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Research Article

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The Investigation of Protective Effect of Quercetin in Rats Exposed to Oxidative Stress by 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

2,3,7,8-Tetraklorodibenzo-*p*-Dioksin ile Oksidatif Strese Maruz Kalan Sıçanlarda Kuersetinin Koruyucu Etkisinin Araştırılması

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ABSTRACT

In this study, 28 Wistar Albino male rats were randomly divided into four equal groups. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was intraperitoneally administered at the dose of 2 µg/kg/week, quercetin was administered at the dose of 20 mg/kg/day by gavages, and quercetin+TCDD were intraperitoneally administered at the doses of 20 mg/kg/day and 2 µg/kg/week, respectively. All applications were performed for 8 weeks. At the end of the eighth week, the rats were sacrificed and their heart and vascular tissues were taken for biochemical analysis (reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS) levels) by spectrophotometric method. As a result of the study, TCDD significantly decreased antioxidant activities and increased lipid peroxidation in rats. In contrast, quercetin significantly prevented the toxic effects of TCDD via increasing SOD, CAT, GSH and GSH-Px levels but decreased the formation of TBARS. Therefore, it can be suggested that quercetin has the potential for treatment against the toxicity caused by TCDD or other environmental contaminants and can decrease the risk of mortality due to cardiovascular diseases, especially in humans.

Keywords

Antioxidant enzymes, cardiovascular system, oxidative stress, TCDD, quercetin.

ÖZ

Bu çalışmada, 28 adet Wistar Albino erkek sıçan rastgele dört eşit gruba ayrıldı. 2,3,7,8-tetraklorodibenzo-p-dioksin (TCDD) 2 µg/kg/hafta dozda intraperitonal olarak uygulandı, kuersetin 20 mg/kg/gün dozda gavajla uygulandı, ve kuersetin+TCDD birlikte sırasıyla 20 mg/kg/gün ve 2 µg/kg/hafta dozlarda uygulandı. Tüm uygulamalar sekiz hafta boyunca gerçekleştirildi. Sekizinci haftanın sonunda sıçanlar sakrifiye edildi ve kalp ve damar dokuları spektrofotometrik yöntemle biyokimyasal analizler (redükte glutatyon (GSH), glutatyon peroksidaz (GSH-Px), katalaz (CAT), süperoksit dismutaz (SOD) ve tiyobarbitürik asit reaktif sübstansları (TBARS) düzeyleri) için alındı. Çalışmanın sonucunda, TCDD sıçanlarda antioksidan aktiviteleri önemli ölçüde azaltmış ve lipid peroksidasyonunu arttırmıştır. Aksine, kuersetinin artan SOD, CAT, GSG ve GSH-Px düzeyleri ile TCDD'nin toksik etkisini önemli ölçüde engellediği ancak TBARS oluşumunu azalttığı belirlenmiştir. Bu nedenle, kuersetinin TCDD ve diğer çevresel kirleticiler tarafından oluşan toksisiteye karşı tedavi potansiyeline sahip olduğu ve özellikle insanlarda kardiyovasküler rahatsızlıklara bağlı ölüm riskini azaltabileceği önerilebilir.

Anahtar Kelimeler

Antioksidan enzimler, kardiyovasküler sistem, oksidatif stres, TCDD, kuersetin.

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INTRODUCTION

Advancing technology and new production techniques have brought biochemical pollution with them. While many toxic substances have effects on the environment and living organisms, dioxins and dioxin-like substances are among the most frequently encountered and have high toxicity. Dioxin compounds are highly toxic environmental pollutants in terms of human and animal health that have a wide spreading range and are stable in nature. Dioxins formed due to chemical events and high temperatures are taken by humans, especially through animal food, and stored in fat tissue [1]. They cause many side effects in humans such as immune and cardiovascular system disorders, cancer, wasting syndrome, hormone and reproductive system dysfunctions [2]. These compounds accumulate in soil, water, air and especially in fatty tissues of living beings. The most toxic compound of the group is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Being an environmental contaminant, TCDD is toxic on the cardiovascular system in human, several animal species and rodents [3]. It has been reported the cases of myocarditis [4], ectasia of the coronary arteries [5] and atherosclerosis [6] among TCDD-exposed individuals. Moreover, it has recently gained much attention worldwide due to its unpleasant effects, such as hepatotoxicity, immunotoxicity, cardiovascular diseases, cytotoxicity and genotoxicity on human health [7-11].

Quercetin (3,3',4',5,7-pentahydroxyflavone), a class of bioflavonoids that can be dissolved in trace amounts of water, is a compound with potent anti-inflammatory and antioxidant properties founded in almost all medicinal plants, fruits, and vegetables that can be eaten [12,13]. Quercetin, a strong antioxidant, is known to act as an antioxidant against various radicals like especially hydroxyl radical, including superoxide radical, lipid peroxyl radical and nitric oxide radical, and also interacts with singlet oxygen and hydrogen peroxide [14]. Quercetin helps prevent cancer effectively by blocking the flow of nutrients and oxygen to cancerous cells. Laboratory studies have shown that quercetin decreases breast cancer cell growth by up to 50 % [15]. Also, quercetin has been shown to inhibit the growth of prostate, colon, breast, liver disease and lung cancer cells in animal studies [16,17]. This data is supported by several animal studies showing that quercetin exhibits various biological effects both in vivo and in vitro [18]. Thus, it increases systemic and coronary vasodilatation and antiaggregant effects in vitro [19,20] and the oxidative status, falls blood pressure and the organ damage in animal models of hypertension [21].

As it is known that plant-based antioxidant molecules such as quercetin have protective effects on the cardiovascular system, they can be successfully used against lipid peroxidation and oxidative stress of TCDD. In our study, we aimed to determine the oxidative stress level of the caused cardiovascular system in rats of 2,3,7,8-TCDD which is used as a model in experimental dioxin intoxication and investigating the inhibition by structured flavonoid quercetin of this damage. In order to evaluate the effect of the oxidant-antioxidant system in the cardiovascular system of rats, TCDD, quercetin and simultaneously TCDD+quercetin were applied, then SOD, CAT and GSH-Px were measured for antioxidant enzyme activities, MDA levels were determined as a marker of lipid peroxidation and finally, GSH levels were measured. In the light of all these evaluations, our study will give an idea on purposeful availability treatment in the cardiovascular system against TCDD toxicity of quercetin which is useful in oxidative stress models.

MATERIALS and METHODS

Chemicals

Analytical grade or the highest grade available chemicals were purchased from Sigma Aldrich (St. Louis, MO) and TCDD (purity > 99%) was obtained from Accustandart, Inc. (New Haven, CT, USA).

Animals and Treatment

8 healthy adult male Wistar Albino rats (between 3–4 months old, 250-350 g in weight) were provided from Inonu University Experimental Animal Institute were used for this experiment. The rats were kept in sterilized special polypropylene rat cages in groups of 4 on standard conditions (12-h light/dark cycle at 21°C). Drinking water and diet from them were given an *ad libitum.* The selection and implementation of experimental animals were carried out based on animal ethics guidelines of Inonu University Institutional Animals Ethics Committee (protocol no: 2010/02) and the study was performed in properly with the ethical rules of standard experimental animal studies. The duration of the experiment was determined to be 8 weeks. Rats were randomly divided into four groups as seven animals in each group. During the study period, group 1 (control) was given 0,5 mL corn oil by gavages daily for 8 weeks. In group 2 (TCDD), TCDD was diluted in corn oil and intraperitoneally administered at the dose of 2 mg/kg/week for 8 weeks. Group 3 (quercetin)

was administered at the dose of 20 mg/kg/day for 8 weeks by gavages and group 4 (quercetin+TCDD) was intraperitoneally administered at the doses of 20 mg/kg/day and 2 µg/kg/week for 8 weeks, respectively. At the end of the 8 weeks after the treatment, the rats were sacrificed under ether anesthesia 60 days.

Biochemical Assay

The tissues taken from deep freezing were weighed to about 1 g and placed in glass tubes. Tris-HCl buffer (pH 7.4) was added to dilute it to 1/10 (g/h) and then homogenized for 3 minutes at a rate of 16,000 rpm in a glass-teflon homogenizer while maintaining the coldness. The homogenates were measured to thiobarbituric acid-reactive substances (TBARS) formed between a thiobarbituric acid and malondialdehyde (MDA). The remaining homogenates were centrifuged at 3500 rpm for 45 min at +4°C to obtain the supernatant. Reduced glutathione (GSH), catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were measured in these supernatants. Behind, the remaining supernatant was added to the reagent 1/1 (v/v) consisting of a mixture of chloroform/ethanol (3/5, v/v). Then, it was centrifuged at 3500 rpm for 45 minutes at +4°C. SOD enzyme activity and protein measurements were performed in the supernatant chloroform/ethanol phase. The levels of TBARS were determined spectrophotometrically at 532 nm by using the method of Yagi [22]. The tissue GSH levels were measured spectrophotometrically at 412 nm using the method of Sedlak and Lindsay [23]. SOD accelerates to dismutation to hydrogen peroxide (H_2O_2) and molecular oxygen of toxic superoxide radicals (O_2^-) formed during the production of oxidative energy. The tissue SOD enzyme activities were determined spectrophotometrically at 560 nm by using the method of Sun et al. [24]. CAT converts water and oxygen by decomposing H_2O_2 with its catalytic activity. The tissue CAT enzyme activities were determined according to the method of Aebi [25]. GSH-Px activity was performed spectrophotometrically at 340 nm according to the method of Paglia and Valentine [26]. The protein quantities from homogenate and supernatant were determined according to the method developed by Lowry et al. [27] using bovine serum albumin as standard.

Statistical Analysis

Statistical analyses were performed using a computer program (SPSS 15.0). When differences between the groups were determined, significance between groups was evaluated using the Mann-Whitney U (Bonferroni) test. Biochemical differences were considered to be significant at $P \leq 0.01$, and statistical analyses were carried out by using the One-Way ANOVA analysis of variance and post hoc Tukey's significant difference test. Results were expressed as mean ± standard error and values of $P \leq 0.01$ were considered statistically significant.

RESULTS

We investigated to effects of quercetin which is an antioxidant, on the cardiovascular system with oxidant-antioxidant system and lipid parameters as an experimental subchronic model in rats. In our study, the heart tissue GSH levels and SOD, CAT and GSH-Px antioxidant enzyme activities in the TCDD group were significantly decreased ($P \leq 0.001$) with TCDD treatment compared with the control group and simultaneous quercetin administration prevented this decrease ($P \leq 0.001$). The heart tissue TBARS levels in the TCDD group were significantly ($P \leq 0.001$) increased with TCDD treatment compared to the control group and simultaneous quercetin administration prevented this increased ($P \leq 0.001$). However, in the quercetin group, GSH and TBARS levels did not significantly change and GSH-Px, SOD, CAT levels significantly increased to compared to the control group. Furthermore, when applied simultaneously TCDD+quercetin together, TBARS levels significantly decreased, however SOD, GSH-Px, GSH and CAT levels significantly increased (Table 1).

The vascular tissue GSH-Px, SOD and CAT antioxidant enzyme activities and GSH levels in the TCDD group significantly decreased ($P \leq 0.001$) with TCDD treatment compared to the control group. While GSH levels and GSH-Px enzyme activity significantly increased ($P \leq 0.001$) with simultaneous quercetin administration ($P \leq 0.001$), SOD and CAT enzyme activities did not statistically significant increase ($P \geq 0.001$). After applying simultaneous quercetin with TCDD, although CAT and SOD enzyme activities increased according to only the TCDD administration group, these increases didn't statistically significant. The vascular TBARS levels in the TCDD group significantly increased ($P \leq 0.001$) with TCDD treatment compared to the control group and simultaneous quercetin administration prevented to this increase ($P \leq 0.001$). However, in quercetin group, GSH and TBARS levels didn't significantly change compared to the control group. SOD, GSH-Px and CAT levels significantly increased

Table 1. The levels of TBARS, GSH, SOD, GSH-Px and CAT in heart tissue.

	Control	TCDD	Q	TCDD+Q
TBARS (nmol g^{-1} tissue)	6.11 ± 0.22[a]	11.28 ± 0.67[b]	5.54 ± 0.40[a]	8.99 ± 0.38[c]
Reduced GSH (nmol mL^{-1})	48.04 ± 2.29[a]	24.30 ± 1.42[b]	50.02 ± 1.81[a]	36.36 ± 1.43[c]
CAT (k g^{-1} protein)	70.33 ± 2.96[a]	46.59 ± 3.04[b]	92.65 ± 2.22[c]	61.95 ± 3.79[a]
SOD (U mg^{-1} protein)	59.92 ± 4.58[a]	36.07 ± 2.47[b]	76.86 ± 4.37[c]	49.21 ± 2.98[a]
GSH-Px (U mg^{-1} protein)	179.7 ± 4.23[a]	105.3 ± 1.99[b]	197.0 ± 9.23[c]	145.4 ± 5.75[d]

a, ***b***, ***c*** and ***d*** within the same row showed significant ($P \leq 0.001$) differences between groups. Q; Quercetin, TCDD; 2,3,7,8-tetrachlorodibenzo-p-dioxin.

compared to control and other groups. Additionally, it was determined that TBARS levels significantly decreased but GSH-Px levels significantly increased and CAT, GSH and SOD levels didn't significantly change compared to TCDD group when rats treated to simultaneous TCDD+quercetin together (Table 2).

As a result of the study; it has been displayed that TCDD caused to oxidative stress by increasing lipid peroxidation in the hearth and vascular tissues, and decreases antioxidant enzyme activities. It has been observed that quercetin decreased lipid peroxidation caused by TCDD on the cardiovascular system and increased generally to antioxidant enzyme activities.

DISCUSSION

Nowadays, depending on the developing industry and technology, environmental pollutants are found in nature and effects on the living of these cause serious problems. Today; it is well known that toxicities caused by these environmental pollutants play a role at the basis of many chronic illnesses, including cancer. Dioxin and similar compounds are highly toxic environmental pollutants that can be found in trace amounts in almost every part of the ecosystem. Toxicologically, many negative effects are depending on the intake of humans and animals of dioxins and similar compounds and the worst is that these negativities have the potential to continue increasing. Because the release to the environment of these compounds increases day by day, it becomes inevitable that poisoning cases lead to much more serious problems [28].

It was known that TCDD exposure could lead to cardiovascular system disorder in human and animals because of elevated tissue damage and oxidative stress in heart and vascular. It leads to irreversible cell damage and inactivation of many enzymes [29]. Some studies have suggested that workers exposed to dioxin and similar compounds have a higher risk of heart rhythm disturbances, hypertension and abnormal peripheral arterial blood flow, and that mortality from ischemic heart disease has significantly increased [30, 31]. In recent years studies have shown that TCDD decreases antioxidant enzyme activities and increases lipid peroxidation. Ciftci et. al. [32] observed that TCDD increased to oxidative stress and lipid peroxidation, conversely, significantly decreased SOD, CAT, GSH-Px enzyme activities and GSH levels in liver tissue. In another study was detected that liver, kidney, heart, and brain SOD activities generally decreased in the TCDD-administered groups compared to control group, CAT activities of TCDD-treated groups decreased significantly in the liver, kidney and brain tissues as compared to the control group and GSH-Px activities

Table 2. The levels of TBARS, GSH, GSH-Px, SOD and CAT in vascular tissue.

	Control	TCDD	Q	TCDD+Q
TBARS (nmol g^{-1} tissue)	6.90 ± 0.39[a]	11.71 ± 0.91[b]	6.69 ± 0.64[a]	9.44 ± 0.34[c]
Reduced GSH (nmol mL^{-1})	21.30 ± 1.25[a]	13.76 ± 0.61[b]	18.85 ± 0.62[a]	16.05 ± 1.12[b]
CAT (k g^{-1} protein)	77.05 ± 5.33[a]	54.38 ± 4.22[b]	89.83 ± 3.70[ac]	64.49 ± 6.19[ab]
SOD (U mg^{-1} protein)	68.21 ± 2.78[a]	47.09 ± 5.10[b]	76.50 ± 3.87[a]	57.32 ± 2.20[b]
GSH-Px (U mg^{-1} protein)	157.1 ± 6.85[a]	124.5 ± 3.32[b]	176.9 ± 3.65[c]	142.0 ± 5.91[d]

a, ***b***, ***c*** and ***d*** within the same row showed significant ($P \leq 0.001$) differences between groups. Q; Quercetin, TCDD; 2,3,7,8-tetrachlorodibenzo-p-dioxin.

decreased significantly in TCDD-treated groups compared to control group [33]. Another study, lipid peroxidation, and antioxidant enzyme activities were measured, the protective effects of quercetin and chrysin on oxidative stress in rat liver of TCDD were investigated and it was observed that TCDD administration decreased the levels of SOD, GSH-Px, CAT and GSH but it increased to MDA levels [34].

In two parallel trials, quercetin has shown a protective effect against nephrotoxicity and cardiotoxicity in rats and significantly decreased the increased lipid peroxidation [35], Hertog et. al., [36] suggested that higher doses of quercetin decreased cardiovascular risk in older men. In this direction, it was expected that quercetin, an antioxidant compound, protects against to the cardiotoxicity effect of TCDD. At the same time, many studies [32,34,37] as in our study showed that quercetin has high antioxidant potential, and these studies confirm our present study.

As a result of, it has been determined that TCDD increases to free radical formation, these radical increases trigger lipid peroxidation, and increases serious in MDA formation. It has been found that the enzymatic and non-enzymatic antioxidant levels for the increase lipid peroxidation are decreased to such a degree that it can be counted as serious. Quercetin, a potent antioxidant, appears to eliminate lipid peroxidation and oxidative damage in tissues significantly. In addition to these important effects; it is also seen that quercetin taken from the outside increases significantly the levels of non-enzymatic antioxidants such as SOD, GSH-Px and CAT activities and GSH levels by showing a characteristic which supports the antioxidant defense system in the body. When all these positive effects are evaluated as a whole, it has been concluded that this potent antioxidant has potential to be used in therapy against oxidative stress which is seen as the most important cause of many chronic diseases, especially cancer nowadays and that new studies should support these findings.

CONCLUSION

The present study revealed to determining of the oxidative stress level caused the cardiovascular system in rats of 2,3,7,8-TCDD which is used as a model in experimental dioxin intoxication and investigated the inhibition of this damage by structured flavonoid quercetin. In this respect, it was measured GSH levels and MDA levels as a marker of lipid peroxidation, SOD, CAT and GSH-Px antioxidant enzyme activities in the cardiovascular system of rats applied quercetin simultaneously with TCDD, quercetin and TCDD in order to evaluate the effect of the oxidant-antioxidant system. As a result of this study, it has been shown that TCDD caused to oxidative stress by increasing lipid peroxidation in the cardiovascular system, whereas decreasing antioxidant enzyme activities in the cardiovascular system. Additionally, it was observed that quercetin decreased lipid peroxidation caused by TCDD on the cardiovascular system and increase generally to antioxidant enzyme activities. Considering these beneficial effects of quercetin, it can be concluded that toxicities caused by TCDD and other environmental pollutants can be used for treatment in the cardiovascular system. In the light of all these evaluations, our study provides an idea of the therapeutic utility in the cardiovascular system against to TCDD toxicity of quercetin which is useful in a variety of oxidative stress models.

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