The protective effect of p-coumaric acid on toluene-induced hepatotoxicity, nephrotoxicity and neurotoxicity in rats

El efecto protector del ácido p-cumárico sobre la hepatotoxicidad, nefrotoxicidad y neurotoxicidad inducidas por tolueno en ratas

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ABSTRACT

Objective. The aim of this study was to determine the protective effect of p-coumaric acid (p-CA) against toluene-induced hepatotoxicity, nephrotoxicity and neurotoxicity in rats. **Materials and methods.** A total of 32 Sprague-Dawley male rats, 8 in each group, were used. 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 b.w toluene and 100 mg/kg 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, blood and tissue samples were collected. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine levels in serum, glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) and glutathione (GSH) levels in the tissue samples were determined. **Results.** In this study, it was determined that there were significant increases in ALT and AST activities, and creatinine levels in toluene-induced group compared to control group. Moreover, there was a decrease in the GSH-Px activities and GSH levels, and an increase in the MDA levels compared to the control group. However, in the toluene+p-CA group, significant decreases in aminotransferases activities, creatinine and MDA levels, and significant increases in GSH-Px activities and GSH levels were determined compared to the toluene group. **Conclusions.** It has been determined that p-CA has a protective effect against toluene-induced hepatotoxicity, nephrotoxicity and neurotoxicity.

Keywords: Brain; coumaric acid; kidney; liver; oxidative stress; toluene (Source: *CAB*).

RESUMEN

Objetivo. El objetivo de este estudio ha sido determinar el efecto protector del ácido p-cumárico (p-CA) contra la hepatotoxicidad, nefrotoxicidad y neurotoxicidad inducida por tolueno en ratas. **Materiales y métodos.** Se utilizaron un total de 32 ratas macho Sprague-Dawley, 8 en cada grupo. Se formaron 4 grupos: el de control, tolueno, p-CA y tolueno + p-CA. Los animales del grupo de control, el grupo de tolueno y el grupo de p-CA recibieron NaCl al 0,9%, 0,9 mg / kg de peso corporal de tolueno y 100 mg / kg de peso corporal de p-CA por vía oral durante 21 días, respectivamente. Los animales del grupo tolueno + p-CA recibieron p-CA durante 3 días y desde el día 4, el tolueno y el p-CA se aplicaron juntos diariamente hasta el día 25. El día 25, se terminó el estudio, se tomaron muestras de sangre y tejido. Se determinaron los

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niveles de aspartato aminotransferasa (AST), alanina aminotransferasa (ALT) y creatinina en suero, actividad de glutatión peroxidasa (GSH-Px) y niveles de malondialdehído (MDA) y glutatión (GSH) en las muestras de tejido. **Resultados.** En este estudio, se determinó que hubo aumentos significativos en las actividades de ALT y AST, y los niveles de creatinina en el grupo inducido por tolueno en comparación con el grupo de control. Además, hubo una disminución en las actividades de GSH-Px y los niveles de GSH, y un aumento en los niveles de MDA en comparación con el grupo de control. Sin embargo, en el grupo de tolueno + p-CA, se observaron disminuciones significativas en las actividades de las aminotransferasas, niveles de creatinina y MDA, y aumentos significativos en las actividades de GSH-Px y los niveles de GSH en comparación con el grupo de tolueno. **Conclusiones.** Se ha determinado que el p-CA tiene un efecto protector contra la hepatotoxicidad, nefrotoxicidad y neurotoxicidad inducidas por el tolueno.

Palabras clave: Cerebro; ácido cumarico; riñón; hígado; estrés oxidativo; tolueno (Fuente: CAB).

INTRODUCTION

Organic solvents such as toluene, benzene and xylene pose a great risk to public health as they are widely used in the industry. Solvents are a class of liquid organic chemicals which have high lipophilicity and volatility (1,2). Toluene, also known as toluol, methylbenzene and methacide, is a colorless, fragrant organic hydrocarbon solvent used widely in fabrication of industrial supplies and synthesis of various products. It is an important component of chemical paints, adhesives, coatings, varnishes, printing inks, fuel additives, glues, thinners and plastics (3,4). Toluene is found in liquid form at room temperature, but because of its low vapor pressure, it can be easily volatilized (5,6). As a result of widespread use of toluene, there are both occupational and non-occupational exposures (7). Exposure to toluene can be occured by a variety of ways, such as consumption with drinking water, food, consumer products or occupational exposure and chemical abuse (8). Toluene has lipophilic properties and is rapidly absorbed after being taken orally or by inhalation and tends to accumulate in adipose tissue and lipid-rich organs such as the brain, kidney, liver, intestines, spleen and adrenal glands (5,9). Toluene is rapidly transformed to benzyl alcohol in the liver by the microsomal enzyme system. Then benzyl alcohol turns into benzoic acid. Benzoic acid reacts with glycine or glucuronic acid. It is excreted in the urine as hippuric acid or benzol glucuronides. A small part of the toluene turns into *o*- and *p*-cresol (6). When living organisms are exposed to toluene, the formation of free oxygen radicals increases and cellular damage occurs. In physiological conditions of living organisms, reactive oxygen species (ROS) are produced. Antioxidant defense systems reduce these ROS and prevent damage caused by them. While toluene is metabolized in the liver, it generates different ROS including singlet oxygen (O_2), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (10). Free oxygen radicals are molecules containing at least one unpaired electron in its outer orbit. Free oxygen radicals react with nucleic acids, free amino acids, proteins, fats, carbohydrates, causing irreversible damage. Thus, they create pathological changes in the cell membrane, cell organelles and DNA (11,12,13).

p-coumaric acid (p-CA) is a phenolic compound usually found in a variety of vegetables (potatoes, tomatoes, peas), fruits (apples, pears, pineapples), foods and beverages (chocolate, tea, coffee, wine, beer) (14,15). p-CA has been reported to have an antioxidant effect by binding metal ions, cleaning reactive oxygen and reactive nitrogen radicals, rearranging endogenous antioxidant enzymes or repairing oxidative damage in biomolecules (14,16). The aim of this study was to determine the protective effect of p-CA on toluene-induced hepatotoxicity, nephrotoxicity and neurotoxicity in rats.

MATERIALS AND METHODS

Ethical aspects. The rats used in the present study were obtained from Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Center and experimental applications were made in the same place. The research was carried out according to decision at the meeting of the Animal Experiments Local Ethics Committee of Burdur Mehmet Akif Ersoy University, Turkey (Ethics No:126-01/04/2015).

Experimental animals & design. In this study, a total of 32 male 10-12 weeks old Sprague-Dawley rats weighing approximately 200-300 g were divided into four groups of 8 animals each (Table 1). All animals were given *ad libitum* pellet feed and water. The doses of toluene (5) and p-CA (14,15) to be given to animals were determined in the light of previous studies. The study was terminated at the 25th day. All animals were anesthetized with 2-3% isoflurane (inhalation). The animals were euthanized by cervical dislocation under anesthesia. Blood and tissue samples were collected.

Table 1. The design of experimental study.

Groups	Treatments	Days
Control	0.9 % NaCl by gavage (1 mg/kg)	4-24.
Toluene	0.9 mg/kg b.w toluene by gavage	4-24.
p-CA	100 mg/kg/b.w p-CA by gavage	4-24.
Toluene+p-CA	3 days ago 100 mg/kg/b.w p-CA by gavage From day 4, 100 mg/kg/b.w p-CA by gavage + 0.9 mg/kg b.w toluene by gavage	1-24.

Biochemical estimations. Blood samples were taken into tubes with and without EDTA. They were centrifuged for 10 minutes at 4000 rpm. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine in blood were measured in Gesan Chem 200 autoanalyzer (Gesan chem 200 Gesan Production srl, Campobello, Italy). Kinetic UV optimized The International Federation of Clinical Chemistry (IFCC) methods were used in ALT and AST measurements and Jaffè method was used in creatinine measurement (17).

Preparation of tissue samples. The tissue samples were prepared for homogenization by washing with 0.9% ice-cold isotonic saline. Buffer solution was prepared as follows: 140 mM KCl, 10 mM $NaHCO_3$, 3 mM KH_2PO_4 and 2 mM K_2HPO_4; dissolved in 950 ml of deionized water and adjusted to pH 7.2 with NaOH (5N) and completed to 1000 ml. The tissue samples were homogenized with the buffer (1/10 w/v). For 45 minutes at 15000 rpm and 4°C, homogenates were centrifuged, supernatants were separated and kept at -20°C until analysis.

Estimations of antioxidant/oxidant parameters in brain, kidney and liver tissues. Glutathione (GSH) levels were measured using the method reported by Sedlak and Lindsay (18) and expressed in µmol/g protein. Glutathione peroxidase (GSH-Px) activities were measured according to the method reported by Paglia and Valentine (19) and expressed in U/g protein. Malondialdehyde (MDA) levels were measured according to the method reported by Ohkawa et al (20) and expressed in µmol/g protein. Determination of total protein was performed based on the Biuret method reported by Gornall et al (21).

Statistical evaluation. Data of the study were evaluated using the "SPSS 22.0" program (*IBM SPSS Statistics for Windows*, *Version 22.0*. *Armonk*, *NY*). Results are expressed with arithmetic mean ± standard error of the mean (SEM). To determine the comparisons of means between groups, one-way analysis of variance (ANOVA) was applied. For determination of the differences between the groups, Tukey's *post hoc* test was used. $p<0.05$ was considered as statistically significant.

RESULTS

Significant ($p<0.05$) increases in serum AST and ALT activities, and creatinine level were detected in toluene group compared to the control. In the toluene+p-CA group, significant ($p<0.05$) decreases in the activities of AST, ALT and level of creatinine, which were increased when toluene was administered alone, were determined. No significant changes were found in the group where p-CA was administered alone compared to the control (Table 2).

Table 2. Serum AST, ALT and creatinine activities of the groups.

Parameters	Groups			
	Control	Toluene	p-CA	Toluene+p-CA
AST	154.57±2.84^a	327.42±6.49^c	150.69±9.98^a	243.17±4.76^b
ALT	83.90±0.82^a	147.33±5.38^c	85.34±2.34^a	96.42±0.99^b
Creatinine	0.50±0.02^a	2.22±0.03^c	0.57±0.03^a	0.89±0.03^b

* Values are expressed as arithmetic mean ± SEM.
** ($^{a, b, c}$) shows differences between groups in the same line, $p<0.05$
p-CA: p-coumaric acid; AST: Aspartate aminotransferase (U/L); ALT: Alanine aminotransferase (mg/dL); Creatinine: mg/dL

Significant ($p<0.05$) increases in the levels of MDA and significant ($p<0.05$) decreases in the levels of GSH and the activities of GSH-Px were detected in the brain, kidney and liver tissues in the toluene group, compared to the control group. In the toluene+p-CA group, significant ($p<0.05$) decreases in the levels of MDA and significant ($p<0.05$) increases in the levels of GSH and the activities of GSH-Px were detected in the brain, kidney and liver tissues compared to toluene group (Table 3,4,5).

Table 3. GSH and MDA levels and GSH-Px activities in liver tissues of the groups.

Parameters	Groups			
	Control	Toluene	p-CA	Toluene+p-CA
GSH	14.02±0.65^c	6.34±0.38^a	14.56±0.64^c	10.03±0.29^b
GSH-Px	132.08±7.22^c	59.80±3.33^a	114.47±6.10^c	88.32±1.65^b
MDA	4.09±0.07^a	9.86±0.69^c	3.73±0.22^a	6.38±0.48^b

* Values are expressed as arithmetic mean ± SEM.
** ($^{a, b, c}$) shows differences between groups in the same line, $p<0.05$
p-CA: p-coumaric acid; GSH: Glutathione (µmol/g protein); GSH-Px: Glutathione peroxidase (U/g protein); MDA: Malondialdehyde (µmol/g protein)

Table 4. GSH and MDA levels and GSH-Px activities in kidney tissues of the groups.

Parameters	Groups			
	Control	Toluene	p-CA	Toluene+p-CA
GSH	0.87±0.02^{c}	0.40±0.05^{a}	0.75±0.04^{c}	0.57±0.04^{b}
GSH-Px	10.90±0.41^{d}	5.21±0.54^{a}	9.45±0.11^{c}	6.97±0.28^{b}
MDA	5.75±0.43^{a}	12.63±0.38^{c}	4.92±0.44^{a}	7.80±0.49^{b}

* Values are expressed as arithmetic mean ± SEM.
** ($^{a, b, c, d}$) shows differences between groups in the same line, p<0.05
p-CA: p-coumaric acid; GSH: µmol/g protein; GSH-Px: U/g protein; MDA: µmol/g protein

Table 5. GSH and MDA levels and GSH-Px activities in brain tissues of the groups.

Parameters	Groups			
	Control	Toluene	p-CA	Toluene+p-CA
GSH	0.91±0.05^{c}	0.23±0.03^{a}	0.82±0.05^{c}	0.64±0.02^{b}
GSH-Px	16.53±1.94^{d}	4.14±0.16^{a}	10.72±0.38^{c}	8.64±0.42^{b}
MDA	7.27±0.39^{a}	16.29±0.70^{c}	6.34±0.35^{a}	11.00±0.48^{b}

* Values are expressed as arithmetic mean ± SEM.
** ($^{a, b, c, d}$) shows differences between groups in the same line, p<0.05
p-CA: p-coumaric acid; GSH: µmol/g protein; GSH-Px: U/g protein; MDA: µmol/g protein

DISCUSSION

The findings of the present study revealed that p-CA has a protective effect aganist toluene-induced tisssue damage. In this study, it was determined that toluene given to rats at a dose of 0.9 mg/kg b.w for 21 days significantly changed the oxidant/antioxidant balance and increased aminotranferases activities and creatinine level. Results of this study showed that toluene could induce oxidative stress in the brain, liver and kidney tissues. On the other hand, p-CA revealed protective effects by improving the antioxidant system defence, suppressing oxidative stress in tissues.

The liver is the primary organ in which drugs and xenobiotics are primarily metabolized. In the present study, significant (p<0.05) decreases in GSH level and GSH-Px activity; a significant (p<0.05) increase in MDA level were detected in liver samples of toluene group compared to control. In previous studies (22, 23, 24, 25) it was emphasized that toluene causes decrease in the efficiency of the antioxidant systems and increases lipid peroxidation in liver tissues. Mattia et al (22,23) administered toluene (1.5 mg/kg intraperitoneally) to rats and detected a significant (p<0.05) decrease in GSH level and increase in MDA level in the liver tissues. El-Nabi Kamel and Shehata (24) gave toluene (650 mg/kg single dose) to rats and found a significant (p<0.05) increase in MDA level and decrease in GSH level in the toluene group. Tas et al (25) reported that liver MDA level in the toluene group were significantly (p<0.05) higher than in control group. In the present study, in liver samples significant (p<0.05) increases in GSH-Px activity and GSH level and decrease in MDA level were detected in toluene+p-CA compared to toluene group. There are studies in which p-CA is used as a protector against oxidative damage induced by different agents. Similar to the current study, p-CA in liver tissues increased GSH levels and GSH-Px activities, and decreased MDA levels in previous studies (26,27,28).

The kidney is the primary excretion organ for most xenobiotics. In this study, MDA level was significantly (p<0.05) increased and GSH-Px activity and GSH level were significantly (p<0.05) decreased in toluene group when compared to the control group in the kidney tissues. El-Nabi Kamel and Shehata (24) administered toluene (650 mg/kg b.w) to rats and found a significant (p<0.05) increase in MDA level and a significant (p<0.05) decrease in GSH level in the toluene group. Ahmadizadeh et al (8) reported that rats administered to rats determined a significant (p<0.05) decrease in GSH level in kidney tissues compared to the control. Afravy et al (3) gave toluene (300, 600 and 900 mg/kg intraperitoneally) to the rats and investigated on toluene-induced kidney damage. In the toluene group compared to the control, the researchers stated that there was a significant (p<0.05) dose-dependant increase in MDA level. The results of the present study are agreed with those of previous studies. In the present study, in kidney samples significant (p<0.05) increases in GSH-Px activity and GSH level and decrease in MDA level were detected in the toluene+p-CA group compared to toluene group. Similar to the present study, p-CA in kidney tissues increased GSH levels and GSH-Px activities and decreased in MDA levels in previous studies (26,27,28,29,30).

Brain is one of the target organs of toluene-induced toxicity due to its lipid-rich characteristics (31). In this study, in brain samples significant (p<0.05) increase in MDA level and significant (p<0.05) decreases in GSH-Px activity and GSH level were detected in toluene group compared to the control group. Mattia et al (22) administered toluene (a single intraperitoneal dose of 1.5 mg/kg b.w) to rats and determined a significant (p<0.05) decrease in GSH levels in brain tissues (hippocampus, cerebellum and striatum). Coskun et al (32) gave toluene (3000 ppm by inhalation for 16 weeks, 8 hour/day, 6 day/week) to rats and detected significant (p<0.05) decrease in GSH-Px activity and significant (p<0.001)

increase in MDA level in sciatic nerve tissues of rats. El-Nabi Kamel and Shehata (24) administered toluene (650 mg/kg a single dose) to rats and found a significant ($p<0.05$) decrease in GSH level and a significant ($p<0.05$) increase in MDA level depending on time in the toluene group. Kodavanti et al (33) treated rats with toluene (4, 12 and 24 months old rats with 0, 0.65 or 1 g/kg b.w single oral) and stated that there was a significant ($p<0.001$) decrease in GSH-Px activities in striatum tissues of 24-month-old rats and hippocampus tissues of toluene-treated 4-month-old rats. Abdel-Salam et al. (31) administered toluene (900 mg/kg b.w/day intraperitoneally) to rats and determined a significant ($p<0.05$) decrease in GSH level and a significant ($p<0.05$) increase in MDA level compared to control group. Montes et al. (34) treated mice with toluene (0 or 4000 ppm) for 4 weeks and 30 min in a day by inhalation. They indicated that there was a significant ($p<0.05$) decrease in GSH/GSSG level in both hippocampus and prefrontal cortex tissues of toluene-treated mice. In the present study, in brain samples significant ($p<0.05$) decrease in MDA level and significant ($p<0.05$) increases in GSH-Px activity and GSH level were detected in the toluene+p-CA group compared to toluene group. Similar to the present study, p-CA in brain tissues increased GSH levels and GSH-Px activities and decreased in MDA levels in previous studies (35,36).

In organ damage, significant changes occur in biochemical parameters. Increases in serum ALT and AST enzyme levels indicate liver damage. Creatinine and urea are markers of kidney damage or failure. Serum aminotransferase levels increase due to hepatitis, cirrhosis, liver cancer, and toxicity of drugs and various xenobiotics (37). In this study, in the toluene group compared to the control, significant ($p<0.05$) increases in serum ALT and AST activities and creatinine level were detected. Bae and Yoon (38) stated that toluene caused a significant ($p<0.01$) increase in ALT and AST levels in rat liver tissues. Tas et al. (25) administered 3000 ppm/hour/day toluene with inhalation to male rats for 4 weeks. It is stated that levels of serum ALT and AST were significantly ($p<0.05$) high in toluene-treated group. Moro et al (39) found that ALT and AST values were significantly ($p<0.05$ and $p<0.001$, respectively) higher in subjects exposed to toluene compared to control. Meydan et al (40) stated that there was a significant ($p<0.001$) increase in serum creatinine levels in toluene group compared to control. Afravy et al (3) applied different doses of toluene to rats (600 and 900) and reported a significant ($p<0.05$) dose-dependant increase in creatinine levels of toluene group. Meydan et al (37) reported that in serum ALT and AST levels there was a significant ($p<0.001$) increase in toluene group compared to the control group. These studies are in line with our results. In the present study, ALT and AST activities in the toluene+p-CA group were significantly ($p<0.05$) higher than in the toluene group. Similar to the present study, p-CA in serum samples decreased ALT and AST activities, and cretainine level (41,42,43,44).

In this study, it was found that the organs most sensitive to lipid peroxidation are brain, kidney and liver, respectively. This shows that toluene, which is a quite lipophilic solvent, accumulates primarily in lipid-rich tissues. The results of this study showed that toluene caused oxidative stress in brain, kidney and liver tissues at the dose administered and the period indicated. Also, it was found that p-CA has not any toxic effects on these tissues. As a result, it has been determined that p-CA (at the indicated dose and period) has a protective effect against toluene-induced hepatotoxicity, nephrotoxicity and neurotoxicity.

Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

Authors declares the contribution of the authors is equal.

REFERENCES

1. Ketan V, Desai K, George L, Highland H. Evidence of oxidative stress, biochemical and histological alterations in kidney and liver on short term inhalation of a specific mixture of organic solvents. IJPHC. 2013; 3(6):113-130. https://doi.org/10.12691/env-3-3-5

2. Meydan S, Nacar A, Ozturk HO, Tas U, Köse E, Zararsiz I, et al. The protective effects of caffeic acid phenethyl ester against toluene-induced nephrotoxicity in rats. Toxicol Ind Health. 2013; 32(1):15-21. https://doi.org/10.1177/0748233713485890

3. Afravy M, Angali K, Khodadadi A, Ahmadizadeh M. The protective effect of Buffalo's milk against toluene induced-nephotoxicity in rats. J Nephropathol. 2017; 6(3):174-179. https://doi.org/10.15171/jnp.2017.30

4. Stajković SS, Borozan SZ, Gađanski-Omerović G. The effect of toluene on oxidative processes in rat blood. J Serb Chem Soc. 2009; 74(1):15-25. https://doi.org/10.2298/JSC0901015S

5. Agency of Toxic Substances and Disease Registry (ATSDR) Toxicological profile for toluene [Internet]. U.S. Department Of Health And Human Services Public Health Service Agency for Toxic Substances and Disease Registry; 2017. Available from: http://www.atsdr.cdc.gov/toxprofiles/tp56.pdf

6. EPA. Toxicological review of toluene. [Internet]. United States Environmental Protection Agency: Washington DC; 2005. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0118tr.pdf

7. Božić TP, Stevanović JŽ, Kovačević MM, Jović SZ, Lukić S, Petakov MD, et al. Toluene mediated oxidative stress and granulo-monocytopoiesis. Acta Vet Scand. 2003; 53(4):201-210. https://doi.org/10.2298/AVB0304201B

8. Ahmadizadeh M, Amirmoezy S, Pole T. Effects of toluene on rat kidney. Jundishapur J Healthy Sci. 2014; 6(1):281-287. https://sites.kowsarpub.com/jjhs/articles/77027.html

9. Benignus VA, Muller KE, Barton CN, Bittikofer JA. Toluene levels in blood and brain of rats during and after respiratory exposure. Toxicol Appl Pharmacol. 1981; 61(3):326-334. https://doi.org/10.1016/0041-008x(81)90353-7

10. Myhre O, Fonnum F. The effect of aliphatic, naphthenic, and aromatic hydrocarbons on production of reactive oxygen species and reactive nitrogen species in rat brain synaptosome fraction: the involvement of calcium, nitric oxide synthase, mitochondria, and phospholipase A. Biochem Pharmacol. 2001; 62(1):119-128. https://doi.org/10.1016/s0006-2952(01)00652-9

11. Karabulut I, Balkanci ZD, Pehlivanoglu B, Erdem A, Fadillioglu E. Effect of toluene on erythrocyte membrane stability under *in vivo* and *in vitro* conditions with assessment of oxidant/antioxidant status. Toxicol Ind Health. 2009; 25(8):545-550. https://doi.org/10.1177/0748233709346758

12. Kumar CA, Das UN. Oxidant stress in preeclempsia and essential hypertension. J Assoc Phys India. 2002; 50:1372-1375. https://pubmed.ncbi.nlm.nih.gov/12583464/

13. Poli G, Leonarduzzi G, Biasi F, Chiarpotto E. Oxidative stress and cell signaling. Curr Med Chem. 2004; 11:1163-1182. https://doi.org/10.2174/0929867043365323

14. Abdel-Wahab MH, El-Mahdy MA, Abd-Ellah MF, Helal GK, Khalifa F, Hamada FM. Influence of p-coumaric acid on doxorubicin-induced oxidative stress in rat's heart. Pharmacol Res. 2003; 48(5):461-465. https://doi.org/10.1016/s1043-6618(03)00214-7

15. Jyoti Roy A, Stanely Mainzen Prince P: Preventive effects of p-coumaric acid on lysosomal dysfunction and myocardial infarct size in experimentally induced myocardial infarction. Eur J Pharmacol. 2013; 699(1-3):33-39. https://doi.org/10.1016/j.ejphar.2012.11.006

16. Huang X, You Y, Xi Y, Ni B, Chu X, Zhang R, et al. p-Coumaric acid attenuates IL-1β-induced inflammatory responses and cellular senescence in rat chondrocytes. Inflammation. 2020; 43(2):619-628. https://doi.org/10.1007/s10753-019-01142-7

17. Jaffè M. Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins. Biol Chem. 1886; 10(5):391-400. https://doi.org/10.1515/bchm1.1886.10.5.391

18. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25(1):192-205. https://doi.org/10.1016/0003-2697(68)90092-4

19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70(1):158-169. https://pubmed.ncbi.nlm.nih.gov/6066618/

20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358. https://doi.org/10.1016/0003-2697(79)90738-3

21. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949; 177(2):751-766. https://pubmed.ncbi.nlm.nih.gov/18110453/

22. Mattia CJ, Adams Jr JD, Bondy SC. Free radical induction in the brain and liver by products of toluene catabolism. Biochem Pharmacol. 1993; 46(1):103-110. https://doi.org/10.1016/0006-2952(93)90353-x

23. Mattia CJ, Ali SF, Bondy SC. Toluene-induced oxidative stress in several brain regions and other organs. Mol Chem Neuropathol. 1993; 18(3):313-328. https://doi.org/10.1016/0006-2952(93)90353-x

24. El-Nabi K, Shehata M. Effect of toluene exposure on the antioxidant status and apoptotic pathway in organs of the rat. Br J Biomed Sci. 2008; 65(2):75-79. https://doi.org/10.1080/09674845.2008.11732801

25. Tas U, Ogeturk M, Meydan S, Kus I, Kuloglu T, Ilhan N, et al. Hepatotoxic activity of toluene inhalation and protective role of melatonin. Toxicol Ind Health. 2011; 27(5):465-473. https://doi.org/10.1177/0748233710389853

26. Amalan V, Vijayakumar N. Antihyperglycemic effect of p-coumaric acid on streptozotocin induced diabetic rats. Indian J Appl Res. 2015; 5(1):10-13. https://www.worldwidejournals.com/indian-journal-of-applied-research-(IJAR)/fileview/January_2015_1421736837__04.pdf

27. Ekinci-Akdemir FN, Albayrak M, Calik M, Bayir Y, Gul I. The protective effects of p-coumaric acid on acute liver and kidney damages induced by cisplatin. Biomedicines. 2017; 5(2):18. https://doi.org/10.3390/biomedicines5020018

28. Adel A, Eman SA, Sanaa MA, Mohamed BA, Ahmed IY. Assessment of the ameliorative effect of p-coumaric acid and gallic acid on oxidative stress and haematological abnormalities in experimental type 2 diabetes. Gen Med Open. 2018; 2(6):1-6. https://doi.org/10.15761/GMO.1000150

29. Tanyeli A, Güzel D. Protective effect of p-coumaric acid as free oxygen radical scavenger in experimental renal ischemia-reperfusion model. Sakarya Medical Journal. 2018; 8(3):625-631. https://doi.org/10.31832/smj.455724

30. Sabitha R, Nishi K, Gunasekaran VP, Annamalai G, Agilan B, Ganeshan M. p-coumaric acid ameliorates ethanol-induced kidney injury by inhibiting inflammatory cytokine production and NF-κB signaling in rats. Asian Pac J Trop Biomed. 2019; 9(5):188-195. https://doi.org/10.4103/2221-1691.258998

31. Abdel-Salam OM, Youness ER, Morsy FA, Yassen NN, Mohammed NA, Sleem AA. Methylene blue protects against toluene-induced brain damage: involvement of nitric oxide, NF-κB, and caspase-3. ROS. 2016; 2(5):371-387. https://doi.org/10.20455/ros.2016.855

32. Coskun O, Yuncu M, Kanter M, Büyükbas S. Ebselen protects against oxidative and morphological effects of high concentration chronic toluene exposure on rat sciatic nerves. Eur J Gen Med. 2006; 3(2):64-72. https://doi.org/10.29333/ejgm/82380

33. Kodavanti PR, Royland JE, Richards JE, Besas J, Macphail RC. Toluene effects on oxidative stress in brain regions of young-adult, middle-age, and senescent Brown Norway rats. Toxicol Appl Pharmacol. 2011; 256(3):386-398. https://doi.org/10.1016/j.taap.2011.04.012

34. Montes S, Yee-Rios Y, Páez-Martínez N. Environmental enrichment restores oxidative balance in animals chronically exposed to toluene: comparison with melatonin. Brain Res Bull. 2019; 144:58-67. https://doi.org/10.1016/j.brainresbull.2018.11.007

35. Guven M, Aras AB, Akman T, Sen HM, Ozkan A, Salis O, et al. Neuroprotective effect of p-coumaric acid in rat model of embolic cerebral ischemia. Iran J Basic Med Sci. 2015; 18(4):356-363. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4439450/

36. Sakamula R, Thong-Asa W. Neuroprotective effect of p-coumaric acid in mice with cerebral ischemia reperfusion injuries. Metab Brain Dis. 2018; 33(3):765-773. https://doi.org/10.1007/s11011-018-0185-7

37. Meydan S, Esrefoglu M, Selek S, Akbas Tosunoglu E, Ozturk O, Kurbetli N, et al. Protective effects of caffeic acid phenethyl ester and thymoquinone on toluene induced liver toxicity. Biotech Histochem. 2019; 94(4):277-282. https://doi.org/10.1080/10520295.2018.1554825

38. Bae SW, Yoon IS. The benefical effects of melatonin for toluene hepatotoxicity in rats. Int J Biomed Sci. 2001; 7:99-102. https://www.koreascience.or.kr/article/JAKO200111921443784.pdf

39. Moro AM, Brucker N, Charão M, Bulcão R, Freitas F, Baierle M, et al. Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of toluene. Mutat Res. 2012; 746(1):42-48. https://doi.org/10.1016/j.mrgentox.2012.02.007

40. Meydan S, Nacar A, Oztürk HO, Tas U, Köse E, Zararsiz I, et al. The protective effects of caffeic acid phenethyl ester against toluene-induced nephrotoxicity in rats. Toxicol Ind Health. 2016; 32(1):15-21. https://doi.org/10.1177/0748233713485890

41. Parvizi F, Yaghmaei P, Rohani SAH, Mard SA. Hepatoprotective properties of p-coumaric acid in a rat model of ischemia-reperfusion. Avicenna J Phytomed. 2020; 10(6):633. https://pubmed.ncbi.nlm.nih.gov/33299819/

42. Moneim AA, Abd El-Twab SM, Ashour MB, Yousef AI. Hepato-renal protective effects of gallic acid and p-coumaric acid in nicotinamide/streptozotocin-induced diabetic rats. Int J Bioassays. 2016; 5(6):4641-4649. https://doi.org/10.21746/ijbio.2016.06.0011

43. Cha H, Lee S, Lee JH, Park, JW. Protective effects of p-coumaric acid against acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol. 2018; 121:131-139. https://doi.org/: 10.1016/j.fct.2018.08.060

44. Mohamadi Yarijani Z, Najafi H, Madani SH. Protective effect of p-Coumaric acid against cisplatin-induced nephrotoxicity and hepatotoxicity in rats. J Mazandaran Univ Med Sci. 2020; 30(185):1-13. http://jmums.mazums.ac.ir/article-1-14504-en.html