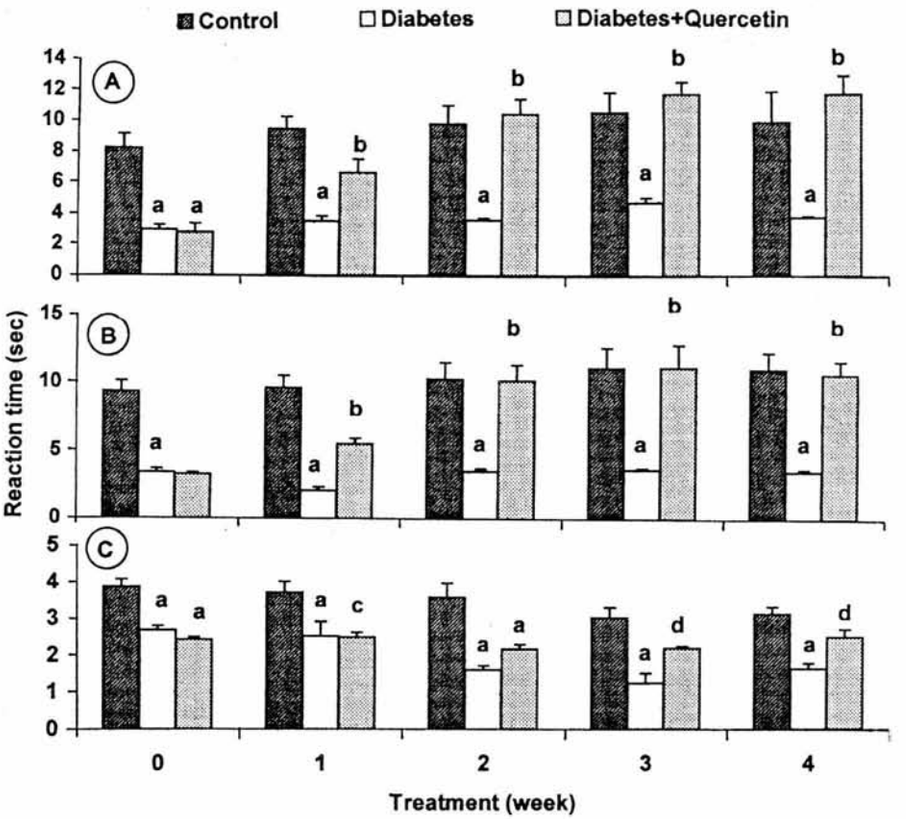


Fig. 1—Effect of chronic treatment of quercetin on pain threshold values in STZ-injected diabetic rats subjected to the tail immersion test in (A) hot water (47° ± 1 °C), (B) cold water (10° ± 0.5 °C) and (C) the hot plate test (55° ± 1 °C). [Values are in mean ± S.E. *P* values; [a]<0.001 as compared with control rats at before and respective week; [b]<0.001 as compared with control diabetic rats at before and respective week; [c]<0.001 as compared with controls at first week and [d]<0.05 as compared with control diabetic rats at respective week]

which quercetin, a natural antioxidant, inhibits lipid peroxidation by blocking the enzyme xanthine oxidase[20], chelating iron[21] and directly scavenging hydroxyl, peroxy and superoxide radicals[1] reveals its antioxidative properties. Quercetin also protects antioxidative defence mechanism by increasing the absorption of vitamin C[22]. Quercetin has been shown to inhibit structural damage to proteins[23], the release and the production of oxidative products generated by the respiratory burst in phagocytes[24]. Hydroxyl radicals generated from decomposition of peroxynitrite or via transition metal-catalyzed Fenton reaction play a potential role in neurovascular dysfunction in diabetes. Quercetin has recently shown to be an iNOS inhibitor, resulting in reduced nitric oxide (NO) and peroxynitrate generation[25]. Moreover, it is an effective metal chelator thereby preventing the Fenton reaction.

Impaired blood flow also seems to contribute to noxious stimulus hypersensitivity. Vasodilator treatment has been demonstrated to reduce allodynia in diabetic rats[26]. Oxidative stress related reduction in perfusion is thought to play a part in cardiac autonomic dysfunction[27]. Similar mechanism could be operating in small fiber sensory neuropathy. Impaired perfusion of neuronal cell bodies could contribute to autonomic dysfunction by restricting the energy supply for synthesis and transport of molecules essential to maintain axonal integrity and neurotransmission[18]. Thus, quercetin may have improved neuronal blood flow through reactive oxygen species scavenging or by its direct vasorelaxant properties[28].

In conclusion, the use of antioxidants as quercetin may constitute a potential therapeutic approach to diabetic neuropathic pain and vasculopathy.

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