Antioxidant Potential of Quercetin: Remarkable Protection Against Hypercholesterolemia in Rats

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Abstract: The aim of the present study was the evaluation of possible protective effects of the flavonoid antioxidant quercetin (QE) against experimentally-induced atherosclerosis in rats. Experimental atherosclerosis was produced by feeding rats a diet enriched in coconut oil (15% by weight) and cholesterol (2% by weight) for 30 days. QE (15 mg kg^{-1} day, intraperitoneal (i.p.) injection) was injected for 3 successive days prior to cholesterol administration for 4 weeks. It has been believed that oxidative stress plays a role in pathogenesis of atherosclerosis. Lipid profile parameters such as plasma total cholesterol, HDL-C, LDL-C, VLDL-C, LDL:HDL ratio and triglycerides were elevated in hypercholesterolemic (HC) rats. Plasma and liver glutathione-S-transferase (GST) enzyme levels were unaffected in all studied groups. In order to determine the changes of cellular antioxidant defense system, antioxidant enzymes such as glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase (CAT) activities, were measured in rat liver homogenate. Moreover, lipid peroxides expressed as thiobarbituric acid substance (TBARS) level in both serum and liver homogenate, were measured. Hypercholesterolemia induced significant increase in lipid profile and lipid peroxidation and decreased the antioxidant enzyme activity. TBARS level in serum and liver were significantly increased (P<0.05) and also the antioxidant levels significantly decreased (P<0.05) in HC group. QE treatment significantly decreased the elevated TBARS (P< 0.05), lipid profiles (p<0.05) and also increased the antioxidant enzyme activities (P< 0.05). QE treatment has shown protective effect possibly through decreasing lipid peroxidation and increasing antioxidant enzyme activity. These findings point out that, QE treatment has protective effect against atherosclerosis by decreasing oxidative stress state associated with HC.

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1. Introduction

Several epidemiological studies have supported the hypothesis that the antioxidant power of flavonoids may reduce the risk of cardiovascular diseases (Sesso et al., 2003). Flavonoids are compounds that are found in many foods, including vegetables, tea, fruit and wine (Hertog et al., 1993; Frankel et al., 1993). They have been recognized for having interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities (Middleton, 1998). One of these flavonoids, quercetin (QE) (3,3′,4′,5,6-pentahydroxyflavone), prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals (Bors *et al*., 1990; Inal *et al*., 2002) protect against lipid peroxidation (Laughton *et al*., 1991) and chelate metal ions (Afanas`ev *et al*., 1989). The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. Accordingly, interest has recently grown in the role and usage of natural antioxidants as a means to prevent oxidative damage in diabetes with high oxidative stress.

Hypercholesterolemia or more specifically elevated plasma low-density lipoprotein cholesterol (LDL-C) is an important risk factor for development and progression of atherosclerosis. The common risk factors for atherosclerosis is increase the production of free radicals by endothelial and vascular sooth muscle. These free radicals initiate processes involved in atherogenesis through several important enzyme systems, including xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADP) oxidases and nitric oxide synthase. Hypercholesterolemic (HC) state lead to increase in free radical production and thereby elevate lipid peroxides (Harrison *et al.,* 2003).

The present study was performed to study the effects of the QE administration against atherosclerosis in experimentally-induced HC rats.

2. Material and Methods

2.1. Chemicals

All chemicals are of highest grade used were supplied from Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Switzerland) companies.

2.2. Treatment of rats and animal dosing

Thirty healthy male Wistar albino rats, weighing 200–250 g, were used in this study. The animals were housed in macrolon cages under standard laboratory conditions (light period 7.00 a.m. to 7.00 p.m. and

21°C). The animals were given standard rat pellets and tap water ad libitum. The rats were randomly divided into three experimental groups: I (control), II (hypercholesterolemic, HC) and III (QE- treated), each containing 10 animals. In groups II and III, hypercholesterolemia was induced by feeding the rats with a HC diet enriched in coconut oil (15% by weight) and cholesterol (2% by weight) for 4 weeks (Anila and Vijayalakshmi, 2002). Group III received QE that freshly dissolved in 0.5 ml of 60% ethanol and injected i.p. for 3 successive days prior to oral cholesterol administration for 4 weeks. The QE dose was chosen on the basis of a previous study (Coskun *et al*., 2005).

2.3. Biochemical analysis

At the end of the experiment, rats were fasted overnight for 12 h, and sacrificed under ether anaesthesia. Blood samples were collected in order to analyze their lipid profile parameters. Plasma triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C were assayed using the commercial kits purchased from Boehringer Mannheim company (Germany). LDL:HDL ratio was calculated. Lipid peroxides; expressed as thiobarbituric acid reactive substance (TBARS) and glutathione-S-transferase (GST) enzyme level were measured in both plasma and liver homogenate. Liver tissues were washed with saline followed by homogenization in phosphate saline (PBS) buffer by means of Ultra Turrax T25 homogenizer. The soluble fraction was prepared by centrifugation at 6000×*g* for 10 min. Then, antioxidant enzyme activities of rat liver homogenate were measured. This included glutathione peroxidase; GSHPx, superoxide dismutase; SOD and catalase; CAT.

2.3.1. Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the concentration of TBARS in plasma and liver homogenate according to the method of Ohkawa *et al.*, 1979. The sample was mixed with 0.2 ml of 8% sodium dodecyl sulphate, 1.5 ml of 20% acetic cid (pH 3.5), and 1.5 ml of a 0.8% aqueous solution of thiobarbituric acid. The volume was made up to 4 ml with distilled water and heated for 60 min at 95^0C. After cooling under tap water, 1 ml of the distilled water and 5 ml of a mixture of *n*-butanol and pyridine (15:1, v/v) were added before shaking the samples vigorously. After centrifugation (8000 rpm, 10 min), absorbance at 532 nm of the organic layer was measured with a spectrophotometer (Genway). Lipid peroxidation was calculated from the standard curve using the TBARS and expressed as nmol TBARS /ml (plasma) and nmol TBARS /g protein. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

2.3.2. Measurement of glutathione-S-transferase

The glutathione-S-transferase (GST) was determined spectrophotometrically using aromatic substrate (1-cholro-2,4dinitrobenzene) and monitor the change in absorbance due to thioester formation. One unit of the enzyme is defined as the amount of GST which needed to catalyze the formation of 1 mol of thioester per minute and the specific activity is expressed as nmol/min/mg protein (Habig *et al.,* 1974).

2.3.3. Measurement of glutathione peroxidase enzyme

The activity of glutathione peroxidase (GSHPx) was determined spectrophotomertrically by measuring the rate of NADPH oxidation at 340 nm, based on the methods of Flohe and Gunzler, 1984 and Carlberg and Mannervik, 1985. The substrates used in this study was H_2O_2. The activity was expressed as U/mg protein.

2.3.4. Measurement of superoxide dismutase enzyme

The superoxide dismutase (SOD) activity of liver tissue was analyzed spectrophotometrically, using nitroblue tetrazolium as a substrate and phenazine methosulphate. The colour intensity of chromogen produced was measured at 560 nm. The concentration of SOD was expressed as U/mg protein (Kakkar *et al.,* 1984).

2.3.5. Measurement of catalase enzyme activity

The catalase (CAT) activity in liver homogenate was measured using H_2O_2 as substrate that can be decomposed by CAT enzyme. One unit of CAT is defined as the amount needed to decompose 1 nmol H_2O_2 of per minute and the specific activity is expressed as U/mg protein (Clairborne, 1985).

2.4. Statistical analysis

All data were expressed as mean ± S.E. The standard error was calculated by dividing the standard deviation by the square root of the number of observations. Paired t-test was carried out to compare populations using GraphPad Prism software (San Diego, CA). A 0.01 level of probability was used as the criterion for significance.

3. Results

3.1. Plasma lipid profile was significantly altered upon feeding atherogenic diet to rats

Feeding using atherogenic diet for 30 days resulted in the development of HC in experimental rats as evident in Table 1. There was a significant increase by 2.5 folds in the levels of triglycerides and cholesterol in atherogenic diet fed rats compared to normal control. The increase in plasma cholesterol was due to a 4 folds increase in LDL cholesterol and a small increase (17%) in HDL cholesterol; $P<0.01$ (Table 1).

Table 1. Effects on plasma lipids profile in rats fed a diet enriched in coconut oil (15%) and cholesterol (2%) for 30 days.

Groups	NC	HC
Triglycerides (mg%)	44.0 ± 2.0	110.0 ± 5.0*
Total cholesterol (mg%)	90.0 ± 3.0	220.0 ± 10.0*
LDL cholesterol (mg%)	30.0 ± 1.0	114.0 ± 3.0*
HDL cholesterol (mg%)	49.0 ± 1.0	59.0 ± 0.6*
VLDL cholesterol (mg%)	25.0 ± 0.3	33.0 ± 0.6*
LDL:HDL ratio	0.6 ± 0.01	2.0 ± 0.1*

Data are expressed as mean±S.E. Number of rats per group n=10

*Statistical significance as compared to the normal control group at P<0.01.HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL= very low density lipoprotein, TG= triglycerides. Normal control; HC: Hypercholesterolemic; HC-QE: Hypercholesterolemic-Quercetin-treated rats.

3.2. Plasma lipid profile was significantly improved upon quercetin administration to hypercholesterolemic rats

Treatment of HC rats with QE in a dose of 15 mg kg^{-1}day, intraperitoneal (i.p.) injection for successive 3 days prior to 4-weeks cholesterol oral administration for 4 weeks, significantly decreased the plasma levels of triglycerides, total cholesterol, LDL-C, HDL-C, VLDL-C and LDL:HDL ratio by 2, 2.5, 3, 0.9, 1.2 and 2.5 folds, respectively in compared to the HC rats; P<0.01 (Table 2).

Table 2. The Effects of quercetin (QE) administration (15 mg kg^{-1}day, intraperitoneal (i.p.) injection) for 3 successive days prior to 4-weeks cholesterol oral administration for 4 weeks, on plasma lipid profile of the HC rats.

Groups	HC	HC-QE
Triglycerides (mg%)	110.0 ± 5.0	55.0 ± 2.0*
Total cholesterol (mg%)	220.0 ± 10.0	88.0 ± 5.0*
LDL-cholesterol (mg%)	114.0 ± 3.0	40.0 ± 2.0*
HDL- cholesterol (mg%)	59.0 ± 0.6	52.0 ± 0.6*
VLDL- cholesterol (mg%)	33.0 ± 0.6	27.0 ± 0.7*
LDL:HDL ratio	2.0 ± 0.1	0.8 ± 0.002*

Data are expressed as mean±S.E. Number of rats per group n=10

*Statistical significance as compared to the normal control group at P<0.01. HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL= very low density lipoprotein, TG= triglycerides. Normal control; HC: Hypercholesterolemic; HC-QE: Hypercholesterolemic-Quercetin-treated rats.

3.3. Lipid peroxidation and antioxidant enzymes were significantly improved upon quercetin administration to hypercholesterolemic rats

The analysis of the effect of QE (in a dose of 15 mg kg^{-1}day, intraperitoneal (i.p.) injection for 3 successive days prior to 4-weeks cholesterol oral administration for 4 weeks, on oxidative stress was performed by measuring the plasma and liver homogenate level of lipid peroxides expressed as TBARS, that was declined after QE intake, by 167% and 150%; P<0.01, respectively compared with the HC control rats. However, the plasma and liver homogenate level of GST enzyme were unaffected by QE treatment (Table 3). The antioxidant enzymes; namely, GSHPX, SOD and CAT were significantly increased by 1.6, 2 and 2 folds, respectively compared with HC rats (Table 4); P<0.01.

Table 3. Effect quercetin (QE) administration (15 mg kg^{-1} day, intraperitoneal (i.p.) injection) for 3 successive days prior to 4-weeks cholesterol oral administration for 4 weeks, on plasma and liver thiobarbituric acid reactive substance; TBARS and glutathione-S-transferase; GST levels.

Groups	NC	HC	HC-QE
Plasma			
TBARS (nmol/ml)	2.0 ±0.02	8.0 ±1.0^{+}	3.0 ±0.02*
GST (mol/hr)	0.7±0.01	0.66±0.01	0.7±0.002
Liver			
TBARS (nmol/g liver)	11.0 ±1.0	30.0 ±2.0^{+}	12.0 ±1.0*
GST (mol/hr/mg protein)	0.9±0.06	0.9±0.05	0.9±0.01

Data are expressed as mean±S.E. $^{+}$ Statistical significance as compared to the normal control group, P<0.01. *Statistical significance as compared to the hyperlipidemic group, P<0.01. Number of rats per group n=10.NC: Normal control; HC: Hypercholesterolemic; HC-QE: Hypercholesterolemic-Quercetin-treated rats.

Table 4. Effects of quercetin (QE) administration (15 mg kg^{-1}day, intraperitoneal (i.p.) injection) successive 3 days prior to 4-weeks cholesterol oral administration for 4 weeks, on antioxidant enzyme activities such as glutathione peroxidase; GSHPX, superoxide dismutase; SOD and catalase; CAT on liver homogenate of hypercholesterolemic rats.

Groups	NC	HC	HC-QE
GSHPX (U/mg protein)	14.0± 0.6	8.0 ±0.5^{+}	13.0 ±0.4*
CAT (U/mg protein)	26.0 ± 1.2	11.0 ±1.0^{+}	24.0 ± 0.7*
SOD (U/mg protein)	50.0 ±2.0	23.0 ±1.0^{+}	44.0 ±2.0*

Data are expressed as mean±S.E. $^{+}$ Statistical significance as compared to the normal control group, P<0.01. *Statistical significance as compared to the hyperlipidemic group, P<0.01. Number of rats per group n=10. Normal control; HC; HC-QE: Hypercholesterolemic-Quercetin-treated rats.

4. Discussion

Treatment of HC rats with QE prior to cholesterol administration, significantly improved the lipid profile of the HC rats as showed by decreased plasma levels of triglycerides, total cholesterol, LDL-C, HDL-C, VLDL-C and LDL:HDL ratio by 2, 2.5, 3, 0.9, 1.2 and 2.5 folds, respectively in compared to HC rats; P<0.01 (Table 2). The possible usefulness of the QE as protective effect against atherosclerosis may arise from the decreasing of oxidative stress and lipid peroxides expressed as TBARS. The preservation of lipid peroxidation may bring about protein damage and inactivation of membrane bound enzymes either through direct attack by free radicals or through chemical modification by its end products, such as TBARS. In this study, significant increases of 38%, 54% and 48% in antioxidant enzyme activities of GSHPx, SOD and CAT, respectively were noticed in comparing with HC rats (Table 4); P<0.01. These findings are consistent with the results of Wolf, 1993; and El-Missiry and El-Gindy, 2000, who indicated an increase in lipid peroxides and a decrease in antioxidant enzymes after QE treatment.

It was obvious that, flavonoids may work by making liver cells more efficient to remove LDL-C from blood by increasing the LDL-receptor densities in liver (Baum *et al.,*1998). Also, it was shown that flavonoids recycle -tocopherol (powerful antioxidant) by donating a hydrogen atom to the -tocopherol radical (Salah *et al.*,1995). This would maintain the -tocopherol (and probably the endogenous antioxidants) concentration in LDL for longer time and delay the onset of lipid peroxidation (Wan *et al.,* 2001). Lee *et al*., 2001, reported that, the antiatherogenic effect of some flavonoids in HC rats was not due to the regulation of plasma lipid profile but through down regulation of some genes. Weggemans and Trautwein, 2003, reported that flavonoids decreased LDL-C and increased HDL-C. The HDL-C may hasten removal of cholesterol from peripheral tissue to liver for catabolism and excretion. Also, high HDL-C levels may compete with LDL receptor sites on arterial smooth muscle cells and thus inhibit the uptake of LDL (Carew *et al.,*1976). The increase in HDL-C concentration could protect the LDL against oxidation *in vivo* because lipids in HDL are preferentially oxidized before those in LDL (Bowry *et al.,* 1992). Anthony *et al.,*1998, reported that the potential mechanisms by which flavonoids might prevent atherosclerosis, include a beneficial

effect on plasma lipids and antioxidant activity. Hermansen *et al.,*2003, reported that LDL:HDL ratio is a strong predictor for cardiac disease. Results in Table 4, showed that this ratio improved significantly by 150%, P<0.01 (Table 2) upon intake of QE compared to HC rats. The present study indicated that flavonoids showed no significant effect on the activity of plasma and liver GST (Table 3), in agreement with the findings of El-Demerdash *et al.,* 2003,Yousef *et al.,* 2004.

The present results showed that formation of lipid peroxides expressed as TBARS was significantly decreased by 167% and 150%; P<0.01, in plasma and liver, respectively upon QE treatment to HC rats (Table 1). These results are in accordance with the work done by Yousef *et al.,* 2004, who studied the effect of flavonoid on lipid peroxides level. Tikkanen *et al.,*1998, indicated that, flavonoids may act *in vivo* to decrease oxidative damage to DNA, protein and lipids leading to reducing the risk of coronary artery disease. This may be attributed to the important role of flavonoids as antioxidants. This power may be attributed to their ability to decompose free radicals before they reach a cellular target (Fran *et al.,*2000).

The present results indicated that the mechanism of antioxidant activity of flavonoids is not through the induction of GST, but might be due to the reduction of free radicals formation. Namely, the results obtained from this study, indicate that the preventive effects of QE may be due to inhibition of lipid peroxidation by its antioxidant nature.

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