

**QUERCETIN ATTENUATES ALTERED COLONIC CONTRACTILITY
AND INTESTINAL TRANSIT IN HFD-FED / STZ-TREATED TYPE 2
DIABETIC RATS.**

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Summary

Diabetic complications involve cardiovascular system, kidneys and nerves. GIT is a prime target of diabetic autonomic neuropathy. Delayed small intestinal transit and megacolon have been demonstrated in streptozotocin treated diabetic rats. Increased lipid peroxidation and accelerated advanced lipoxidation endproduct formation, possibly catalyzed by hyperglycemia and oxidative stress, may play a critical role in the development of neurovascular complications in diabetes. The health-promoting activity of quercetin seems to be related to the antioxidant (free-radical scavenging) activity. Quercetin treatment (100mg/kg/day) for 6 weeks to high fat diet-fed plus low dose streptozotocin diabetic rats significantly reversed both reduced contractile response of distal colon to acetylcholine and delayed transit of charcoal meal in small intestine compared to diabetic control. The significant effect of quercetin in reversing the increased plasma lipid peroxidation level in diabetic rats may be due to its antioxidant property. In conclusion, the present study suggest that quercetin, a bioflavonoid may be useful in preventing type II diabetes induced delay in intestinal motility and since, quercetin is already in clinical use it may be evaluated for preventive diabetic induced delay in intestinal motility in patients at risk of developing autonomic neuropathy.

Keywords: Quercetin, Colonic contractility, Intestinal transit, Diabetic complication, Rat

Introduction

Diabetic complications involve cardiovascular system, kidneys and nerves (1). GIT is a prime target of diabetic autonomic neuropathy. Diabetic animal models exhibit changes in gastrointestinal function that resemble the abnormalities manifested in human disease. Delayed small intestinal transit and megacolon have been demonstrated in streptozotocin treated diabetic rats (2-3).

The pathways contributing to the development of diabetic neuropathy includes increased activation of polyol pathway, oxidative stress, advanced glycation end product formation, nerve hypoxia/ischemia, protein C and reduction of nerve growth factor support.(4-7). Oxidative stress plays a vital role in contributing to neural and vascular complications (8-9) because once the reactive oxygen species are formed they deplete antioxidant defenses (superoxide dismutase, catalase and glutathione peroxidase), rendering the affected cells and tissues more susceptible to oxidative damage. Increased lipid peroxidation and accelerated ALE (advanced lipoxidation endproducts) formation, possibly catalyzed by hyperglycemia and oxidative stress, may play a critical role in the development of neurovascular complications in diabetes (10).

Dietary supplements of antioxidants are required to achieve an increase in antioxidant status which may diminish oxidative stress associated with diabetes mellitus (11). Quercetin (3,5,7,3V,4V-pentahydroxyflavone) is a phenolic compound widely distributed in the plant kingdom. It is found in frequently consumed foods, including apples, berries, onions, tea and brassica vegetables. Quercetin is reported to have many beneficial effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, antiallergic activity, cataract prevention, antiviral activity and anti-inflammatory effects (12-14). The health-promoting activity of quercetin seems to be related to the antioxidant (free-radical scavenging) activity (15). The involvement of oxidative stress in the development of functional changes in gastrointestinal tract and the effect of quercetin on such changes are less documented.

The aim of the present study is to examine the effect of quercetin treatment on altered response of distal colon to exogenous accetylcholine and small intestinal transit of charcoal meal in type 2 diabetic rats.

Methods

Chemicals

Streptozotocin was purchased from Sigma. The feed ingredients such as casein (Himedia laboratories, Mumbai, India), dl-methionine (Loba Chemie, Mumbai, India), vitamin and mineral mix (Sarabhai chemicals, Baroda, India) were procured from the commercial sources. Quercetin (HiMedia laboratories, Bombay, India), lard and heparin were obtained from commercial sources. Pioglitazone & glipizide were obtained from Ranbaxy research laboratories as gift samples. The compounds were administered orally as suspension by mixing with vehicle 1% Na-CMC at a dose volume of 2ml kg⁻¹ body weight of rats.

Preparation of fructose diet

Fructose diet was prepared by the method reported elsewhere (16) and consisted of 660 g fructose, 100 g protein, 80 g fat, 0.04 g zinc carbonate, 5 g vitamins mixture, 5 g mineral mixture and cellulose 150 g, all commercial grades.

Experimental animals

Male Sprague–Dawley (SD) rats (160–180 g) were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22±2 °C) and humidity (55±5%) with 12:12 h light and dark cycle. All the rats were provided with normal pellet diet (Amrut Diet, New Delhi) and water ad libitum, prior to the dietary manipulation. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Development of HFD-fed / STZ-treated type 2 diabetic rats

The model of type 2-like diabetes was established according to the method reported earlier (17) with modification. Briefly, animals were fed high fat diet (HFD), once a day for 2 weeks. After 2 weeks, animals were fasted overnight and were injected intraperitoneally with streptozotocin (35 mg/kg) dissolved in 0.1 mol/l citrate buffer (pH 4.4). The rats with the non-fasting PGL of ≥300 mg dl⁻¹ were considered diabetic and selected for further pharmacological studies.

Experimental protocol

The control rats were divided into two groups of 6-8 rats each (I-II). Group I was treated with NPD (normal pellet diet) and group II was treated with quercetin (100mg/kg, p.o). The fat-fed/STZ diabetic rats (PGL of ≥300 mg dl⁻¹) were randomly divided into 4 groups (III – VI) consisting of ten rats each, such that their basal mean biochemical parameters were similar to each other. The third group of rats (fat-fed/streptozotocin-diabetic rats) were given vehicle 1% Na-CMC (2 ml

kg⁻¹, p.o) served as diabetic control while the groups 4th to 6th were treated with insulin sensitizers, pioglitazone (10 mg kg⁻¹ once daily) (16), insulin secretagogues, glipizide (5 mg kg⁻¹ once daily) (16) and quercetin (100 mg kg⁻¹ once daily) (18) a flavonoid respectively. All the substances above were administered intragastrically for 6 weeks and treatment schedule was started one day before the administration of STZ.

Experimental Procedure

Charcoal meal transit in small intestine

At the end of the treatment period, overnight fasted animals of different groups were administered; po, 2ml/rat with charcoal meal (10% charcoal in 5% gum acacia) and 20 min later the rats were killed by cervical dislocation. The abdomen was opened and the intestine was removed from pyloric junction to caecal end. Then colon was separated and kept in continuously aerated Tyrod's solution. The farthest distance traveled by the charcoal meal through the small intestine and total length of the intestine were measured. Gastrointestinal transit was expressed as the percentage of the distance traveled by the charcoal meal relative to the total length of small intestine (19).

Contractile response of colonic smooth muscle

Immediately after cleansing the colon, 1 cm of distal colon was mounted under a resting tension of 0.5g in an organ bath (40 ml) containing continuously aerated Tyrode's solution. The temperature was maintained at 37^o±1^oC throughout the experiment and the tissue was allowed to equilibrate for 30 min before exposing to acetylcholine. A primary dose of 100 ng of acetylcholine was tested before starting the actual concentration response curve. The contractile responses were recorded isotonicly using a students physiograph. At the end of the initial equilibration period dose response curves were obtained for ascending dose of acetylcholine. ED₅₀ values of acetylcholine were calculated from the graph plotted using percent response against log dose.

Lipid peroxidation in plasma

Lipid peroxidation in plasma was estimated by measuring malondialdehyde (MDA) level in plasma. Amount of malondialdehyde formed was quantified by reaction with thiobarbituric acid as reported previously (20)

Statistical analysis

Data are presented as the mean ± SE from 8 rats per group. Comparison of mean values among the various groups was performed by one way ANOVA. For the single comparison between the groups unpaired Student's t-test was used. P values less than 0.05 were considered significant.

Results

Baseline body weights and blood glucose level were similar in all the groups. Table 1 shows the mean blood sugar level at the end of 6 weeks treatment. Six weeks after injection of streptozotocin, the diabetic rats had significantly higher body weights (data not shown) and there was an increase in blood sugar level when compared with their age matched non-diabetic controls. Treatment with pioglitazone and glipizide significantly decreased blood glucose towards normal levels. Treatment with quercetin had no significant effect on blood glucose levels.

Table 1: Effect of quercetin, pioglitazone and glipizide on blood glucose level and lipid peroxidation (Values are mean \pm SE)

| Groups | Blood glucose Level (mg/dl) | MDA (nmol/100ml of Plasma) |
|----------------------------|-------------------------------|---------------------------------|
| Control | 91.33 \pm 4.83 | 410.5 \pm 19.26 |
| Control plus Quercetin | 84.5 \pm 5.12 | 423.16 \pm 22.19 |
| Diabetic control | 475.5 \pm 6.44 ^a | 825.5 \pm 27.07 ^a |
| Diabetic plus Pioglitazone | 91.33 \pm 3.78 ^b | 445.5 \pm 14.88 ^b |
| Diabetic plus Glipizide | 102.5 \pm 5.43 ^b | 468.0 \pm 10.25 ^c |
| Diabetic plus Quercetin | 466.16 \pm 9.37 | 495.83 \pm 27.81 ^b |

P value: * P < 0.001 when compared with control. # P < 0.001 when compared with diabetic control. † P < 0.01 when compared with diabetic control

ED₅₀ of acetylcholine (Fig 1 A), percent transit of charcoal meal in small intestine (Fig 1 B) and plasma MDA levels (Table 1) showed significant difference among groups. The untreated diabetic rats showed a significant increase in ED₅₀ of acetylcholine (P < 0.001), plasma MDA level (P < 0.001) and a significant reduction of transit of charcoal meal (P < 0.001) compared to normal controls.

Pioglitazone treated diabetic rats significantly reduced ED₅₀ of acetylcholine (P<0.001) and increased the percent distance traveled by charcoal meal (P<0.001) compared to HFD fed /low dose streptozotocin diabetic control. The effect of

quercetin was same as to pioglitazone. Glipizide treated diabetic rats showed less significant effect ($P < 0.01$) when compared to diabetic control.

Effect of quercetin, pioglitazone and glipizide on contractile response of distal colon and small intestine transit of charcoal

Fig 1 A

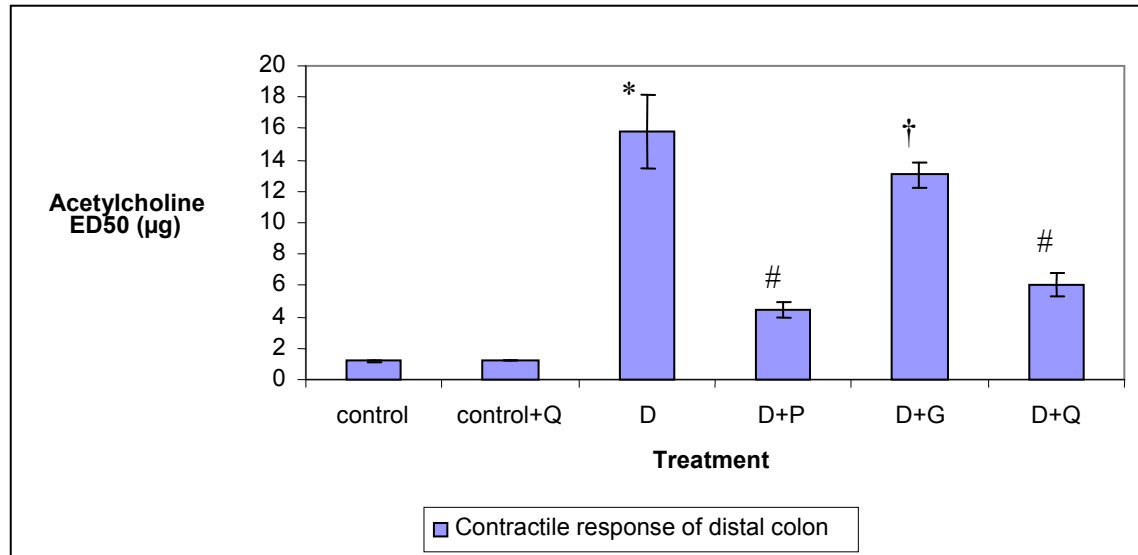
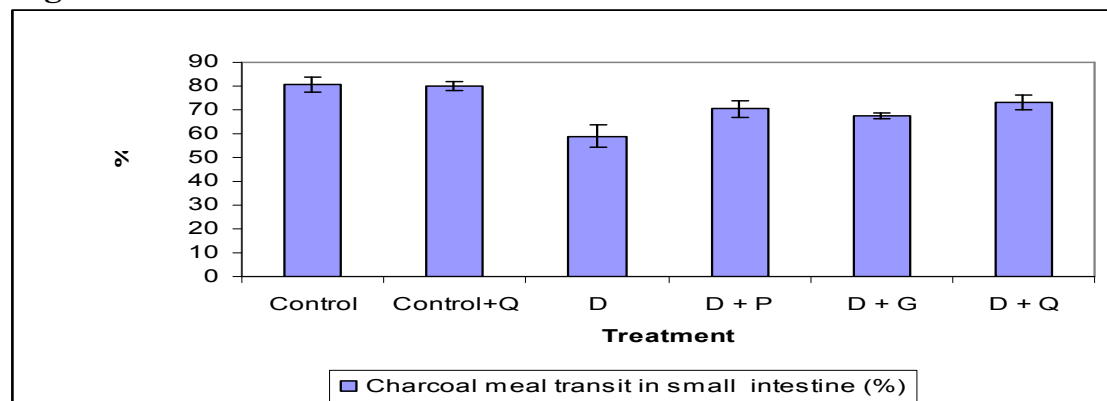


Fig1B



Contractile response of distal colon to exogenous acetylcholine (Fig 1 A) and Small intestinal transit of charcoal meal (Fig 1 B) in non-diabetic and HFD fed/low dose STZ –diabetic rats. D = Diabetic control, D+P = Diabetic +Pioglitazone, D +G = Diabetic +Glipizide, D+Q = Diabetic + Quercetin. P value: * $P < 0.001$ when compared with control. # $P < 0.001$ when compared with diabetic control. † $P < 0.01$ when compared with diabetic control.

Discussion

Metabolic syndrome is characterized by a cluster of pathological changes including obesity, hypertriglyceridemia, impaired glucose tolerance and insulin resistance. A modified diet (fructose diet) was adopted to induce insulin resistant because the role of fructose in the development of diabetic complications was well documented (16) and injection of a single dose of STZ induced a diabetic state similar to prediabetic, insulin resistant state in humans (17). Streptozotocin targets pancreatic β cells, leading to serum insulin reduction. The rodent model induced by high fat diet feeding followed by a low dose streptozotocin injection, stimulates the natural history and the metabolic characteristics of patients with type 2 diabetes (17). Distal colons from untreated diabetic rats were found to be less sensitive to acetylcholine and thereby delay in transit of intestinal content. These observations are in agreement with previous report (2). There is diminished release or production of neurotransmitter, i.e; acetylcholine, to enhanced degradation of the neurotransmitter, or to diminished end organ sensitivities to the neurotransmitter itself (21). There was a parallel increase in lipid peroxidation level in diabetic rats (22). Increased lipid peroxidation and accelerated advanced lipoxidation end product formation, possibly catalyzed by hyperglycemia and oxidative stress, may play a critical role in the development of neurovascular complications in diabetes (10). Treatment with quercetin, glipizide and pioglitazone produced a significant reversal of all the parameters measured, suggesting a role of hyperglycemia and oxidative stress involvement in diabetic complication. Flavonoid like quercetin had profound effect than glipizide. Quercetin has antioxidant- scavenging activity (23-24), delays lipid peroxidation of cell membranes (25), and reduces Cu^{2+} -induced LDL oxidation (26). Quercetin is reported to chelate copper ions and thus inhibit oxidation of LDL (27) and thus beneficial in preventing lipid peroxidation. The reduced contractile response of colonic smooth muscle to exogenous acetylcholine may be the result of excessive degradation of acetylcholine by tissue acetylcholine esterase, diminished muscarinic receptor sensitivity or density or defective interaction between muscarinic receptor and intracellular contractile process. The myogenic phenomenon in distal small intestine of diabetic rats is not affected (21). Studies of the responsiveness of diabetes colonic smooth muscle to acetylcholine are limited, whereas vascular (28) in experimental diabetes show altered sensitivity to acetylcholine. Therefore oxidative stress may induce changes in muscarinic receptor density and binding affinity leading to reduced cholinergic response and thus quercetin may play vital role in abolishing these changes. Impaired cholinergic response of distal small intestinal smooth muscle has been reported²¹ and thus treatment with quercetin improved intestinal motility and enhanced intestinal transit of charcoal meal in diabetic rats.

In conclusion, the present study suggest that quercetin, a bioflavonoid may be useful in preventing type II diabetes induced delay in intestinal motility and since, quercetin is already in clinical use it may be evaluated as preventive therapy in diabetic induced delay in intestinal motility in patients at risk of developing autonomic neuropathy

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