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RESEARCH ARTICLE

The Effects of Ellagic Acid on Some Biochemical Parameters in the Liver of Rats Against Oxidative Stress Induced by Aluminum [1]

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Summary

Antioxidant, anti-inflammatory and anti-carcinogenic effects having Ellagic acid (2,3,7,8-tetrahidroksil [1] benzapiranol [5,4,3-CDE] [1] benzopiran-5, 10-dione) pomegranate, raspberry, strawberry, many fruits such as grapes and walnuts grained derivative is a substance found in vegetable polyphenols. Aluminum is known to cause toxic effects on variety organ systems. In this study, the effect of ellagic acid on the liver of rats against oxidative stress induced by aluminum chloride ($AlCl_3$) was investigated. Adult male Sprague-dawley rats were randomly divided in four groups. Control the first group, the second group Ellagic acid group, the third group $AlCl_3$ group, the fourth group was used as $AlCl_3$ + Ellagic acid. Ellagic acid 12 mg/kg dose with the orogastric probe and $AlCl_3$ 8.3 mg/kg intraperitoneal was administered as a 55 days every other day. Glutathione (GSH) in the liver, malondialdehyde (MDA), vitamin E levels and glutathione peroxidase (GSH-Px) and catalase (CAT) activities were determined. While administration of $AlCl_3$ increased the MDA level and CAT activity, it decreased the GSH-Px activity in liver compared those of the control group. The effect of ellagic acid to $AlCl_3$-treated rats decreased the MDA levels and increased GSH, GSH-Px and CAT activities in these samples. According to the data we obtained, a result $AlCl_3$ application against oxidative damage in the liver, which has the potential protective effect of ellagic acid, can be said.

Keywords: *Ellagic acid, Antioxidant, Aluminum, Oxidative stress, Vitamin E, Rat*

Alüminyum ile Oksidatif Strese Maruz Kalan Sıçanların Karaciğerindeki Bazı Biyokimyasal Parametreler Üzerine Ellagik Asidin Etkisi

Özet

Antioksidan, anti-kanserojen ve antiinflamatuar etkilere sahip olan Ellagik asit (2,3,7,8-tetrahidroksil [1] benzapiranol[5,4,3-cde][1] benzopiran-5, 10-dione) nar, ahududu, çilek, üzüm gibi pek çok taneli meyvelerde ve cevizde bulunan bitkisel polifenol türevi bir maddedir. Alüminyumun, çeşitli doku ve organlar üzerine toksik etkilere sebep olduğu bilinmektedir. Bu çalışmada, alüminyum klorür ($AlCl_3$) ile oksidatif strese maruz kalan sıçanlarda karaciğer üzerine ellagik asit'in koruyucu etkisi araştırıldı. Bu amaçla, erişkin Spraque-dawley ırkı 24 adet erkek sıçan, rastgele dört gruba ayrıldı (n=6). Birinci grup kontrol, ikinci grup Ellagik asit grubu, üçüncü grup $AlCl_3$ grubu, dördüncü grup ise $AlCl_3$ + Ellagik asit grubu olarak kullanıldı. Ellagik asit 12 mg/kg dozda orogastrik sonda ile $AlCl_3$ ise 8.3 mg/kg intraperitoneal olarak 55 gün süresince günaşırı uygulandı. Karaciğerde gulutatyon (GSH), malondialdehit (MDA), Vitamin E düzeyleri ve glutatyon peroksidaz (GSH-Px) ve katalaz (CAT) aktiviteleri belirlendi. $AlCl_3$ kontrol grubuna göre MDA düzeyini ve CAT aktivitesini artırırken, GSH-Px aktivitesini azalttığı belirlendi. $AlCl_3$'ün bu etkilerine karşın, ellagik asidin MDA düzeylerini azalttığı, GSH, GSH-Px ve CAT aktivitelerini artırdığı tespit edildi. Sonuçlarımıza göre, $AlCl_3$ uygulaması neticesinde karaciğerde oluşan oksidatif hasara karşı ellagik asidin koruyucu etki potansiyeline sahip olduğu söylenebilir.

Anahtar sözcükler: *Ellagik asit, Antioksidan, Alüminyum, Oksidatif stres, Vitamin E, Sıçan*

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INTRODUCTION

Ellagic acid (2, 3, 7, 8-tetrahydroxy [1] benzopyrano [5, 4, 3,-cde] [1] benzopran-5, 10-dione) is a polyphenol found at high concentrations in a number of fruits like grapes, strawberries, blacks currants and raspberries. Ellagic acid is a naturally occurring plant polyphenol that exhibits antioxidative properties both *in vivo* and *in vitro* [1,2]. Recently dietary polyphenols are receiving increasing attention as potential protectors against a variety of human diseases like cancer and chemotherapy induced toxicity in animal models [3,4].

Aluminum (Al) is one of the most abundant metals in the earth's crust. Human exposure to Al has been increasing over the last decades. This element appears mainly in food products and in drinking water derived from both natural sources and treatment methods [5]. Oxidative injury has been suggested to contribute to neurodegenerative and metabolic disorders. Al is not a transition metal and therefore cannot initiate oxidative injury. However, several investigations have provided evidence that aluminum has the ability to potentiate the iron-mediate lipid peroxidation [6-10] and to exacerbate reactive oxygen species (ROS) formation [11,12]. Al is known to cause toxic effects to variety organ systems including brain, bone, kidney and blood. It has been suggested that there is a relationship between high level of Al and increased risk of a number of pathogenic disorders, such as microcytic anemia, genotoxic, osteomalacia and, possibly, neurodegenerative disorders including dialysis encephalopathy, Alzheimer's disease and Parkinson's disease [13,14]. Al accumulation in hepatocytes was associated with increased membrane lipid peroxidation. Al could induce membrane lipid peroxidation by several mechanisms. Because Al has a single oxidation state, it is unlikely to induce membrane lipid peroxidation per se. However, Al could interact directly with enzymes known to protect the cell from peroxidation, such as superoxide dismutase, glutatione peroxidase and others. Alternatively, the increased Fe uptake and possibly altered intracellular Fe distribution induced by Al could cause membrane lipid peroxidation by well-described mechanisms [15]. Al salts are also known to accelerate membrane lipid peroxidation stimuled by Fe salts [8,16]. It has been demonstrated in experimental animals that Al exposure has an important impact on liver function [17].

In this study we investigated the effect of ellagic acid on the level of glutathione (GSH), vitamin E, malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities in the liver in the rat against oxidative stress that was induced by aluminum chloride ($AlCl_3$).

MATERIAL and METHODS

Chemicals

$AlCl_3$+$6H_2O$ were purchased from Merck Chemical Co (Germany). Ellagic acid and the other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and Treatment

Twenty-four healthy adult male Spraque-dawley rats (240±10 gr body weight) were used in this study. The animals were obtained from Firat University Experimental Research Centre (FUDAM), Elazig, Turkey. They were maintained at 21±1°C with a 12-h light/dark cycle, and have been given a commercial pellet diet (Elazig Food Company, Elazig, Turkey) and fresh drinking water ad libitum. Animal use protocol was approved by the National Institutes of Health and Local Committee on Animal Research. The rats were randomly divided into four groups; each group containing six rats.

Ellagic acid was dissolved in corn oil and administered to animals by gavage at the dose of 12 mg/kg body weight. $AlCl_3$, were dissolved in distilled water and animals during the 55 days 8.3 mg/kg dose was administered as intraperitoneal (IP). This additional was done for 55 days every other day. The last dose has been administered 12 h before the operation.

Sample Collection

The rats were killed at the end of 55 days. The liver samples were removed and protected against light. Liver samples were washed three times in cold isotonic saline (0.9% v/w) and stored at 20°C until analysis. The remainders of liver samples were immediately used to determine the lipid peroxidation level.

Homogenate Preparation

For the enzymatic analyses, the liver tissue was minced on glass and homogenized by a glass homogenization in cold physiological saline on ice. Then, the tissue was diluted with a 9-fold volume of phosphate buffer (pH 7.4).

Lipid Peroxidation

The levels of lipid peroxidation (as MDA) were measured in liver homogenate with the thiobarbituric acid reaction by the method of Placer et al.[18].

Catalase

The liver tissue CAT activity was determined according to the method of Aebi [19].

Glutathione Peroxidase

Liver GSH-Px activity was determined according to the method of Lawrence and Burk [20].

Glutathione

The GSH content of the liver homogenate was measured at 412 nm using the method of Sedlak and Lindsay [21]. The protein content in the liver was measured by method of Lowry et al.[22].

Vitamin E

Liver (0.5 gr) were homogenized in 3 ml acetonitrile/ methanol/isopropanol (2/1/1, v/v/v)-containing tubes and the samples were vortex for 30 s and centrifuged at 6000×g for 10 min at 4°C. Supernatant was dried under nitrogen in the 37°C, 1 ml redissolved in mobile phase. Samples were transferred to auto sampler vials of the HPLC instrument. For vitamin E, the mixture of acetonitrile/ methanol (3/1, v/v) has been used as the mobile phase and the elution was performed at a flow-rate of 1 ml/min. The temperature of column was kept at 40°C. SupelcosilTM LC 18DB column (250 mm×4.6 mm, 5 µm; Sigma, USA) was used as the HPLC column and detection was performed at 215 nm for δ-tocopherol, α-tocopherol, α-tocopherol acetate. Identification of the individual vitamins was performed by frequent comparison with authentic external Standard mixtures analyzed under the same conditions. Quantification was carried out by external standardization using Class VP software. The results of analysis were expressed as µg/g for tissues [23].

Statistical Analysis

The experimental results were reported as mean ±SEM. Statistical analysis was performed using SPSS Software. Analysis of variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls. One-way ANOVA p value using the post hoc Fischer's LSD test. P-value <0.05 was considered significant.

RESULTS

$AlCl_3$ caused significant increases in the MDA level of liver (P<0.001) when compared to the control group. Significant decreases (P<0.01) observed in the $AlCl_3$+ Ellagic acid group compared to the $AlCl_3$ alone group with respect to MDA levels. MDA level of Ellagic acid group slightly increased (P<0.05) when compared to the control group. CAT activities of all the groups significantly increased (P<0.001) when compared to the control group. The CAT activities of Ellagic acid group were found to be the highest as compared to those of the other groups. CAT activities of $AlCl_3$ + Ellagic acid group increased (P<0.001) when compared to the $AlCl_3$ group. The level of GSH-Px activities in the $AlCl_3$ and $AlCl_3$ + Ellagic acid groups decreased (P<0.001, P<0.05) when compared to the control group. However, GSH-Px activities in Ellagic acid group increased (P<0.001) when compared to the control group. GSH-Px activities of $AlCl_3$ + Ellagic acid and Ellagic acid groups increased (P<0.05, P<0.001) when compared to the $AlCl_3$ group. The GSH level of Ellagic acid and $AlCl_3$ + Ellagic acid groups increased (P<0.05) when compared to the control and $AlCl_3$ groups in liver. Significant difference was not observed between control group and $AlCl_3$ group (P>0.05) with respect to GSH levels. Ellagic acid caused significant increases in the Vitamin E level of liver (P<0.001) compared to the control group *(Table 1)*.

DISCUSSION

In this study is a natural antioxidant substances of ellagic acid against oxidative stress induced by $AlCl_3$ protective role was investigated.

According to the findings obtained, MDA levels in the liver were significantly higher in the $AlCl_3$-induced group when compared to the control group. This increase is a result of oxidative stress and lipid peroxidation. MDA is an indicator of lipid peroxidation [24]. In recent years, a number of authors have demonstrated that $AlCl_3$

Table 1. *Liver biochemical parameters in Control, Ellagic acid and $AlCl_3$ and $AlCl_3$ + Ellagic acid treated groups (n=6)*
Tablo 1. *Kontrol, Ellagik asit ve $AlCl_3$ ve $AlCl_3$ + Ellagic acid uygulanan gruplarda karaciğer biyokimyasal parametreleri (n=6)*

Groups	MDA nmol/mL	CAT (ku/L)	GSH-Px (IU/gr prot)	GSH (nmol/mL)	Vit. E (µg/gr)
Control	14.93±0.35	33.53±0.90	64.04±2.92	2.78±0.05	4.86±0.52
Ellagic acid	16.78±0.66 ^{b}	174.34±6.15 ^{d,t}	81.19±3.80 ^{d,t}	3.24±0.22 ^{b,y}	13.30±1.62 ^{d,t}
$AlCl_3$	24.33±0.43 ^{d,z}	99.15±1.01 ^{d}	45.44±1.15 ^{d}	2.63±0.21	4.83±0.48
$AlCl_3$ + Ellagic acid	21.67±0.62 ^{d}	166.84±4.58 ^{d,t}	53.82±1.07 ^{b,y}	3.35±0.19 ^{b,y}	1.33±0.26 ^{b}

1. *Comparing according to control group, b: P<0.05, c: P<0.01, d: P<0.001 (Kontrol grubuna göre karşılaştırma, b: P<0.05, c: P<0.01, d: P<0.001)*
2. *Comparing between groups, y: P<0.05, z: P<0.01, t: P<0.001 (Gruplar arası karşılaştırma, y: P<0.05, z: P<0.01, t: P<0.001)*

administration increases lipid peroxidation in the rat brain [25-28]. Lipid peroxidation may facilitate in turn Al accumulation which has been shown in rat brain synaptosomes [29]. Ellagic acid has been shown to exert a potent scavenging action on super oxide anion and hydroxyl anion *in vitro,* as well as the protective effect against lipid peroxidation [30]. In this study, it was observed that administration of ellagic acid to $AlCl_3$ treated animals decreased the MDA levels.

The ability of polyphenols to protect cell from oxidative stress has been demonstrated. However, polyphenol compounds could have both antioxidant and prooxidant properties, depending on the concentration and free radical source [31]. In this study, it was determined that the addition of ellagic acid increased the MDA in all groups. It can be suggested that this increase may result from prooxidant effect of ellagic acid related to application time and dose. However, compared to the $AlCl_3$ group in all tissues, the MDA levels in combination groups observed lower. These results show that ellagic acid has a protective effect against oxidative stress induced with $AlCl_3$. Yüce et al. observed that addition of ellagic acid to cisplatin-treated animals decreased the MDA levels [32].

It was observed that GSH levels in liver increased by the addition of ellagic acid when compared to the control group. It was observed that GSH levels were increased in the groups treated with $AlCl_3$ + Ellagic acid combination in liver via effect of ellagic acid. Compared to the control group, statistically significant differences were not determined in the GSH level of liver in $AlCl_3$ group. Some recent studies have shown that ellagic acid was found to be better than quercetin for chemoprevention. When the effect of both of these compounds on GSH, and on lipid peroxidation was investigated in rats, both ellagic acid and quercetin caused a significant increase in GSH and a decrease in NADP-H and ascorbate depend lipid peroxidation. However, ellagic acid was found to be more effective in decreasing the lipid peroxidation and increasing the GSH. It seems that protection rendered by ellagic acid and quercetin is linked with their anti-oxidative properties on the one hand, and induction of GSH, an endogenous antioxidant. GSH is an important non-protein thiol present in the animal cells and is a powerful nucleophile. It can participate in a variety of detoxification reactions, in addition to providing protection against oxidative damage by virtue of its thiol group. As GSH is present in the aqueous environment, it might not be readily accessible to the lipid environment of the membrane; but it cannot be ruled out completely that GSH has a major direct role in the free radical scavenging *in vivo* [33]. It plays a key role as a cofactor with a variety of enzymes including GSH-Px. GSH depletion has been shown to intensify lipid peroxidation and predispose cells to oxidant damage [34].

It was determined that the addition of ellagic acid increases the CAT and GSH-Px activities of all the groups. Especially, we determined that GSH-Px activities in $AlCl_3$ group decreased when compared to the control group in the liver. However, by the addition of ellagic acid, GSH-Px activity increased in the liver of $AlCl_3$ + Ellagic acid group compared to the $AlCl_3$ group. Several studies showed a positive effect of different classes of polyphenols on several enzyme activities, for example GPX, SOD activities. Polyphenolls induced a significant increase in several antioxidant enzyme activities [35-37]. Also it is reported that ellagic acid increased CAT and GSH-Px activities in the tissues of rats induced oxidative stress by cisplatine [38]. In erythrocytes from patients with chronic renal failure, CAT has been shown to be inhibited by Al [39], and a 25% inhibition in rat CAT activity was observed when liver supernatant was incubated with 5 mM $AlCl_3$ [40]. However, in this study, liver CAT activities $AlCl_3$ group were in increased. However, this increase between groups were compared, statistically, were not important. Regarding the increase in CAT, Gomez et al.[41] have reported the addition of $AlCl_3$ increased the CAT enzyme activity in hippocampus. They also have reported that there were no meaningful CAT activity differences between control and $AlCl_3$ group.

It was observed that addition of ellagic acid increased the Vitamin E level especially in liver. Vitamin E inhibits $AlCl_3$ induced activation of glial cells and pro-inflammatory cytokine expression in rat brain areas [42]. Chow [43] reported that Vitamin E can directly regulate H_2O_2 production in mitochondria and suggested that the overproduction of mitochondria ROS is the first event that leads to tissue damage that is observed in Vitamin E deficiency syndromes. Celik [44] reported that Vitamin E can protect fatty acid against H_2O_2. In addition, a study done by giving sodium nitrite generated in rabbits caused acute intoxication in preventing oxidative damage have been reported positive effects of vitamin E [45].

The previously described mechanisms of action of phenolic antioxidants such as ellagic acid, an alternative model has been proposed for the mode of action of ellagic acid from fruits. The antioxidant homeostasis in the cell occurs via the functioning of a diverse array of redox processes, primarily carried out by cellular anti-oxidant such as GSH, ascorbate, tocopherols and array of antioxidant enzymes such as SOD, CAT and GST [46]. However, to maintain the high efficiency of this system, it is important to regenerate the oxidized substrates such as glutathione disulfides (GSSG), dehydroascorbate and other proteins with oxidized sulfhydryl groups. The regeneration of oxidized glutathione, ascorbate and

tocopherol occurs by a group of oxidoreductases which use the cellular reducing equivalents such as $FADH_2$ and $NADPH_2$ and therefore are energy intensive processes [46-48]. To meet the cellular requirement for these reducing equivalents, it is proposed in this model that phenolic antioxidant such as ellagic acid improves the antioxidant response of the cell not only by themselves acting as redox modulators by virtue of their free radical scavenging activity but they are also able to stimulate pathways in the cell that can replenish the need for reducing equivalents that support cellular antioxidant enzyme respons [49-51]. Recent studies have indicated that ellagic acid can prevent or retard experimental selenite-induced cataractogenesis in wistar rats and potential use and benefit of ellagic acid as a modifier of nicotine-induced genotoxicity [52,53].

In conclusion, we have shown that addition of $AlCl_3$ to rats increased lipid peroxidation. Ellagic acid decreased the MDA levels in $AlCl_3$ treated animals. Ellagic acid improved GSH-Px activities and GSH levels in liver. We obtained, according to data application with $AlCl_3$ against oxidative damage in the liver, which has the potential protective effect of ellagic acid and can be said. Nowadays, water, food and exposure to other environmental factors that protect the liver against the harmful effects of Al to this substance ellagic acid containing fruit and vegetables can be helpful.

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