



RESEARCH ARTICLE

The effects of quercetin on antioxidant system and some blood parameters in rats exposed to acute cadmium toxicity

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Akut kadmiyum toksikasyonu oluşturulan ratlarda quercetin'in bazı kan parametreleri ile antioksidan sistem üzerine etkileri

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Öz

Amaç: Bu çalışmada akut kadmiyum toksikasyonuna maruz kalan ratlarda quercetin'in bazı kan parametreleri ile antioksidan sistem üzerine etkilerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu amaç doğrultusunda, canlı ağırlıkları birbirine yakın olarak seçilmiş 30 adet yetişkin erkek "Wistar-Albino" rat kullanıldı. Çalışmada kullanılan hayvanlar Kontrol grubu (K), Kadmiyum grubu (Cd), Quercetin grubu (Q) ve Kadmiyum + Quercetin grubu (CdQ) olmak üzere dört gruba ayrıldı. Cd grubunda yer alan ratlara 3 gün boyunca 4mg/kg/canlı ağırlık/gün dozunda kadmiyum klorür (CdCl₂) subkutan olarak uygulanırken, Q ve CdQ grubundaki ratlara ise 50 mg/kg/canlı ağırlık/gün dozunda quercetin intraperitoneal olarak yine 3 gün boyunca enjekte edildi. Kontrol grubunda yer alan ratlara herhangi bir uygulama yapılmadı. Kan örnekleri çalışmanın başlangıcından sonraki 4. günde toplandı.

Bulgular: Deneme sonunda serum ve plazma örneklerinden SOD, MDA, GSH, katalaz, ALT, AST, GGT, total protein ve albumin düzeyleri belirlendi. Serum MDA düzeyinin sadece Cd uygulanan grupta önemli oranda yüksek olduğu belirlendi (P<0.05). ALT, AST ve GGT enzim düzeylerinin Cd grubunda, K ve Q gruplarına oranla daha fazla arttığı (P<0.05) tespit edildi.

Öneri: Sonuç olarak akut kadmiyum toksikasyonundan negatif etkilenen antioksidan sistem ve bazı kan parametrelerinin quercetin kullanımına bağlı olarak yeniden düzeldiği belirlenmiştir.

Anahtar kelimeler: Antioksidanlar, kan parametreleri, kadmiyum, quercetin, ratlar

Abstract

Aim: The aim of this study was to determine the effects of quercetin on antioxidant system and some blood parameters in rats exposed to acute cadmium toxicity.

Materials and Methods: Adult male "Wistar-Albino" rats (n = 30) were used and divided into four groups as Control (C, n = 6), Cadmium (Cd, n = 8), Quercetin (Q, n = 8) and Cadmium + Quercetin (Cd + Q, n = 8). Cadmium chloride (CdCl₂, 4 mg/kg daily, s.c) were administrated to Cd and Cd+Q groups, and Quercetin (Q, 50 mg/kg daily, i.p) were given to Q and Cd + Q groups for 3 days, respectively. Control group was not received any treatment. Blood samples were collected from all animals at fourth day after treatments.

Results: The levels of serum SOD, MDA, GSH, catalase, and plasma ALT, AST, GGT, total protein, albumin were detected. Although, serum MDA levels were found higher (P< 0.05) in Cd than the other groups, it was similar in Q and C groups. ALT, AST and GGT enzyme levels were observed higher in Cd than C and Q groups (P< 0.05).

Conclusion: Negative effects of acute cadmium toxicity on antioxidant system and some hematological parameters were ameliorated with quercetin treatment in rats.

Keywords: Antioxidants, blood parameters, cadmium, quercetin, rats



Introduction

Recently, the use of toxic and heavy metals in agricultural areas, and increasing chemical activities in the industry cause environmental pollution and health threatening risks in human beings (WHO 2010). Toxic heavy metals like mercury (Hg), cadmium (Cd) and arsenic (As) are the most important environmental pollutants around the world. Particularly, human and animals can be easily exposed to Cd via metal industry, battery production, contaminated food and water, dirty air or inhalation of tobacco (Kanter et al 2013). Cd mainly accumulates in liver and kidney, also in lung, duodenum, pancreas, bone, brain and testicular tissues, so it leads to tissue damages (Gerhardsson et al 2002, Satarug et al 2003, Kocak and Akcil 2006, Karabulut-bulan et al 2008). Cd was identified as class I carcinogen by The International Agency for Research on Cancer (IARC) (Smith et al 1997). In addition, Cd especially causes epigenetic changes in the expression of DNA, damage on the nuclear membrane and mitochondrial cristae, inhibits the cell metabolism and disrupts transport systems in the proximal renal tubules of S-1 segment (Schwartz and Reis 2000, Bernhoft 2013, Gencer et al 2014).

Antioxidants have been used for preventing the adverse effects of Cd intoxication (Karabulut-bulan et al 2004, El-boshy et al 2014). Flavonoids, the most known antioxidants due to biochemical and pharmacological activities, can prevent the formation of lipid peroxide radicals and the other radicals which starts lipid peroxidation. Flavonoids can also prevent the oxidation of lipids by linking metal ions and inhibit the enzyme systems in the occurring of free radicals (Pourmorad et al 2006, Fang 2007).

Quercetin, an antioxidant plant pigment, is a member of bioflavonoids and has metal-binding properties. Grapefruit, onion, apple, black tea, small amounts of leafy green vegetables and beans contain active ingredient quercetin (Erguzel 2006). Quercetin applications lead to increase elimination of oxidative stress and degenerative disorders in various tissues depending on Cd intoxication in many studies (Renugadevi and Prabu 2010, Krishnakumar et al 2012, Wang et al 2013).

It is pointed many of the studies on Cd and its toxicity out related with the chronic accumulation and their elimination (Kocak and Akcil 2006, Andujar et al 2010, Bernhoft 2013). When compared to chronic, effects of acute Cd toxicity on some blood parameters and antioxidant system is poorly understood (Kocak and Akcil 2006, Andujar et al 2010, Hounkpatin et al 2013). Particularly, followed conditions cause to acute toxication of Cd; workers in metal production units via inhalation, to exposure via acidic food and drinks stored in containers coated with large amounts of Cd and carelessness of persons during to laboratory working (ATSDR 2012).

The aim of this study was to determine the effects of quercetin on antioxidant system and some blood parameters in rats exposed to acute Cd toxicity.

Materials and Methods

All animal handling and procedures were approved by Experimental Medicine Research and Application Center of Selcuk University Experimental Animal Ethics Committee (2015/45).

In this study, four months aged and healthy, 30 adult male Wistar albino rats (350 ± 10 g live weight) obtained from Selcuk University Experimental Medicine Research and Application Center (SUEMRAC), were used. Animals were hosted in a standard plastic rattan cages located in SUEMRAC during the study. The room temperature was $23 \pm 2^\circ\text{C}$ and relative humidity was $55\% \pm 10$. Rats were fed ad libitum with standart ration for 12 hours/night in the daylight period. Animals accessed to refreshed daily drinking water (~ 50 mL/day/rat).

Four experimental groups were designed as Control group (C, $n = 6$), Cadmium group (Cd, $n = 8$), Quercetin group (Q, $n = 8$) and Cadmium + Quercetin group (Cd + Q; $n = 8$). Animals were randomized to the experimental groups. Although, treatment groups (Cd, Q and Cd + Q) were recieved either cadmium chloride or quercetin for 3 days, control group had not any treatment. Cadmium chloride (CdCl_2 , 4 mg/kg daily, s.c) was given to animals in Cd and Cd + Q groups, and Quercetin (50 mg/kg daily, i.p) was also administrated to animals in Q and Cd + Q groups.

Cardiac blood samples were taken via cardiac puncture under the general anesthesia (Thiopental anesthesia, 40 mg/kg) at the fourth day after the treatment, and samples were collected using tubes with and without EDTA for plasma and serum, respectively. All of the tubes for both serum and plasma were kept on ice or in a refrigerator before being centrifuged at 3000 rpm for 25 min at 4°C (Hermle z 380, Rösler, Germany); serum and plasma samples were stored at -80°C until the analyses were performed. Animals were terminated by cervical dislocation technique during the anesthesia after the blood collection.

Serum superoxide dismutase (SOD), malondialdehit (MDA), glutathione (GSH), catalase (CAT), and plasma aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyl Transferase (GGT), total protein and albumin levels were determined from plasma and serum samples. Plasma ALT, AST, GGT, total protein and albumin levels were determined by using biochemical analyzers (Architect C-8000, Abbott, USA) with commercial kits according to the manufacturer's instructions. Serum lipid peroxidation product of MDA and the antioxidant GSH, SOD, catalase (Siemens, Oxis, Cayman, USA) enzym levels were determined



Table 1. The comparing of serum MDA and some antioxidant levels in experimental groups (X ± SEM)

Parameters	Groups			
	C (n=6)	Q (n=8)	Cd (n=8)	Cd+Q (n=8)
MDA (nmol/ml)	0.94±0,09 ^b	1.08±0,10 ^b	1.99±0,22 ^a	1.17±0,17 ^b
SOD (U/ml)	0.47±0,42 ^a	0.47±0,02 ^a	0.38±0,01 ^b	0.41±0,18 ^a
GSH (µM)	3.99±0,29 ^a	4.65±0,56 ^a	2.28±0,46 ^b	3.05±0,20 ^{ab}
Catalase (U/ml)	5.71±0,37 ^a	5.29±0,28 ^a	3.88±0,50 ^c	4.07±0,46 ^{bc}

a,b,c; The differences between average values indicated by different letters in the same row of the same parameters are important ($p < 0.05$).

Table 2. The levels of plasma ALT, AST, GGT, total protein and albumine in experimental groups (X ± SEM)

Parameters	Groups			
	C (n=6)	Q (n=8)	Cd (n=8)	Cd+Q (n=8)
T.Protein (g/dL)	5.78±0,16 ^a	5.41±0.16 ^a	4.77±0.17 ^b	5.33±0.10 ^a
ALT (U/L)	38.83±1,95 ^c	37.66±1.20 ^c	84.00±5.17 ^a	71.83±4.74 ^b
AST (U/L)	61.16±4,04 ^c	63.16±5.18 ^c	148.0±16.83 ^a	108.16±9.89 ^b
GGT (U/L)	1.66±0,21 ^b	1.50±0.22 ^b	2.60±0.33 ^a	1.50±0.34 ^b
Albumin(g/dL)	3.71±0,12 ^a	3.57±0.14 ^a	2.95±0.22 ^b	3.54±0.92 ^a

a,b,c; The differences between average values indicated by different letters in the same row of the same parameters are important ($p < 0.05$).

with commercial kits by using ELISA (Biotek ELX 800, Germany).

Statistical differences among the groups were tested by analysis of variance (ANOVA) which is followed by Duncan's test using SPSS for windows version 17.0.

Results

Statistical results of the study concerning with serum MDA, some antioxidants and plasma biochemical levels were presented in Table 1 and 2

Although, serum MDA levels were found to be higher ($P < 0.05$) in Cd group than the other groups, it was similar in Q and C groups. Serum SOD, GSH and catalase levels were determined lower ($P < 0.05$) in Cd group than the other groups, shown in Table 1.

Albumine and total protein levels were detected the lowest ($P < 0.05$) in Cd group. Besides, ALT, AST and GGT enzyme levels were found higher in Cd than C and Q groups ($P < 0.05$), presented in Table 2.

Discussion

Many researchers have been focused on chronic Cd toxicity

and its negative effects on antioxidant systems, organs and tissues (Karabulut-bulan et al 2008, Andujar et al 2010, Hounkpatin et al 2013). Although, acute Cd toxicity has not been widely researched, presented study was aimed to determine effects of acute cadmium toxicity on some blood parameters and antioxidant system (Andujar et al 2010, Hounkpatin et al 2013).

Previous studies indicated that chronic Cd administrations or exposure induced to increase of serum MDA levels in various species (Hussein et al 2009, Gulcen et al 2011, Kanter et al 2013). Serum MDA levels also increased in Cd applied groups in present study which is similar with previous studies. In addition, the levels of MDA were significantly lower in Cd+Q group when compared the Cd group ($p < 0.05$). It can be considered that the application of quercetin ameliorated lipid peroxidation which occurred depends on acute Cd intoxication.

Serum SOD, GSH and catalase levels were determined lower in acute or chronic Cd induced toxicity in previous studies (Bu et al 2013, Kanter et al 2013, Renugadevi and Prabu 2010). Bu et al (2013) also determined that Cd intoxication significantly reduced antioxidant enzyme system activity. Similarly, our present results also indicated that acute cadmium toxicity caused oxidative stress, lipid peroxidation and inhibition of antioxidant enzymes activities (Table 1). In present





study, quercetin were administered to rats (Q and Cd + Q groups) for 3 days and it caused a significantly increase of serum SOD, GSH and catalase levels in Cd + Q group when comparing with Cd group. Similarly serum SOD, CAT, GPx, GST and glutatyon levels were found higher in Cd + Q group than Cd groups in previous sub-acute and chronic toxication studies (Chlebda et al 2010, Bu et al 2013). Moreover, Zargar et al (2015) administered quercetin (100 mg kg⁻¹dose) to animals two hours before cadmium treatment, and it caused significantly increase of enzymatic antioxidant (SOD and CAT) levels. Present results also indicated that quercetin may be effected on preventing the formation of radicals and lipid peroxidation (Pourmorad et al 2006, Fang 2007).

Zohouri Aysen and Tekeli (1999) reported that injection of cadmium chloride (0,1 mg kg⁻¹ dose) to animals lead to significantly decreased of serum albumine levels in Cd group when compared the control. Hussein et al (2009) also detected that plasma albumin and total protein concentrations significantly reduced due to Cd treatment. In present study, plasma total protein and albumin levels were significantly decreased ($p < 0.05$) in Cd when compared the other three groups (C, Q and C + Q) according to Cd treatment (Table 2). These results showed that decreasing total protein and albumin levels pointed out liver damage depending upon the acute Cd intoxication.

Plasma ALT, AST and GGT enzyme levels were defined higher ($p < 0.05$) in Cd groups than C and Q. These enzyme levels were also determined lower in Q + Cd group when compared Cd group in present study. Besides, serum GGT levels were found similar in Cd + Q, C and Q groups that shown in Table 2. In a similar study, acute Cd poisoning caused increase of serum ALT and creatinine levels but decrease of SOD activity (Fahim et al 2012). It was showed that Cd administration lead to liver damage and also elevated the serum protein and albumine levels.

It was reported that quercetin, a member of flavonoids, has an important rol and positive effects on the liver and liver enzyme activities (Pavanato et al 2003, Chen 2010, Marcolin et al 2013). In present study, plasma ALT, AST and GGT levels recovered in Cd + Q group when compared the Cd group (Table 1). Similarly, Cd (5 mg/kg) and quercetin (50 mg/kg) were administered to Wistar rats (Cd + Q group) for 4 weeks, simultaneously. After the treatments, plasma AST, ALT, ALP, LDH enzyme levels and serum GGT values were detected lower in Cd + Q than Cd group by Renugadevi and Prabu (2010). When plasma levels of GGT considered as a determining factor of oxidative stress in cells (Lee et al 2004), the positive effect of quercetin supplementation on oxidative stress was also demonstrated. In addition, decrease of plasma GGT levels in Cd + Q group supports the protective effects of quercetin against acute Cd toxicity.

Conclusions

It was concluded that decreasing of serum MDA and increasing of SOD, GSH and CAT levels demonstrated that, quercetin activated antioxidant system of rats during the acute Cd toxicity.

In addition, quercetin and Cd administration recovered plasma ALT, AST, GGT levels, simultanously. These effects were considered that quercetin had a protective effects on liver which occurred depend on acute Cd toxicity.

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