ISSN: 2347-467X, Vol. 12, No. (3) 2024, Pg. 1093-1105

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Comparison between the Antioxidant Properties of Quercetin and Combined Vitamin E and Selenium to Ameliorate the Oxidative Stress Induced by Cadmium Toxicity

MOHAMMED AL-ZHARANI[1], EMAN ALMUQRI[1], MOHAMMED MUBARAK[1]*, HASSAN RUDAYNI[1], NADA ALJARBA[2], KHADIJAH YASEEN[3], SAAD ALKAHTANI[3], FAHD A. NASR[1]; AMIN A. AL-DOAISS[4] and MOHAMMED S. AL-EISSA[1]

[1]Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia.
[2]Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia
[3]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
[4]Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia

Abstract

Quercetin is the most prevalent flavonoid; its bioactivities have a preventive role in helping protect cells against various damaging effects. Complex vitamin E and selenium help enhance the immune system and maintain brain and heart health, as well as keep normal cell division. Cadmium is a hazardous heavy metal that exists naturally, and both occupational and environmental exposure to cadmium were reported. The present study was undertaken to compare the efficiency of quercetin against combined vitamin E and selenium to ameliorate the oxidative stress induced by cadmium toxicity. Four groups of rats, 20 animals each, were used: control, cadmium, cadmium and vitamin E plus selenium, and cadmium and quercetin. The administration of cadmium, vitamin E, selenium, and quercetin was done through the oral route and there was a gap (10 h) between the administration of cadmium and receiving vitamin E and selenium or quercetin. Concerning the incidence rate of cadmium toxicity, all rats exposed to cadmium exhibited the effect of cadmium toxicity as evidenced by decreased haematological parameters and altered biochemical profile. Compared to decrements of the parameters recorded in cadmium-exposed rats, the haematological parameters estimated in animals exposed

Article History
Received: 23 February 2023
Accepted: 12 July 2024

Keywords
Antioxidant;
Cadmium;
Quercetin;
Selenium;
Toxicity;
Vitamin E.

CONTACT Mohammed Mubarak mohammedahmed_62@yahoo.com Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.3.9

to cadmium and then given vitamin E and selenium or quercetin were relatively improved toward the control levels. Insignificant differences were recorded between cadmium-exposed animals that received vitamin E plus selenium or quercetin. The recorded parameters of the altered biochemical profile of cadmium-exposed animals were relatively brought back toward the control levels in rats exposed to cadmium and then given vitamin E and selenium or quercetin. Based on the encountered haematological parameters and biochemical profile, it was concluded that the ameliorating effect of quercetin compared to that of combined vitamin E and selenium on the cadmium-induced oxidative stress is comparable. Both quercetin and combined vitamin E and selenium as dietary supplements exhibited efficacy in ameliorating cadmium-induced oxidative stress and maintaining the endogenous antioxidant system.

Abbreviations
MDA, malondialdehyde
TAC, total antioxidant capacity

Introduction
Flavonoids are a group of plant pigments that are chemically classified as phenolic substances. Quercetin is one of the natural plant flavonoids, which are grouped under flavanols.[1, 2] Quercetin is considered the most prevalent flavonoid, it exists in a large variety of vegetables and fruits, especially onions, red grapes, apples, citrus fruits, red grapes and plant-sourced beverages (green tea and coffee). Quercetin (polyphenolic substance) should be received from external sources since it is not synthesized in the body.[2] Foods and dietary supplements represent the sources that supply the body with quercetin. Generally, flavonoids are recognized as a group of bioactive molecules that are capable of protecting cells by combating damaging insults.[3] A considerable number of in vivo investigations, as well as *in vitro* studies demonstrated some of the quercetin protective activities.[2-5] The use of quercetin as a prophylactic or therapeutic agent in cardiovascular and neurodegenerative diseases, hyperglycemia, and nephropathic changes was documented in previous studies.[6-8] The preventive role of quercetin is accomplished through certain mechanistic pathways that involve specified biochemical processes.[3, 5] These protective pathways are exemplified by the ability of quercetin to inhibit lipid peroxidation . The diverse bioactivities of quercetin attracted researchers to investigate the relevant mechanistic protective pathways in some human studies.[9-13]

Vitamin E is a fat-soluble vitamin naturally existing in plant oils derived from corn, sunflower, and soya, and in fruits such as almonds and hazelnuts. α-tocopherol and ϒ-tocopherol are the prevalent dietary forms of vitamin E. Vitamin E as a lipid-soluble vitamin acts to sustain many metabolic processes.[14] Vitamin E also has anti-inflammatory action, immune-enhancing effect and inhibitory role on platelets aggregation.

Selenium (Se) is a naturally occurring mineral, its food sources include bread, cereals, wheat, millet, meat, mushrooms and eggs.[15] Selenium is considered a micronutrient integrated into the chemical structure of certain proteins (selenoproteins). The biochemical activities mediated by selenoproteins involve antioxidation and immune enhancement, such activities contribute to preventing and improving the outcome of significant disease conditions such as diabetes, cancer and neurological disorders.[15,16] Complex vitamin E and selenium help enhance immune functions maintain brain and heart health, and keep normal cell division.

Cadmium is a hazardous heavy metal that exists naturally and is engaged in a wide spectrum of industries. Both occupational and non-occupational (environmental) exposure to cadmium were reported. Occupational exposure is linked with the inhalation of industrial fumes, while non-

occupational exposure is related to the ingestion of polluted feed and water.[17] The hazardous effects of cadmium chronic toxicity are progressive since the accumulation of this toxicant heavy metal is gradual (cumulative) in various tissues. Cadmium toxicity is implicated in the causation of a profound oxidative stress associated with oxidative damaging effects in tissues and organs, noticeably liver and kidney.[18]

Among the main damaging insults that take place inside the living body are the oxidative actions induced by certain metabolites or external toxins. Such type of undesired actions may be severe enough to eventually lead to cell death and extensive tissue damage. Counteracting these actions, either by prevention or inhibition, is a crucial role of the relevant antioxidative mechanisms. The naturally operating and efficient endogenous antioxidant system detects oxidative metabolites, such as free radicals, and prevents their damaging effects.[19] The beneficial effect of this essential bioactivity is to maintain the persistent balance between oxidation actions and antioxidative activities to protect cells and preserve the normal functions of cells and tissues. In cases of oxidation: antioxidation imbalance, external antioxidants, preferentially from dietary sources, help support the maintenance of balance between oxidative reactions and the antioxidative activities.

The issue of natural antioxidants which can be received as dietary supplements is presently of great concern. Their usage has steadily grown as a prophylactic measure of some significant cardiovascular diseases, neurodegenerative disorders, cancer, and arthritis, and in the alleviation of ageing changes.[20]

The current study was conducted to compare the antioxidant properties and efficiency of quercetin to that of combined vitamin E and selenium to ameliorate the oxidative damaging effects induced by cadmium toxicity.

Materials and Methods

Experimental Animals

Eighty adult male Wistar rats aged 3 months and weighed 145-210 g, were used in the present study. The rats were housed under convenient laboratory conditions (ambient temperature 24 ± 1 ^{o}C, 12 h dark/light cycle, relative humidity of 35% to 70%). Keep and care of the laboratory animals were done following the rules approved by the "Research Ethics Committee" of Imam Mohammad Ibn Saud Islamic University (IMSIU) (LAB-animals-022-0151).

Cadmium

Cadmium was used as cadmium chloride (Cd Cl2) of analytical grade (Merck, Darmstadt, Germany).

Quercetin

Quercetin was employed as quercetin dihydrate (C15 H10 O7. 2 H2O) (United States Pharmacopeia, pharmaceutical primary standard) (Sigma-Aldrich, Darmstadt, Germany) (Product No 1592409).

Vitamin E and Selenium

Vitamin E (α-Tocopherol) and selenium (Sigma-Aldrich, Darmstadt, Germany) were used as dietary supplements.

Experimental Design

Animals were acclimatized for one week, and then randomly and equally divided into four groups (20 rats/group); Groups 1, 2, 3, and 4. Animals of Group 1 served as control, i.e., neither administered with cadmium nor given quercetin, and only received oral 1 mL distilled water. Group 2 animals were daily administered with cadmium using oral gavage at the dose of 2 mg/kg body weight (1 mL/kg body weight). The cadmium dose was determined based on a previous study.[32] Control animals received an equal volume of distilled water via the same route. Group 3 animals were administered cadmium as done in Group 2, then given daily oral doses of vitamin E (200 mg /kg body weight) and selenium (0.2 mg/kg body weight). Vitamin E and selenium doses were based on previous studies.[20, 21] Group 4 rats were administered with cadmium, and then quercetin was given orally at the dose of 350 mg/Kg body weight. The administered dose of quercetin was determined based on a previous study.[22] Concerning Groups 3 and 4, there was a time gap (10 h) between the administration of cadmium and receiving vitamin E and selenium (Group 3), or quercetin (Group 4).

Feed (dry ration) and drinking water were supplied ad libitum throughout the experiment period (8 weeks).

Behavioural activity, feed consumption, water intake and clinical signs were monitored in all experimental groups.

At the end of the experiment, the animals were anaesthetized and euthanized to obtain the required blood samples from animals in the different groups. Blood samples with anticoagulant (EDTA) were utilized to estimate haematological parameters, including erythrocytic and total leucocytic counts, packed cell volume (PCV) %, and haemoglobin (Hb) concentration.

To assess blood cadmium levels in animals of Groups 2, 3, and 4, 1 mL blood samples were subjected to digestion using a mixture of $HClO_4$- and HNO_3, then blood cadmium levels were determined using an atomic absorption spectrophotometer (CBC 906 AA).

The harvested serum was used to assess the biochemical parameters and to reveal and compare the biochemical profile of the treated animals to that of the control. These parameters included total proteins, albumin, globulin, creatinine, urea and blood urea nitrogen (BUN), as well as serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The antioxidant and oxidant markers; total thiols, catalase, glutathione, malondialdehyde (MDA), H_2O_2, and total antioxidant capacity (TAC) were included.

Erythrocytic and total leukocyte counts were measured using a convenient hemocytometer. The micro-hematocrit method was used to determine packed cell volume (PCV) % and the Cyanmet-hemoglobin method was employed to estimate the concentration of haemoglobin.[23,24]

Colourimetric assay kits were used to measure the levels of total thiols (Cell Biolabs Inc., USA), reduced glutathione (GSH) (ElabScience, USA), and catalase activity (BioVision; Abcam, UK).

TAC was assessed by employing a total antioxidant capacity (TAC) assay kit (Sigma-Aldrich, Germany). The assessment of TAC by this kit is dependent on the concentration of small-molecule antioxidants combined with protein or non-combined small-molecule antioxidants. Cu^{2+} ions are converted to Cu^{+} by small molecules and proteins. However, the insertion of a protein mask as a component of the kit prevents the reduction of Cu^{2+} by proteins, enabling the analysis of only small-molecule antioxidants. The Cu^{+} ions (reduced by the small molecules of antioxidants) were chelated with a colourimetric probe, and the resultant absorbance peak was proportional to the antioxidant capacity.

Colorimetric assay kits were used to estimate the H_2O_2 levels (Elabscience, USA) and MDA levels (Elabscience, USA).

Diagnostic kits (BioMeerieux, France) were employed to measure the levels of ALT, AST, and ALP. A set of colourimetric assay kits were used to determine the levels of urea (BioVision, Biovision Incorporated, UK), BUN (ThermoFisher Scientific, USA), and total proteins, creatinine, bilirubin, albumin and globulin (Interchim Diagnostics Biochemistry Kits, France). Homogenates of the liver and kidney were prepared from animals in the different groups to estimate the levels of glutathione, total thiols, H_2O_2, TAC, catalase, and MDA in the tissues. The diagnostic kits used to assess the levels of these parameters in serum were employed to determine their levels in the prepared homogenates.

Statistical Analysis

The data derived from the conducted assays are expressed by means ± S.D. Analysis of the data was done using one-way ANOVA by the application of statistical software (SPSS Inc. Chicago IL, USA) to compare the means of multiple groups. A post-hoc test (Dunn-Bonferroni test) was also employed. P–values less than 0.05 ($P < 0.05$) were considered of statistical significance.

Results

The cadmium level in the blood of control untreated rats was (0.0023 ± 0.0001 ppm) and significantly increased ($P< 0.05$) in animals administered cadmium to reach the level of (0.573 ± 0.021 ppm). Blood cadmium levels, compared to cadmium-exposed rats, were comparatively decreased in rats administered with cadmium and then given vitamin E and selenium (Group 3) or quercetin (Group 4) to measure (0.217 ± 0.020 ppm) and (0.247 ± 0.022 ppm), respectively.

The haematological profile of rats exposed to cadmium (Group 2), showed varied decrements of its parameters compared to the control levels. Hemoglobin concentration and PCV% were significantly altered. Compared to the decrements of parameters recorded in Group 2, the haematological parameters estimated in animals exposed to cadmium and then given vitamin E and selenium (Group 3) or quercetin (Group 4) were relatively improved to be closer to the control levels. The improvement in Group 3 (vitamin E and selenium) was better compared to Group 4 (quercetin), however the differences were insignificant.

Table 1. Haematological assay in rats administered cadmium, rats administered cadmium and given quercetin, and rats treated with cadmium and received vit. E and selenium in comparison with control rats

Parameter	Control	Cadmium	Cadmium /quercetin	Cadmium/ vitamin E and selenium
RBCs count (106/mm3)	5.77 ± 0.08	4.07* ± 0.09	5.39** ± 0.06	5.43** ± 0.03
Total leucocytic Count ($10^{3}/mm^{3}$)	6.39 ± 0.35	5.13* ± 0.21	6.04** ± 0.09	6.29** ± 0.02
Hemoglobin (Hb) concentration (g/dL)	12.41 ± 0.22	9.68* ± 0.32	11.81** ± 0.37	12.07** ± 0.47
Packed cell volume (PCV %)	45.11 ± 0.28	35.59* ± 0.51	42.46** ± 0.77	43.60** ± 0.83

Values are expressed as means ± S.D., 20 rats/ group,
*Significant differences from control (P < 0.05), ** Significant differences from the group of cadmium.

Table (2). Biochemical assay in rats administered cadmium, rats administered cadmium and given quercetin, and rats treated with cadmium and received vit. E and selenium in comparison with control rats

Table (2 a). Albumin (g/dL), globulin (g/dL), total proteins (g/dL), creatinine (mg/dL), BUN (mg/dL), and bilirubin (mg/dL)

Parameter	Control	Cadmium	Cadmium /quercetin	Cadmium/ vitamin E and selenium
Total proteins	7.51 ± 0.17	5.41* ± 0.12	6.72** ± 0.15	7.22** ± 0.15
Albumin	3.47 ± 0.03	2.78* ± 0.14	3.22** ± 0.11	3.42** ± 0.11
Globulin	3.81 ± 0.09	2.93* ± 0.15	3.18** ± 0.24	3.67** ± 0.24
Creatinine	0.58 ± 0.11	0.98* ± 0.08	0.51** ± 0.29	0.44** ± 0.29
BUN	15.21 ± 1.03	29.22*± 1.31	18.41**± 1.47	17.91**± 1.47
Bilirubin	6.37 ± 0.31	11.67*±0.23	7.59** ± 0.48	7.36** ± 0.48

Values are expressed as means ± S.D., 20 rats/group,
*Significant differences from control (P < 0.05), ** Significant differences from the group of cadmium.

The estimated haematological parameters in rats administered with cadmium, and rats treated with cadmium and then given vitamin E and selenium (Group 3) or quercetin (Group 4) in comparison to the control rats are shown in Table (1).

Concerning the biochemical profile, comparable decrements in the estimated levels of albumin, globulin, and total proteins were encountered in rats of Group 2, which were exposed to cadmium without access to vitamin E and selenium or quercetin. Rats of Group 2 showed a significant increase in the levels of urea, BUN, and creatinine. Total thiols, glutathione and catalase levels were significantly decreased in rats of this group. In addition, levels of MDA, H_2O_2, and TAC of rats in Group 2 were significantly elevated compared to those of the control rats.

The altered components of the recorded biochemical profile were relatively brought back toward the control levels in rats treated with cadmium and then given vitamin E and selenium (Group 3) or quercetin (Group 4). Improvements recorded in Group 3 are better than that of Group 4, however, the differences were insignificant.

Table 2 (b). Aspartate transferase (AST) (IU/L), Alanine transferase (ALT) (IU/L), alkaline phosphatase (ALP) (IU/L), and urea (mg/dL)

Parameter	Control	Cadmium	Cadmium /quercetin	Cadmium/ vitamin E and selenium
ALT	27.77 ± 1.13	66.71* ± 1.14	32.51** ± 1.13	31.31** ± 1.04
AST	42.41 ± 1.19	137.84* ± 3.44	56.13** ± 1.26	54.43** ± 1.18
ALP	24.27 ± 1.11	73.93* ± 1.34	30.19** ± 1.37	29.24** ± 1.53
Urea	40.59 ± 0.48	74.67* ± 0.61	49.16** ± 0.59	48.19** ± 0.61

Values are expressed as means ± S.D., 20 rats/ group,
*Significant differences from control (P < 0.05), ** Significant differences from the group of cadmium.

Table 2 (c). Glutathione (GSH) (µg/mL), total thiols (mmol/L), catalase (IU/L), malondialdehyde (MDA) (nmol/mL), H_2O_2 (mmol/L) and total antioxidant capacity (TAC) (nmol/mL)

Parameter	Control	Cadmium	Cadmium /quercetin	Cadmium/ vitamin E and selenium
Total thiols	2.48 ± 0.27	0.23* ± 0.04	2.03** ± 0.29	2.29** ± 0.37
Glutathione	41.71 ± 1.19	14.13* ± 0.61	36.84** ± 1.49	37.64** ± 1.26
Catalase	52.52 ± 1.47	29.11* ± 1.12	46.79**± 1.27	47.71**± 1.24
MDA	319.37 ± 3.11	439.51* ±3.41	340.28** ±3.51	338.31** ± 3.51
H_2O_2	40.71 ± 1.47	91.22* ± 1.19	52. 16** ± 1.07	51. 21** ± 1.19
TAC	36.39 ± 1.19	15.41* ± 1.12	29.74** ± 1.09	28.81** ± 1.03

Values are expressed as means ± S.D., 20 rats/ group,
*Significant differences from control (P < 0.05), ** Significant differences from the group of cadmium.

The biochemical profile of rats treated with cadmium, and rats administered with cadmium and then given vitamin E and selenium or quercetin compared to the control rats are shown in Table (2) (a, b, and c). Table (3) shows levels of glutathione, catalase, total thiols, malondialdehyde (MDA), H_2O_2 and TAC (total antioxidant capacity) in hepatic and renal homogenates of rats treated with cadmium, and rats exposed to cadmium and then given vitamin E and selenium or quercetin in comparison to the control rats.

Discussion

Reactive oxygen species (ROS) generated in a controlled manner are beneficial for some physiological metabolic activities such as cell respiration and some signalling pathways (messengers).[25, 26] Oxidative stress refers to the loss of balance between the accentuated production of ROS, including free radicals and hydrogen peroxide on one side and the endogenous antioxidant capacity on the other side. If this situation is not resolved, the ultimate sequel is the oxidative damage of lipids (polyunsaturated fatty acids), proteins and DNA.[27]

The antioxidant systems through various protective mechanisms act to maintain the generated ROS at a controlled physiological limit. The generated free radicals within the physiological requirements are scavenged by the antioxidant system; however excess uncontrolled generation of free radicals may lead to extensive lipid oxidation that is associated with tissue damage.[25] Hydroxyl radical (OH) is the most active in lipid peroxidation, this peroxyl radical can interact with free fatty acids to form potentially toxic lipid peroxides. Additionally, the production of excess ROS may inactivate the antioxidant enzymes and result in the overall failure of the antioxidant system.[26]

Oxidative stress in the present experimental study was induced by cadmium toxicity in Wistar rats. Cadmium reaches the various tissues after absorption and accumulates in the liver and kidney. Oxidative damage of cadmium in tissues is an indirect effect through the generated free radicals. The direct damaging effect of cadmium toxicity is mediated by lipid peroxidation of cell membranes.[17] Cadmium-induced cytotoxicity also accounts for lipid peroxidation of mitochondrial membranes which affects drastically the mitochondrial production of ATP and glutathione. Furthermore, antioxidative enzymes are remarkably suppressed by cadmium toxicity with subsequent disturbance of the oxidation: antioxidation equilibrium.[18] Thus, the already existing oxidative stress is accentuated, and eventually, cadmium cytotoxicity causes apoptosis as a result of caspase activation.

The presently decreased RBCs and total WBC counts in rats exposed to cadmium are probably ascribed to cadmium-induced toxic effects on circulating blood cells and/or hematopoiesis.

The elevations of ALP, AST, and ALT exhibited by the presently cadmium-exposed animals reflect the resultant hepatotoxicity. The antioxidant enzyme activity in liver tissue represents one of the prevalent components of the antioxidant capacity, hence any toxic effect on the liver undoubtedly reduces the endogenous antioxidant capacity. Increased serum level of hepatic enzymes is a reliable marker of hepatic damage. Lipid peroxidation of lysosomal membranes causes leakage of hepatocellular enzymes with elevation of their serum levels.[27] Moreover, cadmium-induced nephrotoxicity leads to pronounced impairment of renal functions.

The present results exhibited a significant increase in the components of the biochemical profile, including creatinine and urea, that reflect the status of renal functions.

The presently assessed anti-oxidant markers, including catalase, glutathione, and total thiols, showed a significant decrease in the cadmium-exposed animals. This finding is a reflection of a state of oxidative stress and a compromised antioxidant endogenous system. The oxidation reaction of the generated ROS dramatically affects the cellular antioxidant molecules with the loss of their antioxidant activity.[25] Glutathione and catalase are active scavengers of free radicals. Catalase, a potent antioxidant enzyme, destructs hydrogen peroxide, the major cause of lipid peroxidation, and also maintains homeostasis of the cellular oxidation: reduction rate. The decreased activity of this antioxidant molecule gives the chance for free radicals to induce more pronounced lipid peroxidation. Glutathione, a main non-protein thiol, is produced by all cells, and the liver is the main producer. Besides its role as an endogenous antioxidant, glutathione is a major factor in protein and DNA synthesis, and sustenance of the redox status. Oxidative stress results in the transformation of glutathione into its reduced form.[26] Total thiols were also decreased in the present rats that were administered with cadmium. Thiols constitute the major contributor to antioxidant efficiency, and via acting as receptors for electrons, thiols can actively reduce and eliminate ROS including singlet oxygen and hydroxyl radicals. Under oxidative stress, thiols may become oxidized and their activity to combat ROS is greatly reduced. Decreasing the level of

thiols allows ROS to exert more hazardous effects on cells and tissues.

The elevated level of MDA in rats treated with cadmium reflects the cadmium-induced oxidative damage. MDA is considered an indicator of lipid peroxidation that arises as a consequence of the oxidative activity of free radicals. H_2O_2 was significantly elevated in these rats, it has an outstanding oxidation activity and constitutes a main contributor in the Fenton reaction which yields the hydroxyl radical (OH).[28]

The significantly decreased blood cadmium level in the present cadmium-exposed rats and given combined vitamin E and selenium or quercetin. Indirect chelating activity is suggested by enhancing the binding of cadmium with proteins. Undoubtedly, decreasing cadmium levels in blood remarkably reduces the rate of oxidative stress, since cadmium constitutes the basic trigger. Accordingly, the endogenous antioxidant system started to recover to continue its function against the excess free radicals. Simultaneously, vitamin E and selenium, or quercetin act synergistically with the endogenous antioxidant system to sustain the antioxidant status. This effect was reflected positively in the estimated parameters of rats treated with cadmium.

Vitamin E is a fat-soluble vitamin and it is essential to sustain many metabolic processes as it prevents the damaging effects of lipid peroxidation. Vitamin E (α-tocopherol) inhibits the free radical-induced peroxidation of cell membranous lipoproteins (polyunsaturated lipids). Vitamin E interacts directly with the free radicals, which target cell lipids, to form non-toxic lipids.[29] Vitamin E restricts to a great extent lipid peroxidation of cell membranes through its ability to scavenge lipid peroxyl radicals and then chemically convert them into α-tocopherol radicals . At the early step of ROS generation, vitamin E breaks the reaction chain of the alkyl radical with molecular oxygen and thereby blocks the formation of peroxyl radical.[26] Vitamin E also enhances glutathione peroxidase activity, which is actively implicated in the destruction of lipid peroxides.

There is a profound synergism in several ways between vitamin E and selenium as regards sustaining the antioxidant capacity. Vitamin E and selenium are proposed to function as non-specific biological antioxidants to combat oxidative damage.[29]

These two biomolecules sustain each other to eliminate lipid peroxides, which are the end products of the interaction between fatty acids (polyunsaturated) and ROS. Both vitamin E and selenium can prevent peroxidation of mitochondrial membranes and microsomes. It was demonstrated that both selenium and vitamin E are needed to protect mitochondria and microsomes in hepatocytes from peroxidative damage. Vitamin E can function directly to eliminate lipid peroxides, and selenium can perform the same function but indirectly since it is a co-factor for glutathione peroxidase which is actively engaged in the eradication of lipid peroxides.[30]

There is a profound synergism between vitamin E and selenium as regards sustaining the antioxidant capacity. Vitamin E and selenium biomolecules have the property to function in synergy to eliminate lipid peroxides that contribute to the development of oxidative damage.[14, 29] This synergistic action of selenium and vitamin E can yield similar beneficial effects such as a reduction in lipid peroxidation and increasing glutathione level.[19] In this regard, *in vitro* studies showed that lipid peroxidation, especially in liver microsomes, takes place in association with vitamin E deficiency.[22] Tissues of selenium and vitamin E-deficient animals were found to exhibit an increased rate of peroxidation. Moreover, Adipose tissue of animals suffering from vitamin E deficiency was found to contain lipoperoxides.[24]

Selenium is considered a micronutrient, its main organic form in the living body is selenocysteine, and it is combined with proteins (selenoproteins) or enzymes such as glutathione peroxidase (selenoenzymes).[27] Among selenoproteins (bioproteins), glutathione stands as an outstanding bioactive molecule. The antioxidant property of selenium is ascribed to the fact that one glutathione peroxidase (antioxidant enzyme) molecule contains four selenium atoms. Moreover, selenium acts as a co-factor for glutathione peroxidase which is crucial in the chemical reduction of lipid peroxides to be converted into non-damaging molecules.[29]

Table (3). Glutathione (GSH) (µg/mL), total thiols (mmol/L), catalase (IU/L), malondialdehyde (MDA) (nmol/mL), H_2O_2 (mmol/L) and total antioxidant capacity (TAC) (nmol/mL) in liver and kidney homogenates

Parameter	Control		Cadmium		Cadmium / quercetin		Cadmium/ vitamin E and selenium	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Total thiols	1.83 ± 0.23	1.03 ± 0.21	0.24*±0.03	0.21*±0.05	1.51** ± 0.25	1.09** ± 0.35	1.79** ± 0.25	1.49** ± 0.45
Glutathione	16.21 ± 1.19	14.46± 1.17	5.11*±0.31	4.71*±0.61	14.05**±1.19	14.02**±1.34	14.65**± 1.19	14.73**± 1.49
Catalase	20.33 ± 1.59	19.13 ±1.28	9.65*±1.14	9.13*±1.27	16.62**±1.21	16.43**±1.29	17.03**± 1.21	17.13**± 1.36
MDA	124.14± 3.71	120.33± 3.69	418.11*±3.29	464.31*±3.51	149.23**±3.3	149.78**±3.15	147.44**±3.13	148.08**±3.21
H_2O_2	16.55 ± 1.57	13.29 ± 1.17	89.11*± 1.31	91.11*± 1.42	19.32**±1.41	19.03**±1.11	18.86**± 1.41	18.07**± 1.16
TAC	14.12 ± 1.14	13.81 ± 1.13	6.88* ± 1.14	6.12* ± 1.17	10.76**±1.15	10.77**±1.03	11.87**±1.15	11.07**±1.07

Values are expressed as means ± S.D., 20 rats/ group,
*Significant differences from control ($P < 0.05$), ** Significant differences from the group of cadmium.

It was demonstrated that selenium alone enhances the cellular enzymatic antioxidant activity through increasing levels of glutathione, glutathione peroxidase and superoxide dismutase.[30] It has been shown that selenium deficiency is associated with a dramatic reduction in the activity of glutathione peroxidase in tissues. Selenium and vitamin E as dietary supplements sustain the glutathione peroxidase level and both act synergistically to scavenge ROS. Vitamin E and selenium deficiency may result in a significant lowering of the activities of glutathione peroxidase and glutathione reductase in the liver and brain tissues. Glutathione peroxidase is a crucial antioxidant enzyme (selenium-dependent) to destroy H_2O_2 and hydroperoxides such as lipid hydroperoxides, hence it protects against the effects of oxidative damage on cell membranous structures and other cell structures sensitive to oxidation.[32, 34] Vitamin E performs the same protective role since it prevents the formation of lipid hydroperoxides. Undoubtedly, a decreasing level of glutathione peroxidase indicates reduced activity of the overall endogenous antioxidant system.

The present results demonstrated improved haematological parameters and biochemical profile (blood and tissues) in rats exposed to cadmium and given vitamin E and selenium. These results imply the efficiency of these two biomolecules to counteract the cadmium-induced oxidative stress. Selenium and vitamin E were found to have a significant role in protecting liver tissue against ROS-induced damage.[31]

There was an improvement in the measured parameters in rats treated with cadmium and given quercetin. Quercetin, with a noticeable activity, is supposed to alleviate the cadmium-induced oxidative damage. The antioxidant activity of quercetin is attributed to the hydroxyl groups and double bonds in its chemical structure.[32]

Quercetin exerts its antioxidant activity through a direct scavenging of the excess ROS generated during the process of oxidative stress. The benzene B ring, inserted in the quercetin formula, is an active site for antioxidation, and tends to react with radicals and thus eliminates ROS. Additionally, quercetin promotes glutathione synthesis[31] and enhances the activity of the antioxidant enzymes including catalase, glutathione peroxidase, and superoxide

dismutase.[33] Quercetin, through inhibiting lipid peroxidation, helps protect cellular membranous structures.[34, 35] This was confirmed by the finding that quercetin in case of oxidative stress is associated with a decrease in MDA levels in hepatic and renal tissues, which in turn implies a remarkable inhibitory effect on lipid peroxidation.

Moreover, quercetin can act as a chelating agent to bind $Fe.^{2+}$ [36] Through binding Fe^{2+}, quercetin inhibits lipid peroxidation mediated by Fe^{2+} (Fenton reaction). Quercetin decreases the total available iron ions and blocks the contribution of these ions in the oxidation reactions.[37, 38]

If all aforementioned mechanisms are considered, quercetin can be categorized as a potent antioxidant with a diverse set of actions that involves direct reaction and elimination of ROS, promoting activity of antioxidant enzymes, regulation of glutathione synthesis, inhibition of oxidative enzymes, chelation of iron ions and metal ions (pro-oxidants), and blocking the reactions of lipid peroxidation.

Therefore, it is apparent that quercetin can significantly maintain antioxidant integrity, sustain the antioxidative: oxidative equilibrium, and eventually ameliorate the hazardous effects of oxidative stress.

In the present study, both the combined vitamin E and selenium, and quercetin succeeded in improving the haematological parameters and biochemical profile (blood and tissues) of rats exposed to cadmium. In other words, these dietary supplements were capable of acting as potent antioxidants to alleviate the effects of cadmium-induced oxidative stress

In the current study, haematological parameters and biochemical profile (blood and tissues) of cadmium-exposed rats that were given combined vitamin E and selenium, or quercetin were reversed toward the control levels. These results provide evidence that combined vitamin E selenium, and quercetin can noticeably ameliorate the toxic effects of cadmium on haematological indices, possibly via a chelating effect or indirectly by inhibiting the negative impact of cadmium toxicity on the circulating blood cells. Levels of hepatic serum enzymes and renal function parameters, a reliable marker of tissue damage, were also improved and reversed in rates given vitamin E selenium, and quercetin. This finding represents another evidence that these dietary supplements are capable of alleviating cadmium-induced tissue damage. Reversing of serum enzyme levels implies significant counteracting actions of the administered dietary supplements on the toxic effects of cadmium particularly those acting on liver and kidney tissues. The improvements of catalase, total thiols and glutathione (antioxidant markers) as well as TAC in rats given combined vitamin E and selenium, or quercetin represent direct evidence that the antioxidant mechanisms were restored. Markers indicating active oxidative reactions, including H_2O_2 and MDA levels, were reversed in these rats. All these findings may confirm the efficient antioxidant properties of the employed dietary supplements to inhibit the impact of oxidative reactions and eradicate ROS. Quercetin can alleviate the cadmium-induced oxidative stress and the associated oxidative damage through diverse mechanistic pathways.

Conclusion

Currently, the noticed improvement of the assessed parameters, haematological and biochemical, in rats treated with cadmium might represent evidence of the antioxidant activities of the used dietary supplements in case of toxicities induced by heavy metals. Vitamin E, selenium, and quercetin as dietary supplements presumably perform their antioxidant activities in synergy with the bioactive antioxidant molecules to mitigate oxidative damage and to maintain the oxidation antioxidation balance. The present findings exhibited insignificant differences between the ameliorating effects of combined vitamin E and selenium on one side, and quercetin on the other side in combating the cadmium-induced oxidative stress. However, more research work is recommended to reveal the exact molecular mechanisms that enable dietary supplements to act as potent antioxidants in different types of toxicities.

Acknowledgement

The authors would like to thank Deanship of Scientific Research, Imam Mohammad Ibn Saud Islamic University for their guidance and support to complete this article.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

Keep and care of the laboratory animals were done following the rules approved by the "Research Ethics Committee" of Imam Mohammad Ibn Saud Islamic University (IMSIU) (LAB-animals-022-0151).

Informed Consent Statement

This study did not involve human participants, animal subjects, or any material requires ethical approval.

Author Contributions

- **Mohammed Mubarak:** Conceptualization, methodology and writing the manuscript.
- **Hassan Rudayni:** supervision of lab work, methodology and resources.
- **Mohammed Al-Zhrani:** Methodology, resources and supervision of lab work.
- **Amin Abdullah Al-Doaiss, Nada Aljarba, Kaija Yassen, Eman Almuqri, Saad Alkahtani, Fahd Ali Nasr:** Methodology, lab work, and statistical analysis of data.
- **Mohammed Al-eissa:** Supervision of lab work and resources.

References

1. Magar R.T., Sohng J.K. A review on structure, modifications and structure-activity relation of quercetin and its derivatives. *J Microbiol Biotech*. 2020; 30(1): 11–20.
2. Ulusoy H.G., Sanlier N. A minireview of quercetin: from its metabolism to possible mechanisms of its biological activities. *Critical Rev Food Sci Nutr.* 2020; 60 (19): 3290–3303.
3. Abd-Elsalam H.H., Gamal M., Naguib I., Al-Ghobashy M.A., ZaaZaa H.E., Abdelkawy M. Development of green and efficient extraction methods of quercetin from red onion scales wastes using factorial design for method optimization: A complementary study. *Separations.* 2021; 8: 137.
4. Reyes-Farias M., Carrasco-Pozo C. The anti-cancer effect of quercetin: molecular implications in cancer metabolism. *Int J Mol Sci.* 2019; 20 (13): 3177.
5. Tang P., Diao X., Shu L., Xiong L. Quercetin attenuates the inflammatory response and oxidative stress in LPS-induced RAW264.7 cells: *in vitro* assessment and a theoretical model. *Bio Med Res Int.* 2019; 19: 1–8.
6. Yuan K., Zhu Q., Lu Q. Quercetin alleviates rheumatoid arthritis by inhibiting neutrophil inflammatory activities *The J Nutr Bioch.* 2020; 84: 108454.
7. Lv S., Wang X., Jin S., Shen S., Wang R., Tong P. Quercetin mediates TSC2-RHEB-mTOR pathway to regulate chondrocytes autophagy in knee osteoarthritis. *Gene*. 2022; 820: 146209.
8. Neamtu A.A., Maghiar T.A., Alaya A. A comprehensive view on the quercetin impact on colorectal cancer. *Molecules.* 2022; 27 (6): 1873.
9. Agrawal P.K., Agrawal C., Blunden G. Quercetin: antiviral significance and possible COVID-19 integrative considerations. *Natural Product Communications.* 2020;15 (12): 1934578X2097629.
10. Colunga Biancatelli R.M.L., Berrill M., Catravas J.D., Marik P.E.. Quercetin and vitamin C: an experimental, synergistic therapy for the prevention and treatment of SARS-CoV-2 related disease (COVID-19). *Frontiers in Immunol*. 2020; 11: 1451.
11. Boretti A. Quercetin supplementation and COVID-19. *Natural Product Communications.* 2021; 16 (9): 1934578X2110427.
12. Derosa G.P., Maffioli A., D'Angelo F., Di Pierro. A role for quercetin in coronavirus disease 2019 (COVID-19). *Phytotherapy Res*. 2021; 35 (3): 1230–1236.
13. Zhang J., Pavek P., Kamaraj R., Ren L., Zhang T. Dietary phytochemicals as modulators of human pregnane X receptor. *Critical Rev Food Science Nut.* 2021; 1: 23.
14. Trüeb R.M. Oxidative stress and its impact on skin, scalp and hair. *Int Cosm Sci.* 2021;

43: 9–13.
15. Hariharan S., Dharmaraj S. Selenium and selenoproteins: Its role in the regulation of inflammation. *Infammopharmacol.* 2020; 28: 667–695.
16. Ye R., Huang J., Wang Z., Chen Y., Dong Y. The Role and Mechanism of Essential Selenoproteins for Homeostasis. *Antioxidants*. 2022; 11: 973.
17. Sharma H., Rawal N., Mathew B.B. The characteristics, toxicity and effects of cadmium. *Int J Nanotech Nanosci.* 2015; 3: 1-9.
18. Li S., Tan H., Wang N., Zhang Z., Lao L., Wong C. The role of oxidative stress and antioxidants in liver diseases. *Int J Med Sci.* 2015; 16: 26087-26124.
19. Klaus A., Heribert H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 2004; 55: 373-399.
20. Pham-Huy Z.A., He H., Pham-Huy C. Free radicals and antioxidants in disease and health. *Int J Biomed Sci.* 2008; 4: 89-96.
21. Takhshid M.A., Tavasuli A.R., Heidary Y., Keshavarz M., Kargar H. Protective effect of vitamins E and C on endosulfan-induced reproductive toxicity in male rats. *Iran J Med Sci*. 2012; 37(3):173-181.
22. Aydoğan F., Taştan E, Aydın E., Senes M., Akgedik S., Berkem R., Tekeli A., Haklıgör A., Ustün H. Antioxidant role of selenium in rats with experimental acute otitis media. *Indian J Otolaryngol Head Neck Surg.* 2013; 65 (3): 541-547.
23. Gardi C., Bauerova K., Stringa B., Kuncirova V., Slovak L, Ponist S., Drafi F., Bezakova L., Tedesco I., Acquaviva A., Bilotto S., Russo GL. Quercetin reduced inflammation and increased antioxidant defense in rat adjuvant arthritis. *Arch Biochem Biophys.* 2015; 583:150-157.
24. Benjamin M.M. Outline of Veterinary Clinical Pathology. 1974; 3rd Edn. The Iowa State University Press, USA.
25. Coles E.H. Veterinary Clinical Pathology. 1986; 3rd Edn. W.B. Saunders Co., Philadelphia, USA.
26. Calabrese E.J., Kozumbo W.J. The hormetic dose-response mechanism: Nrf_2 activation. *Pharmacol Res.* 2021; 167: 105526.
27. Taufenberger A., Magistretti P.J. Reactive oxygen species: Beyond their reactive behavior. *Neurochem Res*. 2021; 46: 77–87.
28. Juan C.A., Pérez de la Lastra J.M., Plou F.J., Pérez-Lebeña, E. The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int J Mol Sci.* 2021; 22: 4642.
29. Coline R., Clement V., Anne L., Frederic B. Use of H_2O_2 to cause oxidative stress, the catalase issue. *Int J Mol Sci.* 2020; 21(23): 9149.
30. Hu Q., Zhang Y., Lou H., Ou Z., Liu J., Duan W., Wang H., Ge Y., Min J., Wang F. GPX4 and vitamin E cooperatively protect hematopoietic stem and progenitor cells from lipid peroxidation and ferroptosis. *Cell Death Dis*. 2021; 12: 706.
31. Celebi S. Effect of dietary Vitamin E, selenium and their combination on concentration of selenium, MDA, and antioxidant enzyme activities in some tissues of laying hens. *Pak J Zool.* 2019; 51: 1155–1161.
32. Xu D., Hu M.J., Wang Y.Q., Cui Y.L. Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules.* 2019; 24 (6): 1123.
33. Tsai C.F., Chen G.W., Chen Y.C. Regulatory effects of quercetin on M1/M2 macrophage polarization and oxidative/antioxidative balance. *Nutrients.* 2021;14 (1): 67.
34. Oh W.Y., Ambigaipalan P., Shahidi F. Preparation of quercetin esters and their antioxidant activity. *J Agric Food Chem.* 2019; 67 (38): 10653-10659.
35. Liu S., Zhu Y., Liu N., Fan D., Wang M., Zhao Y. Antioxidative properties and chemical changes of quercetin in fish oil: quercetin reacts with free fatty acids to form its ester derivatives. *Journal Agricultural Food Chemi.* 2021; 69 (3): 1057–1067.
36. Babenkova I.V., Osipov A.N., Teselkin Y.O. The effects of dihydroquercetin on catalytic activity of iron (II) ions in the Fenton reaction. *Bull Exp Biol Med.* 2018; 165(3): 347-350.
37. Dong B., Shi Z., Dong Y. Quercetin ameliorates oxidative stress induced cell apoptosis of seminal vesicles via activating Nrf2 in type 1 diabetic rats. *Biomedicine Pharmacotherapy.* 2022; 151: 113108, 2022.

38. Chen T., Zhang X., Zhu G. Quercetin inhibits TNF-α induced HUVECs apoptosis and inflammation via downregulating NF-kB and AP-1 signaling pathway *in vitro*. *Medicine.* 2020; 99 (38): e22241.