



Düzce University Journal of Science & Technology

Research Article

Role of Protocatechuic Acid (PCA) on Hepatotoxicity and Nephrotoxicity Induced by 2, 3, 7, 8-Tetrachlorodibenzo-P-Dioxin (TCDD) in Rats

 Ahmet SAVCI^{a,*},  İlknur ÖZDEMİR^b

^a Department of Molecular Biology and Genetics, Faculty of Arts and Science, Muş Alparslan University, Muş, TURKEY

^b Chemistry Department, Faculty of Arts and Science, Inonu University, Malatya, TURKEY

* Corresponding author's e-mail address: a.savci@alparslan.edu.tr

DOI: 10.29130/dubited.538712

ABSTRACT

It is known that TCDD, one of the most toxic dioxin compounds, causes oxidative damage by forming free radical in human and animal tissues. In this study, the protective effect of PCA, an important phenolic compound, was examined in rat kidney and liver tissues with TCDD-induced toxicity. For this purpose, 28 Wistar Albino rats (3-4 months old and weighing 280-310 g) were used. Rats were randomly divided into 4 equal groups (control, TCDD, PCA and TCDD+PCA). TCDD and PCA were dissolved in corn oil at doses of 2 µg/kg and 100 mg/kg, respectively. Subsequently, the substances were administered to the rats by oral gavage for 45 days. The test results showed that in both kidney and liver tissues, TCDD increased the level of malondialdehyde (MDA) but inhibited the level of glutathione (GSH) and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). PCA administration was found to increase the enzyme activities and GSH levels, whereas it decreased the TCDD and MDA levels. In conclusion, it was observed that PCA decreased the TCDD-induced lipid peroxidation, increasing the antioxidant activity. Therefore, it might be suggested that PCA is a potential reducing agent for the toxicity caused by TCDD.

Keywords: Antioxidant, Protocatechuic acid, 2, 3, 7, 8-TCDD, Oxidative damage, Rat.

Sıçanlarda 2,3,7,8-Tetraklorodibenzo-P-Dioksin (TCDD) 'nin Neden Olduğu Hepatoksisite ve Nefrotoksisite Üzerine Protokateşik Asitin (PCA) Rolü

ÖZET

En toksik dioksin bileşiklerinden biri olan TCDD'nin insan ve hayvan dokularında serbest radikal oluşturarak oksidatif hasara neden olduğu bilinmektedir. Bu çalışmada, TCDD tarafından toksisite oluşturulan sıçan karaciğeri ve böbreğinde önemli bir fenolik bileşik olan PCA'nın koruyucu etkileri araştırıldı. Bu amaçla 28 adet Wistar Albino cinsi sıçanlar (3-4 aylık ve 280-310 g ağırlığında) kullanıldı. Sıçanlar kontrol, TCDD, PCA ve TCDD + PCA olmak üzere rastgele 4 eşit gruba ayrıldı. TCDD ve PCA mısır yağı içinde çözüldü ve sırasıyla 2 µg / kg ve 100 mg / kg dozunda 45 gün boyunca gavaj yolu ile sıçanlara uygulandı. Çalışmanın sonunda TCDD'nin malondialdehit (MDA) seviyesini arttırdığı, ancak glutatyon (GSH) seviyesini ve süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GSH Px) aktivitelerini azalttığı gözlemlendi. PCA uygulamasının TCDD'nin aksine enzim aktivitelerini ve GSH seviyelerini arttırdığı ve MDA seviyelerini düşürdüğü tespit edildi. Sonuç olarak, PCA'nın TCDD'nin neden olduğu lipid peroksidasyonunu azalttığı ve antioksidan aktivitesini desteklediği görülmüştür. Bu nedenle PCA'nın, TCDD'nin neden olduğu toksisiteye karşı potansiyel bir indirgeyici madde olduğu öne sürülebilir.

Anahtar Kelimeler: Antioksidan, Protokateşik asit, 2, 3, 7, 8-TCDD, Oksidatif hasar, Sıçan.

I. INTRODUCTION

Dioxins are permanent environmental pollutants that pose a potential risk to human health [1]. Among all dioxin compounds, TCDD is a highly toxic environmental contaminant [2]. In the experimental animals exposed to TCDD, various biochemical changes and diseases like dermal toxicity, immunotoxicity, hepatotoxicity, carcinogenesis, teratogenicity, behavioral and endocrine changes are identified [3]-[5]. In addition to such diseases, exposure to TCDD is determined to cause oxidative stress [6]. Oxidative stress is defined as defects in the oxidant-antioxidant balance [7]. In an early study, oxidative stress is identified in various tissues exposed acutely to high doses of TCDD. It is reported that free oxygen species, DNA damage and lipid peroxidation increase in experimental animals exposed to TCDD. Oxidative stress caused by TCDD is ascribed to the aryl hydrocarbon receptor (AHR). The administration of 5 mg/kg TCDD in C57BL / 6J female rats for 8 weeks at three day intervals leads to oxidative stress. Furthermore, the presence of oxidative stress is assessed in brain tissues treated with 0.45 ng TCDD /kg/day. It is seen that there is uncertainty at the point where low-dose TCDD causes oxidative stress [8]. A mechanism for TCDD-mediated free radical production is also proposed for cytochrome P450. It is suggested that CYP1A1 and CYP1A2 are associated with TCDD mediated oxidative stress. The induction of CYP 1A1, CYP 1A2 and CYP 1B1 by dioxin results in the formation of free radicals and an increase in lipid peroxidation [9], [10].

PCA is a simple phenolic compound derived from natural sources. As other simple phenolic acids, PCA plays an important role in human nutrition due to its presence in many plants. Brown rice, onion, plum, gooseberry and other grapes, nuts, almonds, olive oil, white wine, anise, balm, rosemary, thyme, Japanese Ginkgo are rich PCA resources [11]-[13]. PCA is effective on the scavenging of free radicals, including the highly reactive hydroxyl radical [14]. PCA provides inhibition of free radicals through binding with transition metal ions Cu (II) and Fe (II) or by regulating the activity of enzyme catalysis reactions during radical formation. Neutralization of free radicals occurs when they react with hydroxyl groups in PCA. In vitro models demonstrate that PCA prevents DNA damage and lipid peroxidation [15], [16].

The effect of PCA on the TCDD-induced oxidative stress has been widely studied for various tissues (e.g., heart and testis). However, its influence has been rarely examined for liver and kidney tissues, leaving a significant gap on this aspect. The aim of this study was to determine the level of oxidative stress in liver and kidney tissues which was caused by TCDD used as a model in experimental dioxin. Moreover, it aimed to investigate the inhibition of TCDD-induced damage by PCA. For such purposes, the activities of SOD, CAT and GSH-Px, the level of GSH and MDA (as an indicator of lipid peroxidation) were defined in rat's liver and kidney tissues where TCDD, PCA and TCDD+PCA were applied. Consequently, the way that oxidative-antioxidant system of tissues was affected in this process was determined. The results presented in this study indicate that PCA is useful for the inhibition of oxidative stress, and it can be effectively used for the treatment of liver and kidney tissues exposed to TCDD toxicity.

II. MATERIAL AND METHOD

A. CHEMICALS

All chemicals and PCA used in the study were obtained from SIGMA ALDRICH (St Louis, Missouri) and 2,3,7,8-TCDD (purity>99%) from AccuStandard, Inc. (New Haven, USA).

B. ANIMALS and EXPERIMENTAL DESIGN

In this study, 28 male Wistar-albino rats (3-4 months old, 280-310 g) were used. The animals were obtained from Experimental Animal Production and Research Center, İnönü University, Malatya. The

rats were divided into 4 equal groups. Each group of rat was kept in special cages under constant heat and ventilated rooms, 12 hours of daylight and 12 hours of darkness. Freshwater and food were given to the rats as ad libitum.

C. EXPERIMENTAL PROTOCOL

The selection and implementation of experimental animals were carried out on the basis of animal ethics guidelines of İnönü University Institutional Animals Ethics Committee (protocol no: 2011/A-15), and the study was conducted following the ethical rules of standard experimental animal studies.

The rats were divided into 4 equal groups as 7 animals in each group. For Group I: Control, animals were administered on a daily basis with 0.5 ml of corn oil for 45 days. For Group II: TCDD, animals were administered on a weekly basis by oral gavage at a dose of 2 µg/kg of TCDD in corn oil for 45 days. For Group III: PCA, animals were administered daily by oral gavage at a dose of 100 mg/kg of PCA in corn oil for 45 days. For Group IV: PCA + TCDD, animals were treated with PCA by oral gavage at a dose of 100 mg/kg on a daily basis and TCDD at 2 µg/kg in corn oil on a weekly basis for 45 days. Liver and kidney samples were stored at -45 °C in a deep freeze until analyzed.

D. BIOCHEMICAL ASSAY

D.1. Homogenization

The tissues taken from the freezer were weighed approximately 1g and placed in glass tubes. Tris-HCl buffer (pH = 7.4) was added to the dilution of 1/10 (g / v) and then homogenized for 3 minutes at 16,000 rpm using a glass-Teflon homogenizer. In such homogenates, the amounts of malondialdehyde (MDA) were determined in tissues. The remaining homogenates were centrifuged at 3,500 rpm for 45 minutes at + 4 ° C to yield the supernatant. The enzyme activities of GSH-Px and CAT and the level of GSH were measured in these supernatants. The reagent containing a mixture of chloroform/ethanol (3/5, v/v) was added to the remaining supernatant at 1/1 (v/v). It was then centrifuged at 3,500 rpm for 45 minutes at + 4 ° C. SOD enzyme activity and protein measurements were taken in the upper chloroform/ethanol phase.

D.2. Determination of Protein Content

The amounts of protein in the homogenate and supernatants were determined according to the method of Lowry et al. [17]. It is complex with Cu⁺² peptide bonds in the alkaline copper reagent. Each 7 or 8 amino acid residue does bind a copper atom. When the folin-phenol reagent is added to the copper-treated mixture, a purple-blue color is formed. The resulting color is read at 700 nm.

D.3. SOD Activity

Superoxide dismutase accelerates the disruption of toxic superoxide radicals (O₂⁻) to hydrogen peroxide (H₂O₂) and molecular oxygen during metabolic reactions. SOD enzyme activities in the tissues were determined following the method of Sun et al. [18]. In this method, SOD activity involves the inhibition of nitroblue tetrazolium reduction, with xanthine/xanthine oxidase system used as a superoxide generator. The colored formazon formed by superoxide radicals reducing NBT is measured spectrophotometrically. This complex gives maximum absorbance at 560 nm.

D.4. CAT Activity

The catalase converts H₂O₂ into water and oxygen through its catalytic activity. CAT enzyme activities in tissues were determined using the Aebi method [19]. H₂O₂ shows maximum absorbance at a wavelength of 240 nm. The cleavage of H₂O₂ added to the assay medium by the CAT enzyme is

followed as an absorbance reduction in the ultraviolet spectrum. This decrease in absorbance is directly proportional to enzyme activity.

D.5. GSH-Px Activity

GSH-Px uses the GSH and catalyzes the conversion of H₂O₂ into H₂O. GSH-Px activity was performed according to De Valentine [20]. GSH-Px-generated GSSG is converted back into GSH by means of GR in the presence of NADPH. GSH-Px activity is calculated spectrophotometrically through measuring the absorbance difference in optical density at 340 nm by converting NADPH to NADP⁺.

D.6. Measurement of Reduced Glutathione (GSH) Levels

Tissue GSH levels were determined by the method of Sedlak and Lindsay [21]. Glutathione determination is based on the principle of measuring the absorbance of yellow 2-nitro-5-thiobenzoic acid formed by oxidation of GSH by DTNB at 412 nm.

D.7. Measurement of MDA Levels

MDA levels of liver and kidney tissues were determined by the Yagi method [22]. This method is based on the optical density of the pink colored complex formed by MDA at 532 nm.

E. STATISTICAL ANALYSIS

Statistical evaluations were conducted using the One-Way ANOVA test and with the SPSS 10.0 program. Pearson's correlation analysis was made in order to determine the correlation between the parameters. Results were expressed as mean ± standard deviation, and p < 0.001 values were considered to be statistically significant.

III. RESULTS AND DISCUSSION

In this study, PCA was applied to eliminate the harmful effects of TCDD on rat kidney and liver. The enzyme activities of SOD, CAT and GSH-Px and the levels of MDA and reduced GSH were assessed to elucidate the antioxidant effect of PCA. The antioxidant enzyme activities and levels of MDA and GSH in tissues were given in Table I and II. The results showed that SOD, GSH-Px, CAT activities and GSH levels significantly (P ≤ 0.001) decreased, whereas the levels of MDA significantly (P ≤ 0.001) increased in TCDD group when compared with Control group. It was also observed that the lipid peroxidation (MDA) increased significantly in both kidney and liver tissues due to TCDD. A significant increase of GSH-Px and SOD activities was observed in kidney tissue in TCDD + PCA group when compared to TCDD group. The assessment of the MDA levels of liver and kidney tissues indicated that TCDD + PCA group showed a significant decrease compared to TCDD group. Based on the aforementioned test results, it can be said that PCA significantly reduced lipid peroxidation in liver and kidney tissues.

Table 1. MDA and GSH levels; SOD, CAT, GSH-Px activities of liver tissue

	Control	TCDD	PCA	TCDD+PCA
MDA nmol/ml	5.18±0.87 ^a	8.24±0.75 ^b	4.98±0.48 ^a	6.28±0.65 ^c
SOD U/mg protein	35.6±0.90 ^a	21.9±0.89 ^b	33.7±1.21 ^a	23.6±1.50 ^b
CAT k/mg protein	1.15±0.04 ^a	0.82±0.02 ^b	1.16±0.03 ^a	0.95±0.01 ^{ab}
GSH-Px U/mg protein	298.7±8.16 ^a	183.3±12.1 ^b	286.2±9.5 ^a	233.9±7.3 ^{ab}
GSH nmol/ml	26.8±0.89 ^a	16.4±1.04 ^b	24.1±1.11 ^{ac}	22.6±1.18 ^c

a, b, c, ab and ac indicate the significant (P ≤ 0.001) differences between the groups.

Table 2. MDA and GSH levels; SOD, CAT, GSH-Px activities of kidney tissue

	Control	TCDD	PCA	TCDD+PCA
MDA nmol/g tissue	10.1±0.51 ^a	19.4±0.35 ^b	11.1±0.83 ^a	14.9±1.23 ^c
SOD U/mg protein	31.4±1.31 ^a	19.6±1.65 ^b	28.3±1.50 ^{ac}	25.8±1.93 ^c
CAT k/mg protein	1.01±0.023 ^a	0.63±0.021 ^{bc}	0.94±0.021 ^{ac}	0.78±0.022 ^c
GSH-Px U/mg protein	397.1±11.2 ^a	241.4±9.8 ^b	365.8±14.9 ^{ac}	320.9±15.2 ^c
GSH nmol/ml	21.2±0.89 ^a	14.2±1.15 ^b	22.6±0.92 ^a	18.7±1.28 ^c

a, b, c, ac and bc indicate the significant ($P \leq 0.001$) differences between the groups.

Permanent organic pollutants including dioxin-like compounds are agents that adversely affect human health. TCDD is one of the most toxic compounds among the dioxin class of substances, and its toxicity equivalence factor is 1 (the highest toxicity) [23]. Recent studies have shown that TCDD is an important oxidative agent and causes oxidative damage on various tissues [24-26]. In this study, it was observed that TCDD caused the oxidative stress in liver and kidney tissues of rats, and it increased the lipid peroxidation by decreasing the activities of antioxidant enzymes. On the other hand, based on the results of TCDD group, PCA was found to increase antioxidant enzyme activities. Moreover, the activities of SOD, CAT and GSH-Px in liver and kidney tissues significantly decreased ($P \leq 0.001$) in TCDD group when compared with Control group.

A large number of studies are available on the damage of various tissues and the decrease of antioxidant enzyme levels in rats administered TCDD [24, 25, 27-30]. The results presented in this study mostly support the findings of the published work. The present study showed that a decrease of antioxidant enzymes was observed in TCDD-induced tissues, while PCA increased antioxidant enzyme activities. There are several studies in the literature that PCA is a potent antioxidant, which is parallel to the results of this study. It is reported that PCA inhibits lipid peroxidation, which increases GSH-Px and SOD enzyme activities, and reduces free radical production against oxidative damage by H_2O_2 in PC12 cells of rats [31]. In another study, it is determined that the PCA prevents the toxic effects of TCDD against reproductive toxicity of TCDD and increases SOD, GSH-Px, CAT enzyme activities [32]. In this study, we observed that the GSH levels in liver and kidney tissues of rats from TCDD group significantly reduced in the presence of TCDD ($P \leq 0.001$), and the simultaneous administration of protocatechuic acid prevented this decrease ($P \leq 0.001$) when compared to the Control group. The study conducted separately on rat heart tissues shows that TCDD administration decreases GSH levels, and PCA administration significantly increases compared to TCDD group [4]. Because of the high reactivity of free radicals, the membrane interacts with polyunsaturated fatty acids to initiate peroxidation. The lipid peroxides formed in this way are easily destroyed, leading to different secondary products, primarily MDA [33]. In a variety of studies, PCA is shown in different forms to increase the antioxidant defense system enzymes and reduce lipid peroxidation [34].

In this study, MDA levels in liver and kidney tissues in TCDD group significantly increased ($P \leq 0.001$), and simultaneous administration of PCA inhibited such increase ($P \leq 0.001$). Liua et al. examined the protective role of PCA against oxidative damage induced by tert-butyl hydroperoxide (t-BHP) in rat liver [35]. The results show that t-BHP increases the level of MDA in rat liver and decreases the level of GSH. Moreover, PCA co-administered with t-BHP increases the level of GSH by lowering the MDA level. In another study, the protective effects of PCA on toxicity caused by cadmium are investigated in rats' liver and kidney tissues. The results highlight that PCA removes the cadmium-induced toxicity in the tissues [36]. Safaeian et al. investigated the effect of supplementation with PCA on deoxycorticosterone acetate (DOCA)-salt hypertension in rat kidney and heart tissues [37]. It is reported that PCA increases serum CAT activity, total antioxidant capacity and glutathione level, whereas it reduces the levels of MDA and hydroperoxides [37].

IV. CONCLUSION

As a result of this study, it was found that PCA reduced the lipid peroxidation caused by TCDD, while it increased the antioxidant enzyme activities in kidney and liver tissues. Considering the beneficial effects of PCA, it can be concluded that PCA may be used for the treatment of toxicity caused by TCDD in liver and kidney tissues.

ACKNOWLEDGMENTS: This research was supported by IUBAP (Scientific Research Fund of İnönü University) under grant 2011/A-15.

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