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The Antioxidant Effect of p-Coumaric Acid Against Toluene-Induced Oxidative Stress in Rats

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ABSTRACT

This research aimed to investigate the protective effect of p-CA, a derivative of phenolic acid, against toluene-induced oxidative damage. A total of 32 Sprague-Dawley male rats, 8 in each group, were used. A total of 4 groups were formed as control, toluene, p-CA and toluene+p-CA. Animals in the control group, toluene group and p-CA group were given 0.9 % NaCl, 0.9 mg kg-1 b.w toluene and 100 mg kg^{-1} b.w p-CA orally for 21 days, respectively. The animals in toluene+p-CA group were received p-CA for 3 days and from day 4, toluene and p-CA were applied together daily until day 25. On the 25th day, the study was terminated, and blood samples were collected. Catalase (CAT) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) and glutathione (GSH) levels in the erythrocytes and superoxide dismutase (SOD) activity in plasma samples were determined. In this study, CAT and SOD activities and MDA level significantly (p <0.05) increased, GSH level and GSH-Px activity significantly (p <0.05) decreased in the blood samples of toluene group compared to the control group. In toluene+p-CA group, significant (p <0.05) increases in GSH level and GSH-Px activity and significant (p <0.05) decreases in MDA level and SOD and CAT activities were detected compared to the toluene group. It has been determined that toluene caused oxidative stress and lipid peroxidation in blood tissue and against this oxidative damage, p-CA had a protective effect.

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Ratlarda Toluenle İndüklenen Oksidatif Hasara Karşı p-Kumarik Asitin Antioksidan Etkisi

ÖZET

Bu araştırmada, fenolik asidin bir türevi olan p-CA'nın toluen kaynaklı oksidatif hasara karşı koruyucu etkisinin araştırılması amaçlanmıştır. Çalışmada, her grupta 8 olmak üzere toplam 32 Sprague-Dawley erkek sıçan kullanıldı. Kontrol, toluen, p-CA ve toluen+p-CA olarak toplam 4 grup oluşturuldu. Kontrol grubu, toluen grubu ve p-CA grubundaki hayvanlara 21 gün boyunca sırasıyla %0.9 NaCl, 0.9 mg kg^{-1} c.a toluen ve 100 mg kg^{-1} c.a p-CA verildi. Toluen+p-CA grubundaki hayvanlara 3 gün boyunca p-CA verildi ve 4. günden itibaren toluen ve p-CA 25 güne kadar günlük olarak birlikte uygulandı. 25. günde çalışma sonlandırıldı ve kan örnekleri alındı. Eritrositlerde katalaz (CAT) ve glutatyon peroksidaz (GSH-Px) aktiviteleri ile malondialdehit (MDA) ve glutatyon (GSH) seviyeleri; plazma örneklerinde süperoksit dismutaz (SOD) aktivitesi belirlendi. Bu çalışmada, toluen grubunun kan örneklerinde CAT ve SOD aktiviteleri ve MDA düzeyi anlamlı (p <0.05) olarak arttığı; GSH düzeyi ve GSH-Px aktivitesinin anlamlı (p <0.05) olarak azaldığı tespit edildi. Toluen+p-CA grubunda, toluen grubuna göre GSH düzeyi ve GSH-Px aktivitesinde anlamlı (p <0.05) artış; MDA düzeyi ile ve SOD ve CAT aktivitelerinde anlamlı (p <0.05) azalma saptandı. Toluenin kan dokusunda oksidatif strese ve lipit peroksidasyonuna neden olduğu ve bu oksidatif hasara karşı p-CA'nın koruyucu bir etkiye sahip olduğu belirlenmiştir.

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INTRODUCTION

Toluen is an organic solvent, also known as toluol, methylbenzene and phenylmethane, that is frequently used in chemical syntheses and industrial processes such as gasoline and plastic production. Toluene is a volatile at room temperature. It is abused with inhalation form in its pure form or from many commercial mixtures (such as solvents, paints, varnishes, thinners, adhesives, inks). As a result of this inhalation, it passes to the nervous system and causes psychoactive effects (Balster et al., 2009; Cruz et al., 2014).

Toluene has an addictive potential. Exposure occurs at low concentrations by inhalation with consumer products or gasoline. However, significant health risks do not arise where the air circulation is good (Cruz et al., 2014). Abuse or occupational exposure of toluene results in detection of high concentrations, and higher levels of abuse are seen compared to occupational exposure (Tas et al., 2011).

Exposure occurring in workplaces where toluene is used as raw material or solvent occurs approximately five days a week, 5 hours a day. Although there are differences between countries, there are legal regulations and exposure limits are specified by legislation in order to prevent negative effects that may arise from occupational exposures. The safe exposure limit for toluene ranges from 10-100 ppm (Cruz et al., 2014). The limit value, which immediately endangers life and health, has been determined as 500 ppm (OSHA, 2013).

Although toluene primarily accumulates in lipid rich tissues, it is distributed throughout the body (Benignus et al., 1981). Exposure to toluene causes damage to all organs. It is possible to list the main target organs as brain, liver, kidney, heart and lungs (Afravy et al., 2017). It is known that toluene is highly absorbed from the gastrointestinal tract and respiratory tract and slightly from the skin. Its perfusion in the brain is also extremely high. Different cytochrome P450 isoenzymes are incorporated into the toluene metabolism, of which CYP2E1 is known to be the most active and is known to catalyze the conversion of toluene to benzyl alcohol (Tamie and Rui-Sheng, 1994; Cruz et al., 2014). Free oxygen radicals are produced as a result of toluene metabolism and this is the main mechanism of cellular damage caused by exposure (Halifeoglu et al., 2000).

p-Coumaric acid (p-CA), a hydroxycinnamic acid in phenolic nature, is obtained from a number of plants materials (Rafiee et al., 2020). p-CA is found in edible plants such as groundnuts, tomatoes, carrots, sage, and garlic, as well as in beverages such as wine, vinegar, tea, coffee, and beer (Urfalioglu et al., 2017). Several studies have described the antioxidant mechanism of phenolic compounds, such as p-CA. p-CA possesses anti-inflammatory and anti-oxidant activities in various diseases (Shen et al., 2019; Godarzi et al., 2020; Huang et al., 2020; Sabitha et al., 2020). Via scavenging the cytotoxic electrophilic agents and reactive oxygen species (ROS), and responding to pro-inflammatory stimuli, p-CA plays a key role in cellular defense (Kheiry et al., 2019).

The objective of the present study was to assess the protective effect of p-CA on oxidative damage in toluene-induced toxicity.

MATERIALS and METHODS

The rats used in the study were obtained from Burdur Mehmet Akif Ersoy University Animal Experiments Production and Experimental Research Laboratory and experimental applications were made in the same center. The research was conducted within the framework of decision number 126 taken at the meeting of Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee on 01/04/2015.

In this study, a total of 32 Sprague-Dawley male rats (10-12 weeks old), 8 in each group, weighing approximately 200-300 g were used. A total of 4 groups were formed as control, toluene, p-CA and toluene+p-CA. Animals in the control group, toluene group and p-CA group were given 0.9% NaCl, 0.9 mg kg^{-1} b.w toluene and 100 mg kg^{-1} b.w p-CA orally for 21 days, respectively. The animals in toluene+p-CA group were received p-CA for 3 days and from day 4, toluene and p-CA were applied together daily until day 25. All animals were given *ad libitum* pellet feed and water. The doses of p-CA (Abdel-Wahab et al., 2003; Roy and Prince, 2013) and toluene (ATSDR, 2000; Tas et al., 2011) to be given to animals were determined in the light of previous studies. On the 25th day, the study was terminated and all animals were anesthetized with 2-3% isoflurane (inhalation) and blood was taken from the heart. The animals were euthanized by cervical dislocation under anesthesia.

Preparation of blood samples

Blood samples that collected into K_3EDTA containing tubes were centrifuged at 4000 rpm for 5 minutes at 4 °C. The plasmas of blood samples were separated and kept at -20 ° C. The erythrocytes were obtained by centrifugating the blood samples for 5 minutes with addition of 3 times of its volume of saline phosphate buffer solution (pH 7.4) at 4000 rpm and the supernatant discarded. After 3 repeats of this

procedure, 1:1 volume of saline phosphate buffer solution was added to erythrocyte samples. All blood samples were kept at -20 ° C until analysis.

Determination of antioxidant systems

Catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities were measured in blood samples.

CAT activities in erythrocyte samples were measured according to the method reported by Aebi (1984) and expressed in k g^{-1} Hb. GSH-Px levels in erythrocyte samples were measured according to the method reported by Paglia and Valentine (1967) and expressed in U g^{-1} Hb. SOD levels in plasma samples were measured according to the method of commercial kit (Rat Super Oxidase Dismutase ELISA Kit EA0168Ra) and expressed in ng ml^{-1}.

Glutathione (GSH) levels were measured in erythrocytes according to the method reported by Sedlak and Lindsay (1968) and expressed in µmol g^{-1} Hb.

Determination of lipid peroxidation

MDA level in erythrocytes were measured based on the method reported by Yoshioka et al. (1979) and expressed in µmol g^{-1} Hb.

Statistical Evaluation

Statistical calculations were evaluated using the "SPSS 22.0" program. Results were expressed with arithmetic mean±standard error (SE) and compared with one-way analysis of variance (ANOVA) and the differences between the groups were determined by the Tukey test. $p < 0.05$ was considered as statistically significant.

RESULTS and DISCUSSION

When the toluene group was compared to the control group, significant ($p < 0.05$) decreases in level of GSH and activity of GSH-Px were detected in blood samples, whereas significant ($p < 0.05$) increases were determined in activities of CAT and SOD, and level of MDA. When compared to the toluene group, in the group with toluene+p-CA were given together, significant ($p < 0.05$) decreases in the level of MDA and activities of CAT and SOD and significant ($p < 0.05$) increases in level of GSH and activity of GSH-Px were detected in blood samples (Table 1).

In physiological conditions (in the case of homeostatic equilibrium), controlled fluctuations occur in ROS densities in the living organism, these fluctuations are regulated by enzymatic and non-enzymatic antioxidant systems. Oxidative stress occurs when this homeostatic balance is disturbed and excess ROS is produced. During toluene metabolism, oxygen free radicals are produced and these radical species cause tissue and cell damage (Valko et al., 2007; Moro et al., 2012; Cruz et al., 2014).

Antioxidant enzymes (SOD, CAT, and GSH-Px) are recognized as antioxidant enzymes (Meydan et al., 2016). The living organisms try to deal with oxidative stress by showing an increase or decrease in antioxidant enzyme systems. Against ROS, the organism is first protected by GSH, and xenobiotics that cause damage to tissues lead to depletion of GSH store (Mates et al., 2008).

Table 1. SOD, CAT and GSH-Px activities and GSH and MDA levels in blood samples of the research groups.
Çizelge 1. Araştırma gruplarının kan örneklerindeki SOD, CAT ve GSH-Px enzim aktiviteleri ile GSH ve MDA düzeyleri

Parameters	Groups			
	Control	Toluene	*p*-CA	Toluene+*p*-CA
SOD	4.16±0.19[a]	12.54±0.37[d]	5.43±0.08[b]	8.20±0.40[c]
CAT	164.37±5.08[a]	1297.80±127.48[c]	175.00±5.53[a]	456.52±25.17[b]
GSH-Px	114.80±3.87[d]	41.25±3.35[a]	88.14±0.99[c]	66.14±1.67[b]
GSH	1.34±0.53[c]	0.38±0.03[a]	1.22±0.02[c]	0.84±0.05[b]
MDA	0.46±0.11[a]	1.30±0.06[c]	0.57±0.04[a]	0.84±0.03[b]

* Values are expressed as arithmetic mean ± standard error.
** (a, b, c, d) shows differences between groups in the same line, $p < 0.05$
p-CA: p-Coumaric acid SOD: Superoxide dismutase (ng ml^{-1}) CAT: Catalase (k g^{-1} Hb)
GSH-Px: Glutathione peroxidase (U g^{-1} Hb) GSH: Glutathione (µmol g^{-1} Hb) MDA: Malonedialdehyde (µmol g^{-1} Hb)

As a result of peroxidation of membrane lipids, caused by ROS especially superoxide anion, MDA is formed as a final product (Patocková et al., 2003). It is known as one of the main markers of damage that is caused by oxidative stress on lipid-rich tissues and organs (Božić et al., 2003). In many studies, toluene is found to lead to oxidative stress in various tissues (Božić et al., 2003; Stajković et al., 2009; Muti et al., 2016). Also in this study, in blood samples significant ($p < 0.05$) increases in CAT and SOD activities, and MDA level; significant ($p < 0.05$) decreases in GSH level and GSH-Px activity were detected in the OPP group. Božić et al. (2003) gave toluene (3, 7 or 11 days 0.7 µm) to adult female Wistar rats intraperitoneally. They detected that blood MDA level was significantly ($p < 0.05$) higher in toluene group compared to control. Stajković et al. (2009) were administered toluene intraperitoneally (at daily doses

of 0.38 mmol kg^{-1} b.w for 12 days and 5 mmol kg^{-1} b.w for 6 days) to female Wistar rats. When compared to control group, they found significant (p <0.05) increases in SOD and CAT activities and MDA level in toluene group. Muti et al. (2016) administered toluene-2,4-diisocyanate (10%) to rats with intranasal application. After sensitization with toluene-2,4-diisocyanate, researchers detected that MDA level was significantly (p <0.001) increased compared to control. Similar to this study, Stajković et al. (2009) found significant increases in SOD and CAT activities in toluene group. Also consistent with this study, Božić et al. (2003), Stajković et al. (2009) and Muti et al. (2016) found significant increase in MDA level.

There are studies showing the toxic effects of toluene on workers exposed to chemicals containing toluene (Halifeoglu et al., 2000; Dundaroz et al., 2003; Karabulut et al., 2009). Halifeoglu et al. (2000) reported that they observed significant increases in MDA (p <0,001) level and SOD (p <0,05) and GSH-Px (p <0,001) activities in erythrocyte samples of workers in paint industry, compared to control group. Dundaroz et al. (2003) reported that significant (p <0.001) increases in SOD activity and MDA level and significant (p <0.001) decrease in GSH-Px activity in erythrocyte samples of children who exposed to toluene with inhalation. Karabulut et al. (2009) examined blood samples of 10 workers (8 shoemakers and 2 painters) who exposed 20-100 ppm (76.6 mg-383 mg) toluene in their working environment. When compared to control group, significant increase (p <0.05) in MDA level and significant decreases (p <0.05) in CAT and GSH-Px activities.

Phenolic compounds are natural antioxidants with potential benefits to human health. With their free radical scavenging activities, they play an important role in the prevention of many chronic diseases in which oxidative stress plays a role (Boo, 2019; Bento-Silva et al., 2020). Hydroxycinnamic acids are bioactive carboxylic acids, including coumaric acid, caffeic acid, ferulic acid and synapic acid, known as phenolic acids. These compounds deliver the phenoxyl hydrogen atoms to the medium to neutralize the free radicals and form the phenoxyl radical. These phenoxyl radicals show weak reactivity due to the unpaired electrons delocalizing on the ring structure. They create antioxidant activities by providing inhibition of dangerous radical species (Chen, 2016). Coumaric acids are cinnamic acid derivatives which are mono-hydroxylated from phenyl groups, and p-CA is the most abundant isoform (Boo, 2019; Bento-Silva et al., 2020).

The antioxidant properties of p-CA are displayed in several studies (Ekinci-Akdemir et al., 2017a,b; Shen et al., 2019; Rafiee et al., 2020; Sabitha et al., 2020). Also, in this study, in toluene+p-CA group, CAT and SOD activities, and MDA level decreased and there were increases in GSH level and GSH-Px activity when compared to OPP group in blood samples. Shen et al. (2019) administered p-CA (50 and 100 mg kg^{-1} b.w. per day) for 6 weeks to mice which fed with high-fat diet (HFD). CAT level was significantly (p <0.05) increased in serum samples of mice which received p-CA with HFD. There are studies about protective effect of p-CA on oxidative damage induced by different xenobiotics in various tissues (brain, liver, kidney, heart, lung) (Roy and Prince, 2013; Ekinci-Akdemir et al., 2017a, b; Shen et al., 2019; Godarzi et al., 2020; Rafiee et al., 2020; Sabitha et al., 2020). This study is the first to investigate the protective effect of p-CA on toluene-induced oxidative damage. In addition, it is one of the few studies published in which antioxidant effect of p-CA was determined by examining blood oxidative stress parameters.

CONCLUSION

When ROS, which form the basis of the cellular signaling system in a healthily functioning organism, is produced in excess and the antioxidant defense systems cannot cope with this oxidative load, homeostasis is disrupted and oxidative stress occurs. In this study, it was stated that toluene caused oxidative damage in the blood at the chosen dose and the dosage period. This damage is determined according to increases in SOD and CAT activities and MDA levels and decreases in GSH-Px activity and GSH level in blood samples of toluene-treated group. There are endogenous enzymatic and non-enzymatic antioxidant systems to fight oxidative stress that may occur in a healthy organism. Besides, there are also exogenous antioxidant sources. The most important of these are phenolic compounds. They form a good defense against oxidative stress with their radical scavenging effects. p-CA is one of these phenolic compounds. In this study, it was stated that p-CA ameloriated the effects of oxidative damage caused by toluene. This protective effect is determined according to decreases in SOD and CAT activities and MDA levels and increases in GSH-Px activity and GSH level in blood samples of p-CA+toluene-treated group. As a result of this study, we found that toluene caused oxidative damage in the blood and p-CA has a corrective effect on toluene-induced oxidative stress parameters.

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Statement of Conflict of Interest

Authors have declareed no conflict of interest.

Author's Contributions

Authors declares the contribution of the authors is equal.

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