

Quercetin potentially attenuates cadmium induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats

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Abstract. – **BACKGROUND:** Cadmium is one of the potent cardiotoxic heavy metals in the environment, which induces oxidative stress, dyslipidemia and membrane disturbances in heart. Quercetin is an effective antioxidant and free radical scavenger against oxidative stress. This study was designed to evaluate the protective effect of quercetin (QE) on cardiac marker enzymes, lipid peroxidation products, lipid profile, membrane bound ATPases and antioxidant status in cadmium (Cd)-intoxicated rats.

MATERIALS AND METHODS: Twenty four male albino rats were used. Cadmium induced oxidative cardiotoxicity was induced by the oral administration of Cd for four weeks. Quercetin was pretreated along with Cd for four weeks to assess its cardioprotective effect against Cd intoxication. Rats treated with vehicles alone were used as controls.

RESULTS: Rats intoxicated with cadmium (5 mg/kg/day) for 4 weeks in combination with quercetin (50 mg/kg/day) respectively. Cd-induced cardiotoxicity and dyslipidemia was indicated by increased activities of marker enzymes such as creatine kinase-MB, aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase in serum. In addition, the levels of lipid peroxidation products and protein carbonyl contents in heart were significantly ($p < 0.05$) increased and the activities of enzymic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase in the heart and non-enzymic antioxidants such as glutathione, vitamin C and E in the heart were significantly ($p < 0.05$) decreased in Cd intoxicated rats. The levels total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), LDL and VLDL were significantly ($p < 0.05$) increased and the level of HDL was significantly decreased in the serum of Cd-treated rats. Cd intoxication also increased the levels of TC, TG and FFA and decreased the level of PL in the heart tissue. Further Cd treatment significantly ($p < 0.05$) decreased the levels of membrane bound ATPases in heart. QE treatment along with Cd showed significant protective effect on all the biochemical parameters studied. Histopathological findings of QE and Cd treated heart con-

firmed the biochemical findings of this study. Thus, QE protects the myocardium against Cd-induced oxidative stress and dyslipidemia in rats.

CONCLUSIONS: Quercetin may be beneficial in combating the cadmium induced oxidative cardiotoxicity and dyslipidemia in rats.

Key Words:

Cadmium, Quercetin, Heart, Dyslipidemia, Oxidative stress, Rat.

Introduction

Cadmium (Cd), is one of the most toxic pollutants in environment and a well-known human carcinogen¹. Main source of Cd exposure include smoking, foods and water polluted with this metal¹⁻³. Like other toxic heavy metals, Cd is also transported from soil to plants easily and can be greatly concentrated in the food chain². Cd accumulation and toxicity depend on time and the dose. Also, Cd induced cardiac impairment has been reported⁴. Among the various chemical substances, Cd is one of the causes of hypertension⁵. Although several mechanisms have been postulated, the precise mechanism(s) of Cd-induced cardiac impairment is still undefined. It has been reported that Cd may decrease or increase nitric oxide (NO) levels in endothelial cells and may enhance the production of reactive oxygen species (ROS) leading to lipid peroxidation (LPO)⁶⁻⁹. Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress mediated LPO in the tissues^{8,9}.

Long term low-level Cd intake, results in hypertensive and non-hypertensive cardiovascular diseases in humans¹⁰. In human, non-occupational exposure to Cd predominantly results from smoking, air pollution, contaminated food and water¹¹. Cd causes a number of clinical complications in-

cluding cardiovascular diseases, anemia, diabetes and disruption of endocrine system¹²⁻¹⁵.

The toxicity of Cd consists generally, in its ability to disturb numerous cellular functions and causes damage to various cellular structures^{16,17}. Cd²⁺ ions are characterized by high affinity to biological structures containing sulfhydryl (-SH), carboxyl and phosphate groups. They inhibit numerous enzymes and disturb some metabolic processes including lipid metabolism. Both experimental and epidemiological studies indicate that exposure to Cd may alter lipid metabolism and contribute to the development of cardiovascular diseases (CVD), including atherosclerosis, hypertension, stroke and cardiac arrest^{1,18-21}. The main pathologies and specific biochemical changes are related to Cd toxicity and its concentration and the condition of oxidative stress in tissues^{22,23}.

Considering the relationship between cadmium exposure and oxidative stress, attention has been focused on compounds having antioxidant properties in order to combat against cadmium-induced cardiac damage. Plant flavonoids have recently attracted much attention as potential antioxidants of dietary origin. Many epidemiological studies have been performed to evaluate the health impact of flavonoids, and their results have strongly suggested that the consumption of flavonoid-rich diets lowers the risk of degenerative diseases such as cardiovascular diseases (CVD)²⁴. Flavonoids are ubiquitous compounds, occurring in various plants and plant-derived food products, such as tea, herbs, citrus fruits, onion and red wine and many of them are strong free radical scavengers and antioxidants²⁵. Various epidemiological studies have supported the hypothesis that the antioxidant actions of flavonoids may reduce the risk of developing CVD²⁶. Quercetin (QE) (3,5,7,3',4'-pentahydroxyflavone), is a safe and one of the most abundant flavonoids, commonly present in tea, coffee and red wine and in most edible fruits and vegetables²⁷. Its average human consumption is in the range of 25-50 mg per day²⁸. The antioxidant properties of QE might be due to its ability to chelate transition metal ions²⁹, such as Fe²⁺ and Cu²⁺. QE prevents oxidative stress by scavenging superoxide anions. Singlet oxygen and hydroxyl ions with its phenolic hydroxyl groups are responsible for its strongest antioxidant activity³⁰. It exhibits a wide spectrum of pharmacological activities such as anti-inflammatory³¹, anti-tumor³², anti-ulcer³³, immune modulatory³⁴ and vasodilation³⁵. It also prevents the oxidation of low density lipoproteins *in vitro*³⁶. QE was found to be the most active of the 31 tested

flavonoids, with an IC₅₀ value of 0.93 μM in the structure activity relationship (SAR) studies of peroxynitrite scavenging activity³⁷.

The current rate of release of Cd into the environment indicates that Cd intake in human beings will continue to increase and that it will lead to a higher incidence of Cd-related diseases including hypertension, atherosclerosis. Several mechanisms were proposed for the adverse effects of Cd on the cardiovascular system. However, the oxidative stress markers, lipid markers, especially plasma total cholesterol and lipoproteins, are the important risk factors for the development of atherosclerosis. To achieve the greatest possible reduction in coronary heart disease (CHD) risk, treatment strategies should be aimed at reducing the elevated levels of circulatory lipids and lipoprotein components. Considering the beneficial effects of QE in cardiovascular diseases, this study has been designed to investigate the protective efficacy of QE in Cd induced oxidative cardio toxicity and dyslipidemia in rats.

Materials and Methods

Animals

Adult male albino Wistar rats weighing 180 to 200 g obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University, Tamilnadu, India, were used. They were housed in polypropylene cages (47' 34' 20 cm) lined with husk, renewed every 24 h under a 12:12h light/dark cycle at around 22°C. The rats had free access to water and a commercial standard pellet diet (Lipton India Ltd, Mumbai, India). The rat diet consisted of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 55% nitrogen free extract (carbohydrate), and 15067 kJ metabolizable energy. The rats used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The study protocol was approved (Vide No. 160, 2007) by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) at Annamalai University, Annamalainagar, India.

Chemicals

Quercetin and Cadmium chloride were purchased from Sigma Chemical Co., (St Louis, Mo, USA). Tween-80 was purchased from Hi media, Mumbai, India. Commercial diagnostic kits were

obtained from Span Diagnostics (Mumbai, India) for determination of serum cardiac markers, cholesterol, high-density lipoprotein cholesterol (HDL-C) and TG. All other chemicals used in this study were of analytical grade and they were obtained from local firms in India.

Experimental Design

The animals were randomly divided into 4 groups of 6 rats in each.

Group 1: Control rats treated orally administered with 0.1% Tween-80 and normal saline daily for 4 weeks.

Group 2: Rats orally received Cd as cadmium chloride (5 mg/kg bw/day) in normal saline for 4 weeks.

Group 3: Rats were pretreated with QE (50 mg/kg bw/day) dissolved in 0.1% Tween-80 contained distilled water and orally received Cd as cadmium chloride (5 mg/kg bw/day) in normal saline for 4 weeks.

Group 4: Rats were orally administered with QE (50 mg/kg bw/day) dissolved in 0.1% Tween-80 contained distilled water for 4 weeks.

After the last treatment, rats were fasted overnight and all the rats were anesthetized with pentobarbital sodium (35 mg/kg, IP) and euthanized by cervical decapitation. Blood was collected in tubes containing ethylene diamine tetraacetate (EDTA). The plasma was obtained after centrifugation (2000 g for 20 min at 4°C) and used for various biochemical measurements. Heart tissues were excised immediately and rinsed in ice-chilled physiological saline. Known weights of the tissues were homogenized in 5.0 ml of 0.1 M Tris-HCl buffer (pH 7.4) solution. The homogenate were centrifuged and the supernatant was used for the estimation of various biochemical parameters. And the other portions of heart tissues were, washed with ice-chilled physiological saline, and stored at -20°C until analyzed.

Activities of Serum Marker Enzymes

The collected plasma was used for the estimation of cardiac marker enzymes creatinine kinase-MB (CK-MB), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) using commercially available standard enzymatic kits (Span Diagnostics Pvt. Ltd., India). Plasma was also estimated for the lipid profile including total cholesterol, HDL-cholesterol,

triglycerides using commercially available standard enzymatic kits (Span Diagnostics Pvt. Ltd., India) and phospholipids by the method described by Fiske and Subbarow³⁸. Plasma LDL-cholesterol was calculated by the Friedewald formula. $LDL = Total\ cholesterol - (HDL\text{-}cholesterol + (Triglycerides/5))$.

Estimation of Lipid Peroxidation

Lipid peroxidation in heart was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides by the method of Niehiaus and Samuelson³⁹ and Jiang et al⁴⁰ respectively. Protein carbonyl content was determined by the method of Levine et al⁴¹. The levels of conjugated dienes were assessed by the method of Rao and Recknagel⁴².

Determination of Non-Enzymatic Antioxidants

Vitamin C concentration was measured as previously reported Omaye et al⁴³. Vitamin E (α -tocopherol) was estimated by the method of Desai⁴⁴. Reduced glutathione (GSH) was determined by the method of Ellman⁴⁵. Oxidized glutathione was determined by the method of Hissin and Hilf⁴⁶. Total sulfhydryl groups were measured by the method of Sedlak and Lindsay⁴⁷.

Activities of Enzymatic Antioxidants

Superoxide dismutase activity was determined by the method of Kakkar et al⁴⁸. The activity of catalase was determined by the method of Sinha⁴⁹. Glutathione peroxidase activity was estimated by the method of Rotruck et al⁵⁰. Glutathione-S-transferase activity was determined by the method of Habig et al⁵¹. Glutathione reductase was assayed by the method of Horn and Burns⁵². The estimation of glucose-6-phosphate dehydrogenase was carried out by the method of Beutler⁵³. Total protein content of tissue homogenate was estimated as described previously⁵⁴.

Preparation of Lipid Extract

Lipids were extracted from heart tissues by the method of Folch et al⁵⁵ within 24 h. For the lipid extraction, the heart was rinsed in cold physiological saline thoroughly and dried by pressing between folds of filter paper. A known amount of heart (500 mg) was homogenized in a chloroform-methanol mixture (2:1 v/v). Afterward, extraction of the heart and plasma was repeated 4 times with chloroform methanol. The resulting filtrates were combined and

evaporated into dryness. The lipids were purified and the dried lipid residue was dissolved in 5 mL of chloroform-methanol mixture. The redissolved lipid extract was mixed with 1 mL of 0.1N potassium chloride and the contents were shaken well. The lower chloroform phase containing neutral and phospholipids (PL) was again washed 3 times with 2 mL 0.1N potassium chloride-methanol-chloroform mixed at a ratio of 10:10:1, and the upper aqueous phase was removed. The lower chloroform phase was made up to a known volume, and aliquots were taken for the analysis of lipids.

Estimation of Lipids

From the lipid extract, the levels of total cholesterol and TG were estimated by using kits (Span Diagnostics) according to the manufacturers' procedures. For the estimation of total cholesterol, 0.1 mL of the lipid extract was evaporated into dryness and 5 mL of ferric chloride-acetic acid reagent was added. To that, 3 mL of concentrated sulfuric acid was added and the absorbance was read after 20 min at 560 nm⁵⁶. For the estimation of TG, 0.5 mL of lipid extract was evaporated into dryness and 0.1 mL of methanol was added, followed by 4 mL of isopropanol. To this, 0.4 g of alumina was added, shaken well for 15 min and centrifuged. To 2 mL of supernatant, 0.6 mL of the saponification reagent (5 g of potassium hydroxide in 60 mL of distilled water and 40 mL of isopropanol) and 0.5 mL of acetyl acetone reagent (0.75 mL of acetyl acetone in 60 mL of distilled water and 40 mL of isopropanol) were added and placed in a water bath at 65°C for 1 hour. All the tubes were cooled and read at 405 nm⁵⁷.

The level of free fatty acids (FFA) was estimated by the method of Falholt et al⁵⁸. In brief, 0.1 mL of lipid extract was evaporated into dryness. To this, 1 mL of phosphate buffer (pH 6.4), 6 mL of extraction solvent (chloroform-heptane-methanol, 5:5:1) and 2.5 mL of copper reagent were added. 5:1). All the tubes were shaken vigorously, and 200 mg of activated silicic acid was added. After 30 min, the tubes were centrifuged (3000 g) and 3 mL of the copper layer was transferred to another tube containing 0.5 mL of diphenylcarbazide and mixed carefully. The absorbance was read at 550 nm immediately. The PL level was estimated by the method of Zilversmit and Davis⁵⁹ in which the conversion of organic phosphorus to inorganic phosphorus was involved. In brief, 1 mL of concentrated sulfuric acid and 1 mL of concentrated nitric acid were added to 0.1 mL of lipid extract/plasma and di-

gested to a colorless solution. The phosphorus content in this solution was determined by the method of Fiske and Subbarow³⁸.

Membrane-bound ATPases

The sediment after centrifugation was resuspended in ice cold Tris-HCl buffer (0.1M) pH7.4. This was used for the estimations of membrane-bound enzymes and protein content. The membrane-bound enzymes such as Na⁺/K⁺-ATPase, Ca²⁺-ATPase, and Mg²⁺-ATPase activity were assayed by estimating the amount of phosphorus liberated from the incubation mixture containing tissue homogenate, ATP, and the respective chloride salt of the electrolytes⁶⁰⁻⁶². Total protein content was estimated by the method described previously⁵⁴.

Histopathological Studies

For qualitative analysis of heart histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully in different mixture of ethyl alcohol, water, cleaned in xylene and embedded in paraffin. Sections of the tissues (5-6 µm thick) were prepared by using a rotary microtome and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffined xylene medium for microscopical observations. Six rats from each group were sacrificed for analyzing the hepatic histological examinations.

Statistical Analysis

The data were subjected to a one-way analysis of variance using a computer software package (SPSS version 11.0, SPSS Inc, Cary, NC, USA), and the comparisons of significant groups were performed using the Duncan Multiple Range Test (DMRT) at $p < 0.05$. All the data were expressed as mean \pm SD of number of experiments (n = 6).

Results

The effects of Cd and QE treatment on heart weight, body weight and heart weight to body weight ratio are depicted in Table I. There was no significant difference in the body weight between the groups observed, although Cd treated animals showed a slight reduction in body weight which was not significant. The heart weight and the heart weight to body weight ratio were increased significantly ($p < 0.05$) in Cd-administered rats when compared with normal control rats. In rats treated with QE and Cd there was a significant ($p < 0.05$) reduction in the heart weight and the ratio

as compared to Cd treated rats. No significant difference was observed in rats treated with QE alone when compared to normal control rats.

Table II represented the effects of Cd and QE treatment on cardiac marker enzymes including CK-MB, AST, ALT, ALP and LDH. The activities of these enzymes were increased significantly ($p < 0.05$) in Cd treated rats as compared to normal control group rats. QE treatment in Cd treated animals significantly ($p < 0.05$) decreased the CK-MB, AST, ALT, ALP and LDH activities. No significant difference was observed in rats treated with QE alone when compared to normal control rats.

Table III showed the changes in the levels of plasma lipids of control and experimental rats. We observed a significant ($p < 0.05$) increase in the levels of plasma cholesterol, TG, FFA and PL in Cd treated rats when compared with control rats. Upon administration treatment with QE a significant ($p < 0.05$) decrease in the levels of plasma cholesterol, TG, FFA and PL were observed as compared with Cd treated rats. We observed a significant ($p < 0.05$) increase in the levels of LDL-C, VLDL-C, and a significant ($p < 0.05$) decrease in the levels of HDL-C in the plasma of Cd treated rats when compared with control rats. Treatment with QE in Cd intoxicated rats a significant ($p < 0.05$) decrease in the levels of plasma LDL-C, VLDL-C, with a significant ($p < 0.05$) increase in the level of HDL-C were observed when compared with Cd treated rats.

Table IV shows the levels of cardiac lipid status in control and experimental rats. We observed a significant ($p < 0.05$) increase in the levels of cardiac TC, TG and FFA and a significant ($p < 0.05$) decrease in PL in Cd treated rats when compared with control rats. Treatment with QE in Cd intoxicated rats the altered lipid profile in heart tissue reversed to their near normal levels when compared with Cd treated rats.

The perturbations in the levels of cardiac TBARS, LOOH, CD and PC of control and ex-

perimental rats were shown in Table V. We observed a significant ($p < 0.05$) increase in the levels of cardiac TBARS, LOOH, CD and PC in Cd treated rats when compared with control rats. Treatment with QE (50 mg/kg) significantly ($p < 0.05$) reduced the levels of cardiac TBARS, LOOH, CD and PC in Cd treated rats when compared to Cd alone treated rats.

The activities of the cardiac antioxidant enzymes SOD, CAT, GPx, GST, GR and G6PD in normal and experimental rats were listed in Table VI. Cd treated rats showed a significant ($p < 0.05$) decrease in the activities of these antioxidant enzymes, in heart tissue as compared to control rats. Treatment with QE in Cd intoxicated rats the activities of these antioxidant enzymes reversed to normal levels when compared with Cd treated rats.

Cd treatment also resulted in the significant reduction ($p < 0.05$) in the levels of cardiac GSH, TSH, Vitamins C and E and a significant increase ($p < 0.05$) in the level of GSSG in the cardiac tissue of Cd treated rats when compared with normal control rats (Table VII). Treatment with QE in Cd intoxicated rats significantly ($p < 0.05$) revert back these parameters near to control levels as compared to Cd alone treated rats.

Table VIII shows the levels of membrane bound ATPases in the heart tissue of control and experimental rats. The levels of membrane bound ATPases in heart tissue significantly ($p < 0.05$) decreased in Cd intoxicated rats when compared with control rats. Treatment with QE in Cd treated rats the altered levels of ATPases significantly ($p < 0.05$) reversed to normal levels when compared with Cd treated rats.

Discussion

The present study describes the effect of QE against cadmium induced oxidative cardiac im-

Table I. Effect of QE treatment on bodyweight, food intake, organ weight and organ body weight ratio in cadmium induced cardiotoxic rats.

Parameters	Control	QE	Cd	Cd + QE
Body weight (g)	227 ± 5.76 ^a	229 ± 4.94 ^b	204 ± 6.12 ^c	219 ± 3.28 ^d
Heart weight (g)	0.51 ± 0.01 ^a	0.50 ± 0.0 ^a	0.83 ± 0.05 ^b	0.64 ± 0.02 ^c
Heart weight/Body weight ratio (%)	0.224 ± 0.002 ^a	0.218 ± 0.004 ^b	0.407 ± 0.008 ^c	0.292 ± 0.006 ^d
Food intake (g/100 g bw/d)	12.07 ± 0.92 ^a	12.18 ± 0.83 ^b	8.17 ± 0.68 ^c	10.54 ± 0.79 ^d
Water intake (ml/rat/d)	18.20 ± 2.50 ^a	18.60 ± 2.70 ^a	12.40 ± 1.60 ^b	15.20 ± 2.10 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b, c and) differ significantly at $p < 0.05$ (DMRT).

Table II. Effect of QE treatment on cardiac markers enzymes in cadmium induced cardiotoxic rats.

Parameters	Control	QE	Cd	Cd + QE
CK-MB (IU/L)	82.15 ± 3.17 ^a	83.46 ± 2.87 ^a	197.4 ± 11.97 ^b	128.7 ± 8.64 ^c
LDH (IU/L)	178.2 ± 21.14 ^a	170.4 ± 18.21 ^a	321.5 ± 28.51 ^b	246.7 ± 24.71 ^c
AST (IU/L)	43.65 ± 3.38 ^a	44.39 ± 2.13 ^a	102.7 ± 7.42 ^b	71.64 ± 5.75 ^c
ALT (IU/L)	32.07 ± 2.03 ^a	33.68 ± 1.63 ^a	82.17 ± 5.78 ^b	54.80 ± 6.12 ^c
ALP (IU/L)	121.7 ± 7.41 ^a	123.5 ± 5.02 ^a	214.6 ± 18.03 ^b	168.2 ± 11.02 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b, and c) differ significantly at $p < 0.05$ (DMRT).

Table III. Effect of quercetin treatment on lipid profile in serum in Cd induced cardiotoxic rats.

Parameters	Control	QE	Cd	Cd + QE
Total cholesterol (mg/dl)	6.38 ± 2.45 ^a	78.12 ± 2.62 ^a	128.51 ± 6.27 ^b	104.83 ± 4.49 ^c
Triglycerides (mg/dl)	47.88 ± 2.16 ^a	46.27 ± 2.08 ^a	73.09 ± 6.79 ^b	58.23 ± 4.18 ^c
Phospholipids (mg/dl)	97.24 ± 5.83 ^a	95.31 ± 6.01 ^a	142.46 ± 9.34 ^b	112.03 ± 7.12 ^c
Free fatty acids (mg/dl)	71.29 ± 6.37 ^a	70.11 ± 5.78 ^a	122.07 ± 9.81 ^b	83.62 ± 6.83 ^c
LDL-C (mg/dl)	17.41 ± 1.34 ^a	15.72 ± 1.76 ^a	69.14 ± 3.82 ^b	34.41 ± 2.76 ^c
HDL-C (mg/dl)	49.67 ± 4.26 ^a	51.45 ± 4.49 ^a	24.13 ± 1.68 ^b	37.54 ± 3.21 ^c
VLDL-C (mg/dl)	9.57 ± 0.69 ^a	10.28 ± 0.82 ^a	14.61 ± 1.34 ^b	11.64 ± 0.87 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b and c) differ significantly at $p < 0.05$ (DMRT).

Table IV. Effect of Quercetin treatment on cardiac lipid profile in in Cd intoxicated rats.

Parameters	Control	QE	Cd	Cd + QE
Total cholesterol (mg/g tissue)	4.65 ± 0.18 ^a	4.58 ± 0.11 ^a	9.17 ± 0.34 ^b	6.24 ± 0.47 ^c
Triglycerides (mg/g tissue)	4.21 ± 0.24 ^a	4.18 ± 0.19 ^a	6.79 ± 0.41 ^b	5.16 ± 0.29 ^c
Free fatty acids (mg/g tissue)	5.38 ± 0.31 ^a	5.29 ± 0.23 ^a	9.45 ± 0.87 ^b	7.12 ± 0.53 ^c
Phospholipids (mg/g tissue)	21.67 ± 0.54 ^a	19.92 ± 0.37 ^a	11.46 ± 1.25 ^b	15.28 ± 1.08 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b and c) differ significantly at $p < 0.05$ (DMRT).

Table V. Levels of cardiac oxidative stress markers in control and experimental rats.

Parameters	Control	QE	Cd	Cd + QE
TBARS (µmoles/min/mg protein)	1.17 ± 0.07 ^a	1.15 ± 0.08 ^a	1.59 ± 0.11 ^b	1.34 ± 0.09 ^c
LOOH (µmoles/min/mg protein)	1.31 ± 0.12 ^a	1.28 ± 0.09 ^a	1.67 ± 0.08 ^b	1.45 ± 0.11 ^c
CD (A ₂₃₃)	0.52 ± 0.03 ^a	0.49 ± 0.02 ^a	0.68 ± 0.08 ^b	0.57 ± 0.04 ^c
Protein carbonyls (nmol/min/mg protein)	2.19 ± 0.12 ^a	2.16 ± 0.14 ^a	5.82 ± 0.28 ^b	2.89 ± 0.17 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b and c) differ significantly at $p < 0.05$ (DMRT).

Table VI. Levels of cardiac oxidative stress markers in control and experimental rats.

Parameters	Control	QE	Cd	Cd + QE
SOD (Units-50% NBT reduction min/mg protein)	5.75 ± 0.31 ^a	5.84 ± 0.34 ^a	2.94 ± 0.19 ^b	4.21 ± 0.21 ^c
CAT (µmols of H ₂ O ₂ consumed/min/mgprotein)	52.45 ± 5.24 ^a	53.04 ± 5.31 ^a	39.55 ± 3.42 ^b	4.30 ± 4.04 ^c
GPX (µmols of GSH oxidized/min/mg protein)	5.24 ± 0.59 ^a	5.47 ± 0.68 ^b	3.39 ± 0.32 ^c	5.76 ± 0.48 ^d
GR (µmols of NADHoxidized/min/mg protein)	18.78 ± 1.84 ^a	20.05 ± 1.25 ^a	14.30 ± 1.04 ^b	24.48 ± 0.76 ^c
GST (µmols of GSH-CDNB conjugate formed/min/mg protein)	4.31 ± 0.44 ^a	4.92 ± 0.62 ^b	4.01 ± 0.21 ^c	4.67 ± 0.42 ^d
G6PD (nmol/min/mg protein)	52.03 ± 3.74 ^a	56.18 ± 3.81 ^b	26.41 ± 1.63 ^c	3.48 ± 3.43 ^d

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b and c) differ significantly at $p < 0.05$ (DMRT).

pairment and dyslipidemia in rats. Experimental results showed that QE pre-treatment at a dose of 50 mg/kg body weight could prevent the oxidative impairment in rat hearts when supplemented for 28 days along with Cd. Earlier investigations suggested that Cd can initiate peroxidation of membrane poly unsaturated fatty acids by generating excessive free radicals followed by inhibiting the intracellular antioxidant defense system in tissues^{2,63}. Casalino et al⁶⁴ reported that cadmium intoxication released free iron from membrane and participation of this free iron in Fenton type reactions produces ROS. In other reports, it has been suggested that cadmium exposure may increase or decrease the nitric oxide (NO) level in endothelial cells and that may, in turn, increase the generation

of ROS leading to lipid peroxidation^{6,7}. Cadmium intoxication induces the expression of a number of stress genes⁶⁵ and this may cause an important role in affecting the activities of some antioxidant enzymes^{66,67}. After an oxidative stress, ROS can stimulate myocyte hypertrophy, re-expression of fetal gene programs and apoptosis in cardiac myocytes in culture⁶⁸. Reiter and Tan⁶⁹ reported that the production of ROS increased significantly in the myocardium tissue of patients with ischemic-reperfused hearts. In addition to oxidative stress, cadmium toxicity can also include: alteration in metabolic processes⁷⁰ modification in thiols containing proteins⁷¹, alteration in membrane structure and function⁷², as well as direct or indirect modification in DNA structure⁷³.

Table VII. Status of non-enzymatic antioxidants in the heart of control and experimental rats.

Parameters	Control	QE	Cd	Cd + QE
GSH (µg/mg protein)	9.21 ± 0.52 ^a	9.67 ± 0.60 ^b	5.63 ± 0.27 ^c	7.81 ± 0.39 ^d
GSSG (µg/mg protein)	3.67 ± 0.21 ^a	3.62 ± 0.23 ^a	7.98 ± 0.41 ^b	4.37 ± 0.27 ^c
TSH (µg/mg protein)	12.72 ± 0.54 ^a	14.54 ± 0.46 ^b	8.62 ± 0.28 ^c	1.09 ± 0.41 ^d
Ascorbic acid (mg/mg protein)	0.57 ± 0.09 ^a	0.60 ± 0.05 ^b	0.43 ± 0.06 ^c	0.52 ± 0.05 ^d
α-tocopherol (mg/mg protein)	0.94 ± 0.09 ^a	0.95 ± 0.08 ^a	0.78 ± 0.05 ^b	0.85 ± 0.06 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (DMRT).

Table VIII. Effect of QE treatment on membrane bound ATPase enzymes in Cd induced cardiotoxic rats.

Parameters	Control	QE	Cd	Cd + QE
Na ⁺ k ⁺ -ATPase (µmol of piliberated/min/mg protein)	7.46 ± 0.15 ^a	7.51 ± 0.13 ^a	3.17 ± 0.08 ^b	5.12 ± 0.27 ^c
Ca ²⁺ -ATPase (µmol of piliberated/min/mg protein)	5.24 ± 0.12 ^a	5.27 ± 0.11 ^a	12.01 ± 0.26 ^b	8.41 ± 1.09 ^c
Mg ²⁺ -ATPase (µmol of piliberated/min/mg protein)	5.32 ± 0.59 ^a	5.24 ± 0.47 ^a	3.02 ± 0.14 ^b	4.67 ± 0.36 ^c

Values are expressed mean ± SD for six rats in each group; ^{a,b,c,d}Values are not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (DMRT).

Cd challenge markedly increased the activities of serum marker enzymes of cardiac damage. Actually these enzymes are considered as reliable markers of early and late cardiac injury. Similar elevations in cardiac marker enzyme activities have been reported by Manna et al⁷⁴ in rats following chronic Cd administration. Pre administration of QE ahead of Cd notably reduced the activities of the cardiac enzymes in the serum of rats and brought them back to the normal levels. This may be mainly due to the anti-lipoperoxidative, anti oxidant and membrane stabilizing properties of QE⁷⁵.

Cadmium induces a wide spectrum of toxicological effects and biochemical dysfunctions constituting a serious hazard to health. Lipoprotein abnormalities resulting in the disruption of serum and cellular lipid levels account for the genesis of Cd mediated cardiovascular diseases⁷⁶. Several lines of studies suggests that exposure to Cd may alter lipid metabolism and contribute to the development of cardiac related disorders^{20,21}. In our study, oxidative stress induced by Cd in the heart tissue of rats might be responsible for the observed abnormalities in antioxidant, lipids and lipoprotein profiles.

Cd induced CVD is associated with increased levels of lipids in serum. We have observed increased levels of plasma TC, TG, FFA and PL in the plasma of Cd treated rats. Cd induced changes in the plasma concentrations of the estimated lipid compounds noted in the present study are in consistent with the findings of other Authors^{20,77,21}. The changes in the cholesterol profile and other lipid compounds noted in Cd treated rats may be explained by Cd ability to increase the activity of hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) via releasing inflammatory cytokines and interleukins⁷⁸. The increase in the serum concentration of cholesterol may also result from Cd ability to decrease its uptake by macrophages that play an important role in the processing of cholesterol⁷⁷. Moreover, Cd may contribute to the development hypercholesterolemia via increasing the production of inflammatory cytokines such as tumor necrosis factor α (TNF- α) and interleukin1 β (IN-1 β) in the heart⁷⁹. Several studies have also shown that cytokines are involved in the increase in serum TG levels and VLDL production by stimulating hepatic lipogenesis and suppressing fatty acid oxidation^{80,81}. The increased concentration of serum FFA might result from the inhibition of β oxidation via Cd and their increased accumula-

tion in the heart mitochondria with their subsequent discharge into circulation^{20,82}. The Cd induced decrease in the hepatic concentration of PL with the simultaneous increase in the serum concentration may result from their enhanced degradation by PL by phospholipases. Cd is noted as the stimulator of the activity of phospholipase A₂ responsible for this process⁸³. Pretreatment with QE in Cd intoxicated rats was shown to be normalizing the elevated levels of serum lipids. This hypolipidemic effect of QE is mainly attributed with their antilipoperoxidative activity which prevents the oxidation of membrane lipid components and thus preserving the cellular and organeller membranes from the oxidative injury elicited by Cd. In addition, quercetin is reported to exert anti-inflammatory effects⁸⁴, thereby, reducing the inflammatory reactions of cytokines, which in turn suppress their influence on lipid metabolism.

The observed increase in the levels of plasma VLDL-C and LDL-C with a decreased level HDL-C in Cd intoxicated rats clearly reflects the abnormalities in lipoprotein metabolism. Our findings of elevated total cholesterol and lipoproteins by Cd are in tune with the previous reports^{20,85}. Lowered level of plasma HDL-C implies the altered metabolism of the major HDL apoprotein A-I⁸⁶. An increased oxidative stress and disturbance of cell function due to Cd accumulation²⁰ may cause a defect in synthesis and secretion of certain lipoproteins from the heart. The overproduction of hepatic VLDL-C and impaired catabolism of TG-rich particles in Cd toxicity may lead to hypertriglyceridemia. The changes in the activity of HMG-CoA reductase may depress LDL receptor gene expression. Defects in LDL receptor interfere with cholesterol uptake from the blood stream, which in turn causes excess cholesterol synthesis in heart and high levels of plasma cholesterol and LDL-C⁸⁷. Prior oral administration of QE in Cd intoxicated rats significantly has normalized the upregulated lipoprotein levels in plasma through their antilipoperoxidative and antioxidant actions, which inhibit the extensive accumulation of cholesterol and oxidized lipid components as well as prevent hypercholesterolemia.

Elevated levels of FFA in heart could be due to a disturbance of mitochondrial function induced by Cd⁸², that leads to the inhibition β -oxidation and increased accumulation of FFA in heart. Usually elevated FFA in circulation promotes the conversion of FFA into PL and cholesterol in the

heart. These FFA and PL along with excess TG formed at the same time in heart may be discharged into blood in the form of lipoproteins⁸⁸. In contrast, the excess FFA available in heart of Cd exposed rats increased cholesterogenesis and decreased PL content. This change in PL level probably results from enzymatic disorders induced by Cd, especially with regard to calmodulin-dependent enzymes under the effects of Cd^{89,90}. The increased cholesterol content in heart may also affect the activation of membrane-bound enzymes, membrane fluidity and permeability of ions and increase the degradation of PL⁹¹. In fact, PL are rich in polyunsaturated fatty acids, which are easily susceptible to ROS⁹². Formation of such ROS in Cd treated rats¹⁶ may be responsible for the increased degradation and the decreased level of PL. Moreover, oxidation of lipids, notably oxidative modification of LDL-C, is implicated in the development of cardiovascular and cerebrovascular diseases^{23,93}.

Pretreatment with QE in Cd treated rats significantly reversed the Cd induced alterations in lipid metabolism. QE is reported to augment the endogenous antioxidants, which is an important event that protects the heart and endothelial cells from oxidative injury. It is already reported that QE is the potent scavenger of ROS and antilipoperoxidative agent that inhibits the oxidation of LDL-C, which prevent endothelial cell injury induced by oxidized LDL-C⁹⁴. QE and is reported to inhibit Cd induced lipid peroxidation⁷⁵ which reduces the amount of circulating oxidized LDL-C and subsequent accumulation of cholesterol in macrophages, smooth muscles and blood vessel walls, may inhibit atherogenic fatty streaks.

Altogether, the accumulation of Cd in tissues leads to oxidative stress abnormalities in lipid and lipoprotein levels as a consequence of metabolic disturbances in heart, which potentiates the risk for cardiovascular diseases. Pretreatment with QE has normalized the oxidative stress, the levels of lipids and lipoproteins in Cd intoxicated rats. This could be due to the ability of QE by inhibiting the activity of the HMG-CoA reductase and increasing the activity of LCAT⁹⁵. The antioxidant property of QE and AT indirectly helps to lower the lipids by inhibiting lipid peroxidation. Thus the lipid lowering effect of QE could reduce the incidence of atherosclerosis upon exposure to environmental toxicants such as Cd. Our findings support the views that dietary compounds have the ability to lower the risk of cardiovascular diseases. Further investigations are

necessitated to establish the exact molecular mechanisms of QE in the amelioration of Cd induced anomalies in the metabolism of lipids and oxidative cardiotoxicity.

The major forms of cellular damage in heart induced by cadmium are lipid peroxidation, oxidation of proteins and thiol depletion. This study demonstrates the elevated levels of lipid peroxidation, lipid hydroperoxides, conjugated dienes and increased formation of protein carbonyls in the heart of cadmium treated rats might be due to the over production of free radicals and erosion of antioxidant defense by cadmium, which leads to oxidative modifications of proteins and lipids in the heart tissue. Cd due to its peroxidative properties, causes oxidative damage to various macromolecules, including lipids^{77,76,96}. Cellular membranes are recognized to be the major target sites for Cd action and this toxic metal induced lipid peroxidation. It is an important mechanism in the damage to some tissues and organs including primarily the heart and kidneys^{16,97,21}. Moreover, the Cd induces oxidative stress may also be involved in the development of cardiovascular diseases (CVD)^{98,96}. A large body of evidence indicates that the peroxidation of lipids in cellular membranes and lipoprotein oxidation in the serum leads to CVD^{99,100}. Restoration in the levels of these cardiac oxidative stress markers to their near normal levels by the pretreatment with QE in Cd treated rats clearly revealed the antilipoperoxidative and anti oxidant effects of QE.

Antioxidant enzymes are considered to be the first line of cellular defense that prevents cellular ingredients from oxidative damage. Among them SOD and CAT mutually function as important enzymes in the elimination of ROS. Reduction in cardiac SOD activity has been observed in the cadmium exposed animals. Since SOD is a metallo-enzyme; reduction in its activity may be attributed to dysfunctional conformational change which may be due to the replacement of Zn²⁺ present in SOD by cadmium leading to loss of enzymatic activity^{101,102}. Like SOD, another antioxidant enzyme, CAT also experienced significant reduction in activity in the cardiac tissue of the cadmium exposed animals under experimental set of conditions. At physiological pH of 7 (the pH at which CAT was assayed) the nitrogen atom of imidazole ring in His-74 is deprotonated and thus interacts with the Cd²⁺ ion causing reduction in CAT activity¹⁰¹. Both GR and GPx are FAD dependent and Se dependent oxido reductase which protect cellular biomolecules as well as structure

from toxin-induced oxidative damage. Reduction in the activities of these two enzymes represents the cadmium-induced cardiac oxidative stress. Cadmium exposure also reduced the activities of GST and G6PD. Pretreatment with QE significantly elevated the activities of all these antioxidant enzymes indicating that cadmium-induced cardiac injury can be prevented by QE treatment.

Thiol based antioxidant system plays second line of cellular defense against reactive free radicals and other oxidant species mediated oxidative damage. The levels of GSH, GSSG, total thiols and vitamins C and E are additional indicators of cardiac oxidative injury. GSH with its sulfhydryl group acts as a catalyst in disulfide exchange reaction. It functions by scavenging free radicals as well as detoxifying various xenobiotics and consequently converted to its oxidized form, glutathione disulfide (GSSG). GSH being the most important biomolecule against metal induced oxidative stress and can participate in the elimination of reactive intermediates by reaction of hydroperoxides in the presence of GSH-dependent enzymes. GSH also functions as a free radical scavenger and in the repair of radical caused biological damage¹⁰³. Following cadmium intoxication, cardiac glutathione red-ox status was greatly impaired, as indicated by a significant decrease in the levels of total thiols, GSH along with the increased level of its metabolite GSSG. The depleted level of cardiac GSH following Cd intoxication depicts the increased utilization of GSH for the detoxification of Cd. Treatment with QE prior to the cadmium administration could prevent the depletion of these non enzymatic antioxidants, indicating that QE might play an important role in the metabolism of GSH which in turn increase the concentration of thiol groups and thus increases intracellular antioxidant power²⁸.

A significant decreased activity of Na⁺/K⁺-ATPase and Mg²⁺ ATPase and a significant increased activity of Ca²⁺ ATPase in the heart were observed in Cd treated rats. Decreased activity of Na⁺/K⁺-ATPase could be due to enhanced lipid peroxidation by free radicals on Cd induction, since Na⁺/K⁺-ATPase is a 'SH' group containing enzyme and is lipid dependent. Decreased activity of Na⁺/K⁺-ATPase can lead to a decrease in sodium efflux, thereby altering membrane permeability. Ca²⁺-ATPase regulates the calcium pump activity. Enhanced Ca²⁺-ATPase activity observed in Cd treated rats is due to the activation of adenylate cyclase by Cd. Calcium overload in the myocardial cells during ischemia acti-

vates the Ca²⁺-dependent ATPase of the membrane depleting high energy phosphate stores, thereby, indirectly inhibiting Na⁺ and K⁺ transport and inactivation of Na⁺/K⁺-ATPase. Mg²⁺ ATPase activity is involved in other energy requiring process in the cell and its activity is sensitive to lipid peroxidation. Pretreatment with QE normalized the activities of Na⁺/K⁺-ATPase, Mg²⁺-ATPase and Ca²⁺-ATPase in Cd treated rats. Restoration of Na⁺/K⁺-ATPase activity due to QE pretreatment in Cd treated rats could regulate the intracellular Ca²⁺ levels, thereby, protecting the myocardium from excess damage by maintaining the membrane integrity. Elevated levels of Na⁺ concentration resulted in depressed effects of Ca²⁺ and augment Ca²⁺ influx. This could be due to the ability of QE to protect the "SH" groups from the oxidative damage through the inhibition of peroxidation of membrane lipids. This effect is due to the membrane stabilizing properties of quercetin⁷⁵. The membrane stabilizing property might be due to the blocking of lipid peroxidation in cell membranes.

Histological examination of the cardiac segment reveals that cadmium treatment caused abnormal ultra structural changes in the cardiac tissue. In myofibril impairment observation, no significant difference has been observed between control and QE pre-treated groups. This histological finding indicates that QE is capable of preventing the myofibril damage induced by cadmium. Therefore, it may be suggested that QE might inhibit Cd-induced cardiac damage.

It has been observed that cadmium toxicity increased the extent of both lipid peroxidation and protein carbonylation which may be regarded as an indicator of increased ROS production during the toxin exposure period. Treatment with QE prior to cadmium exposure, however, effectively inhibited the metal toxicity by decreasing the levels of lipid peroxidation as well as protein carbonylation. The status of intracellular ferric reducing antioxidant power has been significantly impaired due to cadmium toxicity and that could be prevented by QE treatment before the toxin administration. The triglycerides, total cholesterol as well as HDL-cholesterol are the indicators of cardiac dysfunction. QE administration, prior to toxin exposure could reverse the alterations of those indices in serum suggesting its prophylactic role in Cd-induced cardiac dysfunction.

The following mechanisms have been proposed for the antioxidant effects of QE: (1) being a direct antioxidant, QE could quench and detox-

ify several reactive intermediates, like hypochlorous acid (HOCl) generated by myeloperoxidase, nitric oxide, H₂O₂, and hydroxyl radical (•OH)¹⁰⁴, (2) as an indirect antioxidant, QE could prevent the changes in membrane permeability due to oxidative injury via intercalating into the membrane and stabilizing it¹⁰⁵. Renugadevi and Miltonprabu⁷⁵ reported that the membrane stabilizing effect of QE is linked to an action on permeability of ions and water. Further investigation on the protective mechanism of QE is needed to shed light on these phenomena.

Conclusions

Altogether, we would like to mention that foods with QE supplements could protect cardiac tissue against heavy metal induced especially, cadmium-induced oxidative cardiac impairment and dyslipidemia. At this stage the precise mechanism of protection played by QE is not fully clear. Further researches are necessary to investigate the detailed molecular protective mechanism played by QE against cadmium-induced cardiac dysfunction and dyslipidemia.

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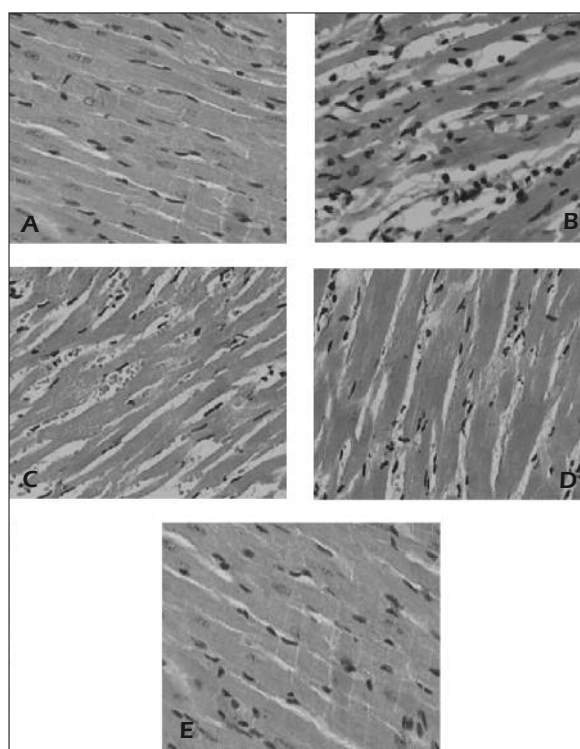


Figure 1. Hematoxylin and eosin stained cardiac sections of (A) control rat (400 ×) showing normal histoarchitecture of cardiac muscle, (B-C) Cd treated rat (400 ×) showing extensive degeneration, swelling, hypertrophy, inflammatory cell infiltration and fibre destruction in cardiac muscle, (D) Cd+QE treated rat (400 ×) showing occasional loss of muscle fibre, (E) QE treated rat (400 ×) showing normal structure of myocytes.

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