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Effects of Ellagic acid and Hesperetin on Levels of Some Elements in Livers of Aluminum-Induced Rats

[Aluminyuma Maruz Kalmış Sıçanların Karaciğerlerindeki Bazı Elementlerin Seviyeleri Üzerine Hesperetin ve Ellagik Asitin Etkileri]

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ABSTRACT

Objective: Ellagic acid and hesperetin are well known as having free radical scavenging activity in some rat tissues. Aluminum is known to cause toxic effects on various organ systems. In this study, the protective role of ellagic acid and hesperetin has been investigated on macro and trace elements in liver of rats treated with $AlCl_3$.

Method: Thirty-six healthy adult male *Spraque-dawley* rats (240±10 g body weight) were used in this study. The rats were randomly divided into six groups and Ellagic acid, Hesperetin, $AlCl_3$, $AlCl_3$+ Ellagic acid and $AlCl_3$+Hesperetin were treated to each group except from the control group. Determinations of iron, calcium, copper and zinc were performed using a Flame Atomic Absorption Spectrometer and determination of aluminum and manganese were carried out with Inductively Coupled Plasma Atomic Emission Spectrometer.

Results: Levels of aluminum, calcium and iron in $AlCl_3$ treated group were found to be increased compared to the control group ($p<0.01$, $p<0.001$). In $AlCl_3$+ Ellagic acid and $AlCl_3$+Hesperetin treated groups, the levels of aluminum and calcium were found as decreased ($p<0.05$, $p<0.001$). When compared to the control group, level of manganese in liver were found to be increased in ellagic acid treated group ($p<0.01$). The zinc level of Hesperetin group increased when compared to the other groups ($p<0.05$).

Conclusion: Our results suggest that ellagic acid and hesperetin may have a protective role upon major and trace elements against $AlCl_3$-induced stress conditions.

Key Words: Aluminum, Ellagic acid, Hesperetin, Trace elements, Calcium

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: Ellagik asit ve hesperetinin bazı sıçan dokularında serbest radikal seviyelerini azaltıcı aktiviteye sahip oldukları iyi bilinmektedir. Alüminyumun çeşitli organ sistemleri üzerine toksik etkisinin olduğu bilinmektedir. Bu çalışmada $AlCl_3$ uygulanmış sıçanların karaciğerlerinde makro ve eser elementlerin seviyeleri üzerine ellagik asit ve hesperetinin koruyucu etkileri araştırılmıştır.

Yöntem: Bu çalışmada 36 adet sağlıklı erkek *Spraque-dawley* sıçanı (240±10 g ağırlığında) kullanılmıştır. Sıçanlar rastgele seçilerek 6 grup oluşturulmuş, bir grup kontrol grubu olarak kalmış, diğer grupların her birine Ellagik asit, Hesperetin, $AlCl_3$, $AlCl_3$+ Ellagik asit ve $AlCl_3$+Hesperetin uygulanmıştır. Demir, kalsiyum, bakır ve çinko miktarları Alevli Atomik Absorbsiyon Spektrofotometresi ile aluminyum ve mangan miktarları ise indüklenmiş eşleşmiş plazma atomik emisyon spektrofotometresi ile tayin edilmiştir.

Bulgular: $AlCl_3$ uygulanan grupta alüminyum, kalsiyum ve demir düzeylerinin kontrol grubuna göre arttığı gözlenmiştir ($p<0.01$, $p<0.001$). $AlCl_3$+ Ellagik asit ve $AlCl_3$+Hesperetin gruplarında alüminyum ve kalsiyum seviyesi düşük bulunmuştur ($p<0.05$). Kontrol grubu ile kıyaslandığında ellagik asit karaciğerde mangan seviyesini artırdı ($p<0.01$). Diğer gruplarla kıyaslandığında Hesperetin grubunun çinko seviyesi artmıştır ($p<0.05$).

Sonuçlar: Sonuçlarımıza göre, ellagik asit ve hesperetinin, $AlCl_3$ kaynaklı stress koşullarına karşı makro ve eser elementler üzerinde koruyucu bir etkisi olabileceğini düşündürmektedir.

Anahtar Kelimeler: Aluminyum, Ellagik asit, Hesperetin, Eser Elementler, Kalsiyum

Çıkar Çatışması: Yazarların mevcut olan konuyla ilgili herhangi bir çıkar çatışması bulunmamaktadır.

Introduction

Elements are required for the proper functioning of the human system. Deficiency of those elements causes serious metabolic abnormalities and the increase of those creates toxicity. The levels of trace elements in some diseases, such as chronic kidney, liver and lung diseases, have been studied and important results have been obtained, as well. Aluminum is known to cause toxic effects in a variety of organ systems, including brain, bone, kidney and blood. It has been suggested that there has been a relationship between high level of aluminum and increased risk of a number of pathogenic disorders, such as microcytic anemia, neurodegenerative disorders including dialysis encephalopathy, Alzheimer's disease and Parkinson's disease. Aluminum ions have strong affinity to bio-membranes; they are capable of increasing the cellular oxidative milieu by potentiating the pro-oxidant properties of transition metals [1-3].

Hesperetin (HES) and Ellagic acid (EA) are polyphenolic compounds found in a wide variety of fruits. It has been reported that HES (3, 5, 7-trihydroxy-4-methoxy flavanone) shows a wide spectrum of pharmacological effects such as, anti-carcinogenic, anti-atherogenic and anti-hypertensive effects [4, 5].

EA(2,3,7,8-tetrahydroxy[1]benzopyrano[5,4,3-cde][1]benzopyran-5,10-dione) is known to increase antioxidant activity in lipid peroxidation and protect cells from oxidative damage. In addition, EA exhibits important health promoting effects via its antioxidant, anti-proliferative, chemo-preventive, anti-atherogenic, anti-apoptotic, anti-inflammatory activities and it also repairs DNA damage [6-9]. In our previous study, we observed positive effects of EA on some biochemical parameters in oxidative stress induced rats [10]. It has been stated that deficiency and redundance of the elements is important for the metabolism [1-3]. We have determined in our literature review that effects of EA and HES upon trace element and major elements in aluminum induced rat tissues has not been researched before.

The aim of this study was to analyze effects of EA and HES on trace element and major element levels in $AlCl_3$ treated rat liver.

Materials and Methods

Chemicals

$AlCl_3.6H_2O$, EA, HES and other chemicals were purchased from Merck Chemical Co (Germany).

Animals and Treatment

Thirty-six healthy adult male *Spraque-dawley* rats (240±10 g body weight) were used in this study. The animals were obtained from Fırat University Experimental Research Centre (FUDAM), Elazıg-Turkey. They have been kept 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 Institute of Health and Local Committee on Animal Research (Number:27.07.2006-3). The rats were randomly divided into six groups with each group containing six rats.

First group: Control (C)

Second group: EA alone administered group

Third group: HES alone administered group

Fourth group: $AlCl_3$ (A) administered group

Fifth group: A+EA administered group

Sixth group: A+HES administered group

EA and HES were dissolved in corn oil and administered to animals by gavage at the dose of 12 mg/kg EA [10] and 75 mg/kg HES [11] body weight. $AlCl_3$ was dissolved in distilled water and administered to the animals through intraperitoneal route at a dose of 8.3 mg/kg for 55 days [10]. The last dose was administered 12 h before the operation.

Analysis of Tissue Samples

The rats were decapitated and their livers were removed by surgery. Liver samples (0.50-0.80 g) were washed with isotonic salt solution (0.9 % v/w) and homogenized. A 0.30 g homogenized sample was digested by microwave digestion system with closed system pots polytetrafluorethylen (PTFE) according to our previous study [12,13]. The same procedure was applied to the blank solution. Determinations of iron (Fe), calcium (Ca), copper (Cu) and zinc(Zn) were performed using a Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer, Analysis 400) and determination of aluminum (Al) and manganese (Mn) were carried out with Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Perkin Elmer 3100).

Statistical Analysis

The experimental results were reported as mean ± SD Statistical analysis was performed using SPSS 10.0 Software. Analysis of variance (ANOVA) and an LSD (Least Significant Difference) test were used to compare the experimental groups with the controls

Results

The levels of elements in rat livers are shown in Table 1 and Table 2.

$AlCl_3$ has been increased in Fe, Ca and Al levels in liver ($p<0.01$, $p<0.001$) when compared to the control group. No statistically significant difference was observed in Fe, Ca and Al levels among EA, HES and control groups ($p>0.05$). Administration of ellagic acid and hesperetin to $AlCl_3$-treated rats has prevented the $AlCl_3$-induced increase in Al ($p<0.001$) and Ca ($p<0.05$) levels in liver tissues. Ellagic acid treatment has caused significant

Table 1. Concentrations (μg/g) of Macro and Trace Elements in Liver of Rats Treated with $AlCl_3$ and/or EA

Groups	Fe	Ca	Zn	Cu	Mn	Al
C	117,73±3,15[a]	91,53±3,66[a]	31,51±1,21[a]	2,22±0,26[a]	1,21±0,08[a]	4,73±0,46[a]
EA	131,65±4,42[a]	86,74±8,92[at]	32,13±1,18[a]	2,49±0,31[a]	1,56±0,11[cz]	7,78±1,26[at]
A	144,94±5,70[c]	135,26±11,70[d]	32,50±1,80[a]	1,87±0,53[a]	1,14±0,08[a]	41,63±5,35[d]
A+EA	139,94±6,29[b]	95,24±8,22[az]	29,66±1,71[a]	2,65±0,30[ay]	1,15±0,05[a]	25,21±5,11[dt]

Values are expressed as means ± SD; n=6 for each treatment group. a: $p>0.05$
Statistical significancy compared to the control group: b: $p<0.05$, c: $p<0.01$, d: $p<0.001$
Statistical significancy compared to the $AlCl_3$ group: y: $p<0.05$, z: $p<0.01$, t: $p<0.001$

Table 2. Concentrations (μg/g) of Macro and Trace Elements in Liver of Rats Treated with $AlCl_3$ and/or HES

Groups	Fe	Ca	Zn	Cu	Mn	Al
C	117,73±3,15[a]	91,53±3,66[a]	31,51±1,21[a]	2,22±0,26[a]	1,21±0,08[a]	4,73±0,46[a]
HES	125,28±3,43[ay]	103,42±3,30[az]	37,47±1,20[by]	2,68±0,07[ay]	1,23±0,07[a]	7,15±0,44[at]
A	144,94±5,70[c]	135,26±11,70[d]	32,50±1,80[a]	1,87±0,53[a]	1,14±0,08[a]	41,63±5,35[d]
A+HES	136,28±8,61[b]	110,71±4,65[ay]	35,96±2,18[a]	1,54±0,06[b]	1,05±0,04[a]	22,28±3,10[dt]

Values are expressed as means ± SD; n=6 for each treatment group. a: $p>0.05$
Statistical significancy compared to the control group: b: $p<0.05$, c: $p<0.01$, d: $p<0.001$
Statistical significancy compared to the $AlCl_3$ group: y: $p<0.05$, z: $p<0.01$, t: $p<0.001$

increase in Mn ($p<0.01$) levels of liver tissues and Zn levels of HES group increased when compared to control group ($p<0.05$).

Discussion

A number of studies have shown that animals exposed to $AlCl_3$ have increased Al concentrations in liver, plasma, urinary, kidney [14], testis and serum [15], hippocampus [16], and brain [17,18]. In the present study, there has been a significant increase in liver Al level after $AlCl_3$ exposure. Yousef *et al.* reported that $AlCl_3$ which is capable of causing marked alterations in some biochemical parameters has induced oxidative damage and inhibited the activities of antioxidant enzymes [19]. Wilhelm *et al.* reported that A1 accumulation in the liver can be caused by A1 exposure and at high concentrations this metal can have toxic effect upon the issue [20]. In our results, Al levels increased as response to $AlCl_3$ treatment, implying the increased oxidative damage to the tissues. It was observed that Fe level in liver of $AlCl_3$ administered group was found to be increased compared to the control group. Some studies have shown that animals that are exposed to Al have had increased Fe concentrations in brain, urinary excretion [17] and testis [15]. Ward RJ *et al.* noted that in an animal model of Al overload, increases in tissue iron in kidney, liver, heart and spleen, and also in various brain regions, such as frontal, temporal and parietal cortex and hippocampus, has been parallel to the increase at tissue A1 content [21]. The ionic radii of Al^{3+} most closely resemble to those of Fe^{3+}, therefore the appearance of Al^{3+} in Fe^{3+} sites is possible. Al is known to be bound by Fe^{3+} carrying protein transferrin, thus reducing the binding of Fe^{2+}. The increase in free intracellular Fe^{2+} causes peroxidation of membrane lipids and thus causes membrane damage [22]. We observed that administration of EA and HES of $AlCl_3$ treated animals decreased the Al levels in liver tissues. In addition, EA and HES combination caused a relative reduction in Fe levels. EA and HES are flavonoid-derived substances. Fernandez *et al.* reported that flavonoids could be very effective antioxidants which could also operate through antiradical and chelating mechanisms [23]. We have considered that the reason why EA and HES have decreased the level of Aluminum can be metal chelation and antioxidant effects of those flavonoid-derived substances. Moreover, in our previous study, EA decreased the high MDA levels in aluminum treated rat livers and EA also regulated GSH-PX enzyme activity which was decreased by the effect of aluminum [10].

Intracellular calcium ions have been assumed to increase its importance as a regulator of a variety of cellular processes, including muscle contraction, secretion and cell division [24, 25]. We found that Ca concentrations of the A group increased in the liver tissues, suggesting that Ca levels were significantly affected by the Al toxicity. Phenolic phytochemicals such as EA and flavanoids from fruits have been known as good chelators; therefore, these phytochemicals can effectively chelate the ions such as Ca in the extra cellular matrix or the cytosol and alter the net concentration of free Ca [26-29]. We observed that the increased liver Ca concentration due to Al exposure was reduced by EA and HES treatments. We think that EA and HES may have a corrective effect upon Ca concentration. Our results are in agreement with the literature [24-29].

In the current study, we have found an increase in Mn concentrations in liver tissues of EA administered rats. Esparza *et al*. demonstrated that Mn concentrations were higher in the melatonin- treated group than the one in Al-treated group in liver [30]. Moreover, in this study, it has been suggested that the protective effects of melatonin against cellular damage caused by Al-induced oxidative stress, together with its low toxicity, make melatonin worthy of investigation as a potential supplement to be included in the treatment of neurological disorders in which the oxidative effects must be minimized.

Furthermore, Mn acts as a cofactor of the antioxidant enzyme superoxide dismutase. It has been found that Mn deficiency stimulates lipid peroxidation [31]. However, we could not observe similar results in HES group.

Levels of Zn in HES-treated group were significantly higher than that in other groups ($p<0.05$) and there was not any statistical significant difference amongst of C, A, A+HES and A+EA groups ($p>0.05$). Zn is necessary for proper liver function and Zn deficiency has been related to the pathogenesis of multiple liver diseases [32-35]. Although liver Zn levels were observed to be increased by HES in HES treated groups, there were no statistically significant changes in EA administered groups. These effects of HES may be considered to be associated with liver enzymes.

It was found that Cu levels were relatively decreased in A group when compared to C group. The Cu levels of A+EA groups were increased in contrast to A group ($p<0.05$). Therefore, it can be considered that EA have a regulatory impact upon Cu concentrations. Gumus *et al*. found that EA has modulator effects on Cu and Zn levels in liver and serum of cholestatic rats [36]. Level of Cu in A+ HES-treated group was lower than the control group ($p<0.01$) and there was not any statistically significant difference among C, HES and A groups ($p>0.05$).

In conclusion, we observed that administration of $AlCl_3$ to rats**,** increased liver tissue Al and Ca levels. EA and HES decreased Al and Ca levels in $AlCl_3$ treated animals. Moreover, whereas EA has increased the levels of Cu and Mn in EA treated individual group, HES has provided increase at Zn level. At present, aluminum found in food, water and environment may have harmful effects on human metabolism; none the less, we consider that this harmful effect can be minimized consuming EA and HES that exist in fruits and vegetables.

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Ethical Considerations: The study protocol was approved by the National Institute of Health and Local Committee on Animal Research (Date: 27 July 2006, number: 3). All subjects were informed about the details of the study and the written consent of each subject was received.

Conflict of Interest: The authors declare no conflict of interest.

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