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RESEARCH ARTICLE

Investigation of oxidative stress index in pyridine and ellagic acid treated mice

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Piridin ve ellagik asit uygulanan farelerde oksidatif stres indeksinin araştırılması

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Öz

Amaç: Bu çalışmada, piridin intoksikasyonunda paraoksonaz (PON1) aktivitesi, oksidatif stres indeksi (OSİ), total antioksidan (TAS) ve total oksidan (TOS) seviyeleri üzerine ellagik asitin etkisinin araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmada 28 adet erkek Swiss albino fare 4 eşit gruba ayrıldı. Grup I'e (Kontrol grup, n:7, 0.9% NaCl, IP, SID, 7 gün), Grup II'e piridin (n:7, 100 mg/kg, IP, SID, 7 gün), Grup III'e ellagik asit (n:7, 10 mg/kg, SC, SID, 7 gün) ve Grup IV'e ise piridin (n:7, 100 mg/kg, IP, SID, 7 gün) ile ellagik asit (n:7, 10 mg/kg, SC, SID, 7 gün) verildi. Deneysel uygulamaların sonunda serum PON1 aktivitesi, OSİ, TAS ve TOS seviyeleri spektrofotometrik olarak analiz edildi.

Bulgular: Piridin verilen farelerde kontrol grubuna göre PON1 aktivitesi ve TAS seviyeleri azalırken, OSİ ve TOS seviyelerinin önemli oranda arttığı saptandı.

Öneri: Ellagik asit, piridin tarafından oluşturulan oksidatif strese karşı koruyucu etki gösterebilir.

Anahtar kelimeler: Fare, piridin, paraoksonaz, oksidatif stres, ellagik asit.

Abstract

Aim: In the present study, effect of ellagic acid on levels of paraoxonase (PON1), oxidative stress index (OSI), total antioxidant (TAS) and total oxidant (TOS) in pyridine intoxication was investigated.

Material and Methods: In the study, 28 male Swiss albino mice were divided into 4 equal groups as following. Control I (Control group, n:7, 0.9% NaCl, IP, SID, 7 days), Group II was applied with pyridine (n:7, 100 mg/kg, IP, SID, 7 days), Group III was applied with ellagic acid (n:7, 10 mg/kg, SC, SID, 7 days) and Group IV was treated with pyridine (100 mg/kg, IP, SID, 7 days) plus ellagic acid (10 mg/kg, SC, SID, 7 days). Serum PON1 activity, OSI, TAS and TOS levels were determined by spectrophotometric methods at the end of the experiment.

Results: OSI and TOS levels significantly increased, whereas serum PON1 activity and TAS levels reduced in pyridine treatment group (Group II) when compared to control group.

Conclusion: Ellagic acid may show the protective effects against pyridine-induced oxidative stress.

Keywords: Mice, pyridine, paraoxonase, oxidative stress, ellagic acid.

Introduction

Pyridine (C5H5N) is a heterocyclic aromatic compound structurally related to benzene, with one methine group instead of a nitrogen atom. It is a colorless liquid which freezes at -41.6°C and boils at 115.2°C. Pyridine which easily dissolves in water, alcohol, ether, acetone, and benzene is used as solvent and base for chemically reactions (Hotchkiss et al 1993, Zalat and Elsayed 2013). Pyridine and its compounds are regulators to development in the pharmaceutical as a building block for drug production and used as an insecticide or herbicide in the agriculture industry. Also, pyridine and its compounds that contaminate groundwater and soil in the industrial and agricultural regions are created serious health risks due to its harmful functions (Jori et al 1983).

Paraoxonase (PON1, aryldialkyl phosphatase, E.C.3.1.8.1) enzyme which adheres to HDL and catalyzes to hydrolysis of the organophosphates as the pesticide group prevents the accumulation of lipid peroxides in the soft tissues such as blood, liver and kidney (Mackness et al 1991). In addition to the paraoxonase, lactonase and arylesterase activities of PON1, it was reported that showed an activity like to peroxidase due to its effective on the lipid peroxides and hydrogen peroxide (Başkol and Köse 2004, Draganov et al 2005, Ferretti el al 2005). The activity like to peroxidase of PON1 may be contribute may be a significant proportion to the antioxidative defense system. The most important feature of the antioxidant defense system, all system components is to serve in the manner to create a synergy against oxidants (Chaudiere and Ferrai-Iliou 1999). Therefore, all antioxidants are vital importance in maintaining homeostasis (Doyotte et al 1997). As a result of together interaction of a proportion of oxidants and antioxidants in the blood occur more oxidant and antioxidant effect from consisted by itself for each. Therefore, in the determination of the oxidant/antioxidant balance is reported that analysis of TOS and TAS may be more useful instead of individual measurement of oxidants and antioxidants (Erel 2004, Erel 2005).

Ellagic acid included in the group of phenolic acids of plants and hydroxybenzoic acid derivative is stated that has significant biological activities such as antioxidant (Priyadarsini 2002) and anticarcinogenic (Narayanan 1999) characteristics. 3',4'-O-dihydroxy groups of ellagic acid from the phenolic compounds is claimed to be effective against oxidation (Meyer et al 1998). In addition, ellagic acid for free-radical scavenging activity was reported that was ideal chemical structure and displayed more antioxidative capacity than vitamin E and C as in vitro (Pari and Sivasankari 2008, Rice-Evans et al 1997).

In this study, it was investigated the effect of ellagic acid in terms of oxidative on these with levels of the PON1 activity, TAS, TOS, and OSI in pyridine treated mice.

Material and Methods

The ethical approval of the study was confirmed by Kafkas University Animal Care and Use Committee (KAÜ HADYEK: 2014-34/036). Animals were housed in a room maintained at 18±2°C and 60-65% moisture with a rotating 12 h dark-light cycle. Food and water were administrated as ad libitum. In the experiments, 28 Swiss albino mice (male, 30-35 g, 14-16 weeks) were used. Mice were composed 4 equal groups as; Group I: Control group (0.9% NaCl, IP, 7 days), Group II: Pyridine (C5H5N, Sigma-Aldrich Corp., USA) was treated at the dose of 100 mg/kg/day (IP, 7 days), Group III: Ellagic acid (E2250, Sigma-Aldrich Corp., USA) was treated at the dose of 10 mg/kg/day (SC, 7 days), and Group IV: Pyridine (100 mg/kg/day, IP, 7 days) plus ellagic acid (10 mg/kg/day, SC, 7 days) were treated.

At the end of the 7th day, blood samples were collected into tubes from the hearts via cardiac puncture under ether anesthesia for levels of the PON1 activity, TAS, TOS, and OSI analysis and then kept at -20°C until the analyzes were carried out. The levels of the PON1 activity, TAS, TOS, and OSI were analyzed by the methods stated below.

In the measurement of the serum PON1 activity, the paraoxon was used as substrate. The level of absorbance of the colour, emerging as a result of hydrolysis of paraoxon at 37°C by adding 20 µL serum into 1 ml Tris tampon (0.1 M, pH: 8.0)

Table 1. The levels of the serum PON1, TAS, TOS and OSI of the groups (mean±SD).

Parameters	Control	Pyridine	Ellagic acid	Pyridine plus ellagic acid
PON1 (U/L)	155.85±13.59^{a}	96.27±10.55^{b}	151.27±16.79^{a}	119.76±11.57^{b}
TAS (mmol Trolox eq/L)	1.14±0.09^{a}	0.64±0.57^{b}	1.16±0.13^{a}	0.98±0.11^{a}
TOS (µmol H_2O_2 eq/L)	7.28±0.57^{ab}	11.03±1.29^{c}	6.98±1.07^{a}	9.06±1.42^{b}
OSI (AU)	0.64±0.05^{a}	1.89±0.21^{c}	0.61±0.04^{a}	0.93±0.08^{b}

PON1: Paraoxonase 1, TAS: Total antioxiddant status, TOS: Total oxidant status, OSI: Oxidative stress index, U/L: Unit/liter, H_2O_2: Hydrogen peroxide, Eq: Equivalent, AU: Arbitrary unit, $^{a, b, c}$: Values with different letters within the same row indicates significant changes.

that contains 2 mM $CaCl_2$ and 6 mM paraoxon, at 412 nm. PON1 activity measured as basal activity and the results are given as U/L (Eckerson et al 1983). Total antioxidant status (TAS diagnostic kit, Rel Assay, Gaziantep, Turkey) and total oxidant status (TOS diagnostic kit, Rel Assay, Gaziantep, Turkey) levels were measured with and autoanalyzer (Aeroset®, Abbott, Illinois, USA). The rate of TOS and total antioxidant status (TAS) were used as oxidative stress index (OSI). Units of TAS were converted into mmol/L, and OSI values were calculated according to following formula; OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L) / TAS (μmol Trolox Eq/L) (Demirbağ et al 2007).

Study data were expressed as mean ± standard deviation (SD). Data was analyzed by the variance analysis (ANOVA) and Tukey multiple comparison test. The correlation relationship between parameters belong to groups was determined by Pearson's correlation analysis (SPSS 20.0 software Windows version, IBM). $P<0.05$ level was accepted statistically significance level.

Results

The levels of PON1, TAS, TOS and OSI are shown in Table 1. It was determined that the pyridine or ellagic acid treatment caused statistically meaningful changes on the serum PON1 activity, TAS, TOS, and OSI. Correlation analyses showed that the PON1 activity had a negatively correlation with the OSI and TOS ($r= -0.716$ and -0.767; $P< 0.01$, respectively) and a positively correlation with the TAS in the serum ($r=0.624$, $P< 0.01$).

Discussion

Investigation of oxidant/antioxidant balance and paraoxonase activity in pyridine treated or exposed organisms are important in terms of both environmental and clinical due to extensively used in agriculture sector (Jori et al 1983, Başkol and Köse 2004). In spite of intensive researches to improve of new methods which work against to biochemical changes induced by pesticides like pyridine in blood, the effective application that would prevent its negative effects on the metabolism have yet established (Acet 1985, Salama et al 2013). In this study, effects of ellagic acid were analyzed to against pyridine toxicity. The findings indicated that negative biochemical changes in mice could decrease by ellagic acid treatment. In the biochemical measurements, levels of TAS and PON1 activity were lower when levels of OSI and TOS of pyridine alone treated mice compared to control group were higher. Ellagic acid application was detected to normalize as near to the control animals, speciality in terms of the level TAS and TOS.

It is reported that a radical center on the pyridine ring may be formed (Agrios and Pichat 2006). Thus, degradation of pyridine or pyridine derivatives is relied on the generation of reactive free radicals, especially hydroxyl radicals (•OH) (Zalat and Elsayed 2013). In last years, by means of TOS level is provided monitoring of total quantities of change oxidant species like •OH and superoxide anion radical (O_2^-) (Erel 2005). In the present study, it was concluded that the reason of high levels of TOS and OSI in pyridine treated mice may be related to free radical generation from pyridine. It is reported that the method developed by Erel (2004) for levels of TAS is a more sensitive method for monitoring the antioxidative effects of vitamin C, bilirubin, polyphenols, uric acid and proteins.

Total protein which also has antioxidative effective free sulfhydryl groups in serum is claimed to contribute 52.9% to the analyzed serum total antioxidant status in healthy subjects (Erel 2004). It is possible that PON1 activity participating pesticide catabolism (Mackness et al 1991) and related to HDL according to the findings of this study can contribute to the TAS because positive correlation ($r= 0.624$; $P< 0.01$) among them.

Kim et al (1988) was recorded that chronic pretreatment with 100 mg/kg/day intraperitoneally pyridine injection for 4 days on male Sprague-Dawley rats caused 2.5-fold rise in cytochrome P450 levels relative to uninduced microsomes. The uncoupling caused production of H_2O_2 and O_2^- in the course of catalytic cycle of the cytochrome P450 can lead to escape of O_2– being from ROS (Gonzalez 2005). In a study performed before, in vitro incubations revealed that 100 μmolar of ellagic acid prevented P450 2B1, 1A1 and 2E1 activities by 18, 55 and 87% respectively (Ahn et al 1996).

In beside study, pyridine and its metabolites were also demonstrated to stimulate cytochrome P450 1A1 and P450 1A2 (Hotchkiss et al 1993, Iba et al 2002). In addition, it has been saved that pyridine created inductor effect to cytochromes and injury on the other tissues such as the liver, hearth, and kidney (Tunca et al 2009). According to our results related to the levels of TAS, TOS, and OSI in ellagic acid and pyridine treated mice can be supported with connected earlier studies because ellagic acid and pyridine its antagonistic responses on cytochrome P450. In the present study, it is shown that especially decreased serum OSI level in the pyridine plus ellagic acid treated mice is possible to prevent by the ellagic acid of the inductor effect on cytochrome P450 of pyridine.

Conclusion

The findings of this study show that pyridine may be cause oxidative stress in the serum of mice. It is concluded that ellagic acid belong to group of the phenolic acids is possible contribute to the PON1 activity and TAS reducing the oxidative stress related to pyridine in the serum.

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