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Antioxidant Potential of Quercetin: Remarkable Protection Against Hypercholesterolemia in Rats

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Author's contribution

This whole work was carried out by author HHB.

Original Research Article

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ABSTRACT

Aims: To explore protective role of the antioxidant flavonoid quercetin (QE) towards experimentally-induced atherosclerosis in rats.
Study Design and Methodology: This study was carried out during Feb. 2014. Experimental atherosclerosis in rats was induced through oral intake of the coconut oil enriched diet (15%) and cholesterol (2%) for successive 30 days. QE (15 mg kg^{-1} day) was injected intraperitoneally (i.p.) for three consecutive days before cholesterol intake for 30 days.
Results: The triglycerides, total cholesterol, LDL-C, HDL-C, VLDL-C and LDL/HDL ratio levels in plasma were estimated in hypercholesterolemic (HC) rats. Glutathione-S-transferase enzyme level in plasma and liver homogenate were unchanged in all rats. Superoxide dismutase, glutathione peroxidase and catalase activities, were measured in rat liver homogenate. Serum and liver lipid peroxides were determined. HC induced significant elevation in lipid profiles and lipid peroxides and decreased antioxidant enzymes activities. Serum and liver TBARS were significantly increased. The antioxidant enzymes were decreased significantly in HC rats. QE treatment significantly decreased lipid profiles and elevated the antioxidant enzymes.
Conclusion: QE intake protect against HC, by reducing lipid peroxides and elevating antioxidant enzymes levels. Data revealed that, QE could protect against atherosclerosis by decreasing the oxidative stress state.

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Keywords: Quercetin; hypercholesterolemia; oxidative stress; rats.

1. INTRODUCTION

It was reported that, the antioxidant potential of flavonoids might decrease the incidence of cardiac diseases [1]. Many foods, including vegetables, tea, fruits and wine, contains flavonoids [2,3], that have been recognized to guards against inflammation, allergy, bacteria, viruses and cancer [4]. Quercetin (QE) (3,3′,4′,5,6 pentahydroxyflavone), is a flavonoid that decline cell death and oxidant injury through scavenging oxygen free radicals and chelating metal ions to protect against lipid peroxidation. So that, interest has recently grown towards ambient use of antioxidants of natural origin to minimize oxidative damage in diseases with elevated oxidative stress state [5-8].

Hypercholesterolemia (HC) characterized by high plasma LDL-C as an important risk factor of atherosclerosis, that associated with free radicals production and initiating atherogenesis through several enzyme systems, such as nitric oxide synthase, NADP oxidases and xanthine oxidase. HC can lead to elevation of free radical production and subsequently, increase lipid peroxides [9].

The current work was performed to investigate and gain insight QE effects to protect experimentally-induced HC in male rats.

2. MATERIALS AND METHODS

2.1 Chemicals

The chemicals were supplied from Sigma-Aldrich company (USA) and Acros company (Belgium).

2.2 Treatment of Rats and Animal Dosing

The current study was conducted during Feb. 2014 and approved by local Medical Ethics Committee. Thirty normal Wistar albino male rats (200–250g), were used. They were placed in polycarbonate standard cages and dieted with rat standard pellets(purchased from Lab Diet Co., USA).The tap water was ad libitum. Animals were randomly classified into 3 experimental groups (n=10): I (control), II (HC) and III (QE-treated). In groups II and III, HC was induced by feeding rats for 30 days HC diet (15% coconut oil and 2% cholesterol) [10]. Group III received QE that freshly dissolved in of 60% ethanol (0.5ml) and injected i.p. for 3 successive days prior to HC diet intake for 30 days. The dose of QE was chosen according to a previous study [11].

2.3 Biochemical Parameters

Rats were fasted for 12 hours at the end of the experiment, followed by scarification under halothane anaesthesia. Liver was excised and washed with saline followed by homogenization in phosphate buffer saline (PBS) buffer using the Bullet Blender homogenizer (purchased from Next Advance Inc., USA) to obtain the rat liver homogenate

supernatant that was used to measure levels of glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase (CAT). Blood samples collected to analyze lipid profiles. Plasma total cholesterol, LDL-C, HDL-C, VLDL-C and triglycerides were assayed using commercial kits purchased from Boehringer Mannheim Company (Germany). LDL/HDL ratio was calculated. Lipid peroxides and glutathione-S-transferase (GST) levels were estimated in both plasma and liver homogenate.

2.4 Lipid Peroxides Estimation

Lipid peroxidation (nmol thiobarbituric acid reactive substance (TBARS)/ml for plasma and nmol TBARS/g protein) was estimated by measuring plasma and liver TBARS levels. Each sample was added to 0.8% thiobarbituric acid (1.5 ml), 8% sodium dodecyl sulphate (0.2 ml), 20% acetic acid pH 3.5 (1.5 ml), and dist. H_2O (1 ml). The mixture heated for 60 min at 95ºC. After cooling, dist. H_2O (1 ml) and 15:1 *n*-butanol/pyridine (5 ml) added followed by shaking samples vigorously and centrifugation at 8000 rpm for 10 min. Absorbance of organic layer at 532 nm was recorded spectrophotometrically. TBARS standard calibration curve was used to calculate lipid peroxides [12]. Protein was estimated using serum bovine albumin as a standard [13].

2.5 Glutathione-S-transferase Enzyme Estimation

The glutathione-S-transferase (GST) activity as μmol/min/mg protein or μmol/hr was determined using aromatic substrate (1-cholro-2,4-dinitrobenzene) and recorded the absorbance change attributed tothioester bond formation using spectrophotometer at 360 nm. A 1U GST is required to form 1 μmol of thioester/min [14]

2.6 Measurement of Glutathione Peroxidase Enzyme

Glutathione peroxidase (GSHPx) activity as U/mg protein was determined spectrophotometrically *via* measuring NADPH oxidation rate at 340 nm using H_2O_2 substrate.

2.7 Superoxide Dismutase Enzyme Estimation

Superoxide dismutase (SOD) activity of liver tissue as U/mg protein was analyzed using nitro blue tetrazolium as a substrate and phenazine methosulphate to produce a coloured conjugate that measured at 560nm using spectrophotometer [16].

2.8 Catalase Enzyme Estimation

Catalase (CAT) activity of liver tissue as U/mg protein was measured using H_2O_2 as substrate that can be degraded by CAT enzyme. A 1U of CAT is the amount needed to degrade 1 nmol H_2O_2/ min [17].

2.9 Statistical Analysis

Data in this study presented as mean±S.E. Data were manipulated using GraphPad Prism software V.5 (USA) to compare populations using the Paired t-test. A 0.01 probability level was used to test for significance.

3. RESULTS

Plasma lipid profile was significantly changed upon feeding atherogenic diet to rats. Feeding using atherogenic diet for 30 days induced HC in experimental rats (Table 1); $P<0.01$. A 2.5 folds significant elevation of triglycerides and cholesterol levels in rats dieted with atherogenic diet as compared to normal control. Plasma total cholesterol increment was due to 4 folds LDL-C increase and 17% HDL-C elevation (Table 1); $P<0.01$. Plasma lipid profile was improved significantly after QE administration to HC rats. Treatment of HC rats with QE (15 $mgkg^{-1}$day, i.p. for 3 successive days) before intake of cholesterol-containing diet orally for 30 days, significantly decreased triglycerides, total cholesterol, LDL-C, HDL-C, VLDL-C and LDL/HDL ratio levels by 2, 2.5, 3, 0.9, 1.2 and 2.5 folds, respectively, compared to HC rats (Table 1); $P<0.01$.

Lipid peroxidation and antioxidant enzymes were improved significantly upon QE administration to HC rats. The analysis of the effect of QE (15 $mgkg^{-1}$ day, i.p.. for 3 successive days) before intake of cholesterol-containing diet orally for 30 days, on cell wall oxidation was conducted by measuring plasma and liver homogenate of lipid peroxides level, that declined after QE intake, by 167% and 150%; $P<0.01$, respectively, compared to HC rats. However, plasma and liver homogenate level of GST enzyme were unaffected by QE treatment (Table 2); $P<0.01$. The liver GSHPX, SOD and CAT levels were elevated significantly by 1.6, 2 and 2 folds, respectively, compared to HC rats (Table 3); $P<0.01$.

Table 1. The Effects of quercetin (QE) administration (15 $mgkg^{-1}$ day, i.p. injection) for 3 successive days before intake of diet rich in coconut oil (15%) and cholesterol (2%) for 30 days, on plasma lipid profile of the HC rats

Groups	NC	HC	HC-QE
Triglycerides (mg%)	44±2	110±5*	55±2*
Total cholesterol (mg%)	90±3	220±10*	88±5*
LDL cholesterol (mg%)	30±1	114±3*	40±2*
HDL cholesterol (mg%)	49±1	59±0.6*	52±0.6*
VLDL cholesterol (mg%)	25±0.3	33±0.6*	27±0.7*
LDL:HDL ratio	0.6±0.01	2±0.1*	0.8±0.002*

*Data (mean±S.E.). #Rats/group; n=10. *Significance difference compared to NC group; P<0.01. +Significance difference compared to HC group; P<0.01. NC=Normal control, HC=Hypercholesterolemia, QE= Quercetin, LDL= Low density lipoprotein, HDL= High density lipoprotein, VLDL= Very low density lipoprotein, TG = Triglycerides*

Table 2. The Effects of quercetin (QE) administration (15mgkg^{-1}day, i.p. injection) for 3 successive days before intake of diet rich in coconut oil (15%) and cholesterol (2%) for 30 days, on plasma and liver lipid peroxides (as 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 (μmol/hr)	0.7±0.01	0.66±0.01	0.7±0.002
Liiver			
TBARS (nmol/g liver)	11±1.0	30±2.0^{+}	12±1.0*
GST (μmol/hr/mg protein)	0.9±0.06	0.9±0.05	0.9±0.01

*Data (mean±S.E.). #Rats/group;n=10. *Significance difference compared to NC group; P<0.01. $^{+}$Significance difference compared to HC group; P<0.01. NC=Normal control, TBARS= Thiobarbituric acid reactive substance, HC=Hypercholesterolemia, QE=Quercetin*

Table 3. The Effects of quercetin (QE) administration (15 mgkg^{-1}day, i.p. injection) for 3 successive days before intake of HC diet for 30 days, on glutathione peroxidase; GSHPX, superoxide dismutase; SOD and catalase; CAT on liver homogenate of HC rats

Groups	NC	HC	HC-QE
GSHPX (U/mg protein)	14±0.6	8±0.5^{+}	13±0.4*
CAT (U/mg protein)	26±1.2	11±1.0^{+}	24±0.7*
SOD (U/mg protein)	50±2.0	23 ±1.0^{+}	44±2.0*

*Data (mean±S.E.). #Rats/group; n=10. *Significance difference compared to NC group; P<0.01. $^{+}$Significance difference compared to HC group; P<0.01. NC=Normal control, HC=Hypercholesterolemia, QE=Quercetin*

4. DISCUSSION

The flavonoid QE is present in many plants including edible fruits and vegetables. QE is poorly absorbed from GIT when it was administered orally [18]. QE exist in regular human diet with daily intake of 50–500 mg [19]. The intake of QE in a single dose of 15 mgkg^{-1}, i.p.to rat (equivalent to1000mg administered to human), was shown to be able to reduce oxidative stress [20].

The investigation of protective effects of QE, against experimentally-induced HC in rats, was carried out. Experimental HC was achieved *via* feeding rats 15% coconut oil and 2% cholesterol-rich diet for 30 days (data showed in Table 1). QE (15 mgkg^{-1} day, i.p.) administered for 3 successive days before 30-days oral cholesterol-rich diet intake. Oxidative stress elicited crucial role in progression and pathogenesis of HC. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx) have been shown to protect cells against lipid peroxides accumulation, which is considered as the initial step in HC [21]. Decreased antioxidant levels as a consequence of free radical production in several diseases such as HC and atherosclerosis, have been reported by some authors [22,23].

Treatment of HC rats with QE pre-cholesterol administration, significantly improved 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 1). 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 lipid peroxidation tend to initiate protein breakdown and inactivate enzymes bounded to the membrane *via* invade cells directly by with free radicals or modification of cell membrane chemically by the end products of lipid peroxides. This study elaborated significant increases of 38%, 54% and 48% in antioxidant enzymes levels of GSHPx, SOD and CAT, respectively (Table 3); $P<0.01$, that were in accordance with the results reported by others, who revealed elevation of lipid peroxides and a decline in antioxidant enzymes during oxidative stress state [24-27].

It was obvious that, flavonoids render liver cells more susceptible to remove LDL-C by accumulating hepatic LDL-receptors [28]. Flavonoids can reform α-tocopherol (powerful cell antioxidant) *via* borrowing one hydrogen to α-tocopherol radical [29]. This would maintain the α-tocopherol (endogenous antioxidants) level in LDL for a longer time and delay lipid peroxidation onset [30]. Antiatherogenic effect of some flavonoids in HC rats was not due to lipid profile regulation but through down-regulation of some genes to help to decrement LDL-C and elevate HDL-C [31]. The HDL-C may activate the cholesterol scavenging from several tissues to liver [32]. The elevated HDL-C level interfere with LDL receptors in arterial smooth muscle cells, inhibit LDL uptake [33] and increase HDL-C which, guard against LDL oxidation *in vivo* [34]. LDL/HDL risk ratio is a potent predictor of heart diseases [35]. The major mechanism by which flavonoids might protect against atherosclerosis, include potential positive effect on plasma lipids and antioxidant activity. Results in Table 3, showed that this ratio improved significantly by 150%, $P<0.01$ (Table 1) upon intake of QE compared to HC rats.

This study revealed that, flavonoids showed insignificant effects regarding the level of plasma and hepatic GST (Table 2), in agreement with other previous researches [36,37]. The genesis of lipid peroxides was significantly decreased by 167% and 150%; $P<0.01$, in plasma and liver, respectively, upon QE intake to HC rats (Table 1), That were compiled with other work, that investigated flavonoid effect on lipid peroxides formation [37]. It was indicated that, flavonoids may act *in vivo* to decrease DNA, lipids and protein oxidative damage that result in minimize the risk of heart diseases attack[38], that may be referred to the crucial antioxidant role of flavonoids through QE ability as free radicals scavengers and thereby decrease incidence of oxidative stress [39].

5. CONCLUSION

The current results revealed that, QE can protect against HC in rats due to inhibition of lipid peroxidation, free radicals scavenging and increasing cellular antioxidant enzymes potentials.

CONSENT

Not applicable.

ETHICAL APPROVAL

Animals were manipulated according to European Communities Council Directive (86/609/ECC). Protocol was officially authorized by local Medical Ethics Committee- Faculty of Applied Medical Sciences, Taibah University, Madinah, Saudi Arabia.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Sesso HD, Gaziano JM, Liu S, Buring JE. Flavonoid intake and risk of cardiovascular diseases in women. Am. J. Clin. Nutr. 2003;77:1400-1408.
2. Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer. 1993;20:21-29.
3. Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet. 1993;341:454-457.
4. Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. Adv. Exp. Med. Biol. 1998;439:175-182.
5. Bors W, Heller C, Michel C, Saran M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol. 1990;186,343-355.
6. Inal M, Akgun A, Kahraman A. Radio protective effects of exogenous glutathione against whole-body gamma-ray irradiation: age- and gender-related changes in malondialdehyde levels, superoxide dismutase and catalase activities in rat liver. Methods Find. Exp. Clin. Pharmacol. 2002;24:209-212.
7. Laughton MJ, Evans PJ, Moroney MA, Hoult JR, Halliwell B. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives: Relationship to antioxidant activity and to iron ion-reducing ability. Biochem. Pharmacol. 1991;42:1673-1681.
8. Afanas'ev IB, Dorozhko I, Brodskii AV, Korstyuk VA, Potapovitch A. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem. Pharmacol. 1989;38:1763-1769.
9. Harrison D, Griendling K, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am. J. Cardiol. 2003;91:7A-11A.
10. Anila L, Vijayalakshmi NR. Flavonoids from *Emblica officinalis* and *Mangifera indica*-effectiveness for dyslipidemia. J. Ethnopharmacol. 2002;79(1):81-7.

11. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacological Res. 2005;51(2):117-123.

12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric reaction. Anal. Biochem. 1979;95:351-35.

13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951;193:265-269.

14. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 1974;249:7130-7139.

15. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984;105:114-121.

16. Kakkar P, Das B, Viswanathan P. A modified method for assay of superoxide dismutase. Ind. J. Biochem. Biophys. 1984;21:131-132.

17. Clairborne, A. (1985). Catalase activity. In Handbook of methods for oxygen radical research, Greenwald, R.A., ed., 383, CRC press, Boca Raton, FL, USA.

18. Murota K, Terao J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. Arch. Biochem. Biophys. 2003;417:12–17.

19. Deschner EE, Ruperto J, Wong G, Newmark HL. Quercetin and rutin as inhibitors of azoxymehanol-induced coloni neoplasia. Carcinogenesis. 1991;12:1193-1196.

20. Nuraliev IN, Avezov GA. The efficacy of quercetin in alloxan diabetes. Exp. Clin. Pharmacol. 1992;55:42-44.

21. Pritchard KA, Patel ST, Karpen CW, Newman HA, Panganamala RV. Triglyceride-lowering effect of dietary vitamin E in streptozocin-induced diabetic rats. Increased lipoprotein lipase activity in livers of diabetic rats fed high dietary vitamin E. Diabetes. 1986;35:278–281.

22. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic. Biol. Med. 1990;8:281-291.

23. Grankvist K, Marklund S, Taljedal L. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. Biochem. J. 1981;199:393–398.

24. Wolff SP. Diabetes mellitus and free radical. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br. Med. Bull. 1993;49:643–649.

25. El-Missiry MA, El-Gindy AM. Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of Eruca sativa seeds. Ann. Nutr. Metab. 2000;44:97-100.

26. Zahedi M, Ghiasvand R, Feizi A, Asgari G, Darvish L. Risk factors and Inflammatory Biomarkers in women with type 2 diabetes: A double-blind randomized controlled clinical trial. International Journal of Preventive Medicine 2013;4(7):777-85.

27. Jung CH, Cho I, Ahn J, Jeon T, Ha T. Quercetinreduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. Phytotherapy Res. 2013;27(1):139-143.

28. Baum JA, Teng H, Erdman JW, Weigel RM, Klein BP, Persky VW, Freels S, Surya P, Bakhit RM, Ramos E, Shay NF, Potter SM. Long term intake of soy protein improves blood lipid profile and increases mononuclear cell low-density lipoprotein receptor messenger RNA in hypercholesterolemic postmenopausal women. Am. J. Clin. Nutr. 1998;58:545-551.

29. Salah N, Miller NJ, Paganga G. Polyphenolic flavanols as scavenger od aqueous phase radical and as chain-breaking antioxidants. Arch. Biochem. Biophys. 1995;322:339-346.

30. Wan Y, Vinson JA, Etherton TD, Porch J, Lazarus SA, Kris-Etherton PM. Effects of cocoa powder on LDL oxidative susceptibility and prostaglandin concentration in humans. American J. Clin. Nutr. 2001;74(5):596-602.

31. Lee C, Jeong T, Choi Y, Hyun B, Oh G, Kim E, Kim J, Han J, Bok S. Anti-atherogenic effect of citrus flavonoids, naringin and naringenin, associated with hepatic ACAT and aortic VCAM-1 and MCP-1 in high cholesterol-fed rabbits. Biochemical and Biophysical Res. Comm. 2001;284:681-688.

32. Weggemans RM, Trautwein EA. Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta analysis. European J. Clin. Nutr. 2003;57:940-946.

33. Carew TE, Koschinsky T, Mayers SB, Steinberg D. A mechanism by which high-density lipoproteins may slow the atherogenic process. Lancet. 1976;1:1315-1317.

34. Bowry VW, Stanley KK, Stocker R. High density lipoprotein is the major of lipid hydroperoxides in human blood plasma fasting donors. Proc. Natl. Acad. Sci. USA. 1992;89:10316-10320.

35. Hermansen K, Dinesen B, Hoie LH, Morgenstern E, Gruenwald J. Effects of soy and other natural products on LDL: HDL ratio and other lipid parameters: a literature review. Advanced Therapeutics. 2003;20:50-78.

36. El-Demerdash FM, Yousef MI, Al-Salhen KS. Protective effects of isoflavone on biochemical parameters affected by cypermethrin in male rabbits. J. Environmental Sciences and Health. 2003;38:365-378.

37. Yousef MI, Kamel KI, Esmail AM, Baghdadi HH. Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. Food and Chemical Toxicology. 2004;42:1497-1503.

38. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoesterogen intake on low-density lipoprotein oxidation resistance. Proc. Natl. Acad. Sci. USA. 1998;95:3106-3110.

39. Fran K, Donald E, James G. Research trends in healthful foods. Food Technology. 2000;54(10):45-52.

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