Kafkas Univ Vet Fak Derg
18 (5): 823-828, 2012
DOI:10.9775/kvfd.2012.6517

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Protective Effect of Ellagic Acid Against Renal Ischemia-Reperfusion Injury in Male Rats

Yasar BOZKURT * Ugur FIRAT ** Murat ATAR * Ahmet Ali SANCAKTUTAR *
Necmettin PEMBEGUL * Haluk SOYLEMEZ * Hatice YUKSEL *** Harun ALP ****
Mehmet Nuri BODAKCI * Namık Kemal HATIPOGLU * Sadık BUYUKBAS *****

* Department of Urology, Faculty of Medicine, Dicle University, TR-21280 Diyarbakır - TURKEY
** Department of Pathology, Faculty of Medicine, Dicle University, TR-21280 Diyarbakır - TURKEY
*** Department of Biochemistry, Faculty of Medicine, Dicle University, TR-21280 Diyarbakır - TURKEY
**** Department of Pharmacology, Faculty of Medicine, Mustafa Kemal University, TR-31040 Hatay - TURKEY
***** Department of Biochemistry, Faculty of Medicine, Mustafa Kemal University, TR-31040 Hatay - TURKEY

Makale Kodu (Article Code): KVFD-2012-6517

Summary

The aim of this study was to evaluate the possible protective effect of ellagic acid (EA) on rats following renal ischemia–reperfusion (I/R) injury. Twenty-four Wistar rats were divided into three groups. Sham group underwent laparotomy then waited for 45 min without ischemia. I/R group were subjected to left renal ischemia for 45 minutes followed by 60 min of reperfusion. I/R+EA group were subjected to the same renal ischemia/reperfusion as the I/R group, were also given 85 mg/kg EA perorally 30 min prior to the ischemia. Malondialdehyde (MDA), total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were determined on the blood samples and kidney tissues. Histopathological analyses were conducted on the kidney tissues. I/R damage significantly increased serum MDA levels in the I/R group when compared with Sham group. Serum TAC level was significantly lower in I/R group than I/R+EA group. A significantly increase on OSI levels and decrease on TAC levels was found in the kidneys in I/R group. In I/R + EA group, EA reversed the negative effects of I/R injury. EA pretreatment was effective in decreasing tubular necrosis score. In conclusion; EA pretreatment ameliorated the oxidative damage and histopathological changes occurring following renal I/R injury.

Keywords: *Antioxidant, Ellagic acid, Ischemia-reperfusion, Rat, Kidney*

Erkek Ratlarda Renal İskemi-Reperfüzyon Hasarına Karşı Elajik Asitin Koruyucu Etkisi

Özet

Bu çalışmanın amacı ratlarda renal iskemi-reperfüzyon (I/R) hasarına karşı elajik asitin (EA) olası koruyucu etkisini değerlendirmektir. Yirmi dört erkek Wistar rat; Sham, I/R ve I/R+EA olmak üzere üç gruba ayrıldı. Sham grubuna laparotomi yapıldı ve iskemi uygulanmaksızın 45 dakika bekletildi. I/R grubuna 45 dakikalık sol renal iskemiyi takiben 60 dakikalık reperfüzyon uygulandı. I/R+EA grubuna da I/R grubundakilere benzer şekilde iskemi/reperfüzyon prosedürü uygulandı, fakat iskemiden 30 dakika önce ratlara 85 mg/kg EA ağızdan verildi. Kanda ve böbrek dokusunda malondialdehit (MDA), total antioksidan kapasite (TAC), total oksidan statusu (TOS) ve oksidatif stres indeksi (OSI) bakıldı. Böbrek dokusu histopatolojik yönden incelendi. Sham grubu ile kıyaslandığında I/R grubunda serum MDA düzeyi anlamlı derecede yüksek idi. Ayrıca I/R+EA grubu ile kıyaslandığında I/R grubunda serum TAC düzeyi anlamlı derecede düşük bulundu. I/R grubunda böbrek dokusunda OSI düzeyi yüksek TAC düzeyi düşük bulunurken, I/R+EA grubunda iskemi ile oluşan etkilerin EA ile düzeldiği görüldü. EA verilmesinin tubuler nekroz skorunu azaltmada etkili olduğu bulundu. Çalışmanın sonucuna göre; EA, renal I/R hasarı sonrası meydana gelen oksidatif hasarı ve oluşan histopatolojik değişiklikleri iyileştirmektedir.

Anahtar sözcükler: *Antioksidan, Elajik asit, İskemi-reperfüzyon, Rat, Böbrek*

INTRODUCTION

Injury to kidney cells following ischemia-reperfusion (I/R) is one of the reasons for renal failure. This injury occurs following trauma, sepsis, renal transplantation and during some vascular surgeries [1]. For organ transplant patients I/R

İletişim (Correspondence)
+90 412 2488001
dryasarbozkurt@hotmail.com

injury plays an important role in the pathogenesis of rejection.

Several mechanisms have been proposed to explain the pathogenesis of I/R injury, but knowledge about the treatment remains limited. Renal ischemia initiates a series of incidents such as cellular dysfunction and necrosis [2]. Reperfusion, paradoxically, can create more tissue injury by promoting a further complex of cellular events that result ultimately in apoptosis and necrosis of renal cells. The mechanisms underlying I/R damage to renal cells are likely to be multifactorial and involve hypoxia, inflammation, and free radical damage. High levels of oxygen free radicals play a critical role in injury and this process leads to cell damage [3].

Number of agents have been experimentally investigated and showed some ability to prevent or reverse induced changes [4]. Ellagic acid (EA), a derivative of hydroxybenzoic acid, is one of the most important phenolic acids. It is found in a number of foods including raspberries, strawberries, walnuts, and pomegranates [5]. EA has been shown to induce apoptosis in pancreatic and leukemia cancer cells [6-8]. Several in vivo and *in vitro* studies have confirmed its anti-oxidative, anti-inflammatory, and anti-tumorigenic properties [9-11]. EA has a protective effect on gastric lesions and on I/R injury of the stomach. This protective effect is probably due to its antioxidant activity [12,13]. EA is also known to inhibit leukocyte recruitment and adhesion to the endothelium through inhibition of the generation of reactive oxygen species (ROS) and cytokine-induced ROS, inflammation, and expression of adhesion molecules [14]. The concentration–time profile was fitted with an open two-compartment system with lag time and its max concentration of ellagic acid in plasma was 213 ng/ml only 0.55 h after oral administration extract 0.8 g/kg [15]. Teel and Martin have found that the levels of ellagic acid were highest in blood 30 min [16]. Also, Boyuk *et al*. reported that EA have been given as dose 85 mg/kg orally for EA and I/R + EA groups, and found that EA treatment protected the rats lung tissue against intestinal I/R injury [17].

To the best of our knowledge, the effects of EA on renal I/R injury has not yet been studied. The aim of the present study was therefore to determine the possible protective effects of EA against I/R injury in the kidney of male rats by determining biochemical parameters and by histological examination.

MATERIAL and METHODS

Animals and Experimental Design

This study was approved by Dicle University Animal Ethical Committee (Form date and number: 09.07.2009-2009/12) and was carried out in accordance with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Dicle University, Animal Ethical Committee." This experimental study was performed with 24 mature, male, 3-month Wistar albino rats weighing 200-250 g. All animals were housed under standard conditions at an ambient temperature of 25±2°C and 12/12 hours of light-dark cycle in animal cages and treated in compliance with the National Institutes of Health guidelines. All experimental procedures in compliance with the animal use regulations of Dicle University Experimental Research Center, Diyarbakır, Turkey.

The ellagic acid was obtained from Sigma Chemicals (St. Louis, MO, USA). Ellagic acid solution was prepared as follows: 12.5 mg ellagic acid was suspanded in 1 ml distilled water [18,19].

The rats were randomly divided into three groups. Sham group (S, n=8): Rats were underwent exposure of left renal pedicles without I/R. I/R group (I/R, n=8): Rats were subjected to left renal ischemia for 45 min followed by 60 minutes of reperfusion. I/R + EA group (I/R + EA, n=8): EA (85 mg/kg) was given perorally [15,17] to the rats 30 minutes before the ischemia and then rats were subjected to left renal ischemia for 45 minutes followed by 60 min of reperfusion**.**

Surgical Procedure

The animals were anesthetized with an intramuscular injection of ketamine and xylazine (90 mg/kg and 10 mg/kg). After disinfection with povidone iodine solution, the abdomen was entered through a midline small incision, and the left kidney was isolated. In the S group, the abdomen was only closed. In the I/R and I/R + EA groups, the left renal artery was occluded using nontraumatic microvascular clamps for 45 min, and occlusion of blood flow was confirmed by visual inspection of the kidneys. The animals received 50 mL/kg of warm saline into the abdominal cavity during the entire procedure. After declamping, renal blood flow was confirmed to be restored prior to closing the incision. After waiting 60 min following the reperfusion, rats were sacrificed by taking 5 ml blood from the cardiac cavity. The abdomen was opened again and left nephrectomy was carried out; half of the left kidney was used for biochemical analysis and the other half was stored in 10% formalin for histopathological examination.

Measurement of Malondialdehyde

Malondialdehyde (MDA) levels were estimated by the double heating method of Draper and Hadley [20]. The principle of this method is spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. A 2.5 mL volume of trichloroacetic acid solution (10%) was added to 0.5 mL serum in a centrifuge tube, and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1000 g for 10 minutes, and 2 mL of the supernatant was added to 1 mL of TBA solution (6.7 g/L) in a test tube. The tube was placed in a boiling water bath for 15 minutes. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer (Shimadzu UV-1208, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA–TBA complex

(absorbance coefficient of 1.56x105 cm^{-1} M^{-1}) and is expressed as nmol/mL.

Measurement of the Total Antioxidant Capacity

The total antioxidant capacity (TAC) of serum and super-natant fractions was determined using a novel automated measurement method developed by Erel [21]. In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution present in Reagent 1 is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals such as brown colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. This method measures the antioxidant effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical. The results are expressed as nmol Trolox equivalent/mg protein for tissue results and µmol Trolox equivalent/L for serum results.

Measurement of Total Oxidant Status

Total oxidant status (TOS) of the supernatant fractions was determined using a novel automated measurement method developed by Erel [22]. Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of nmol H_2O_2 Equivalent /mg protein.

Determination of Oxidative Stress Index

The percent ratio of TOS level to TAC level was accepted as the oxidative stress index (OSI). The OSI value was calculated according to the following formula: OSI (Arbitrary Unit) = TOS (nmol H_2O_2 Equivalent/mg protein)/TAC (nmol Trolox Equivalent/mg protein) [23].

Histopathological Assessment

Kidneys were fixed in a 10% formalin solution and embedded in paraffin for histopathological assessment. Embedded tissues were cut into 5 µm-thick sections with a microtome and stained with hematoxylin and eosin (H&E). An experienced pathologist, blinded to the treatment conditions and the groups, examined the histological preparations with a light microscope (Nikon ECLIPSE 80i, Japan). Renal injury was graded[24] as follows: grade 0, no diagnostic change; grade 1 demonstrated tubular cell swelling, brush border loss, nuclear condensation with up to 1/3 of tubular profile showing nuclear loss; grade 2 is as grade 1, but greater than 1/3 and less than 2/3 of tubular profile showing nuclear loss; grade 3, greater than 2/3 of tubular profile showing nuclear loss. A minimum of 10 fields for each kidney slide were examined.

Statistical Analysis

All data were expressed as mean and standard deviation (SD). Differences between groups were evaluated by Kruskal-Wallis variance analysis followed by a Mann-Whitney U-test with Bonferroni correction for binary comparisons. The Pearson Chi-Square test was used to compare kidney pathology grades. P values <0.05 were considered statistically significant. All data were processed using the SPSS 15.0 for Windows (SPSS INC., Chicago, IL, USA) statistical package.

RESULTS

Plasma

I/R damage significantly increased serum MDA levels (P<0.038) in the I/R group when compared with Sham group. Lower MDA levels were seen in the group treated with EA prior to I/R injury compared to the I/R group, but the difference was not statistically significant. When TAC levels were compared between these two groups, the levels of TAC were significantly lower in the I/R group (P<0.000) than in the EA treated group *(Table 1)*.

Kidney

I/R damage significantly increased OSI (P<0.002), and decreased TAC levels (P<0.007) in rat kidney tissues in the I/R group when compared with S group. Administration of EA prior to I/R revealed the negative effects of I/R injury. The

Table 1. *Oxidant and antioxidant parameters in rat groups*
Tablo 1. *Rat gruplarındaki oksidan ve antioksidan parametreler*

Oxidant and Antioxidant Parameters	S (n=8)	I/R (n=8)	I/R+EA (n=8)
Serum			
MDA (nmol / mL)	0.44±0.11	0.67±0.22*	0.54±0.09
TAC (µmol Trolox equivalent/L)	2.52±0.39	1.03±0.53*a2	2.30±0.45**
Kidney Tissue			
TAC (µmol Trolox Equivalent/mg prot.)	2.30±0.22	1.57±0.44*	2.41±0.20**
TOS (µmol H_2O_2 Equivalent/mg prot.)	140.62±25.61	174.19±21.66*	145.69±16.97
OSI (H_2O_2/Trolox)	62.01±15.31	118.42±34.90*	60.59±7.67**

Data were given as mean ± SD, ***MDA:*** *Malondialdehyde,* ***TAC:*** *Total Antioxidant Capacity,* ***TOS:*** *Total oxidant status,* ***OSI:*** *Oxidative stress index.* ***S:*** *Sham,* ***I/R:*** *Ischemia-reperfusion,* ***EA:*** *Ellagic acid, * P<0.05 versus S group, ** P<0.001 versus S group*

TOS levels were higher in the I/R group compared with the S group (P<0.015). Administration of EA for EA + I/R group inhibited some of the increase in TOS seen following I/R treatment, but these lower TOS levels were not significantly different.

Kidney Histopathological Analysis

Histopathological evaluation revealed that the renal tissues of the sham group had normal structure with no pathological changes *(Fig. 1a)*. The kidney tissues of the I/R group exhibited severe tubular cell damage in 4 rats and moderate damage in 4 rats *(Fig. 1b)*. In I/R+EA group, no severe tubular cell damage was observed, but moderate tubular cell damage was seen in 3 rats *(Fig. 1c)*. In I/R + EA group, the histopathological scores were significantly lower than in the I/R group *(Fig. 2)*.

DISCUSSION

In this study, our results showed that TOS levels and OSI index were increased after renal I/R injury, indicating oxidative stress. While MDA levels were significantly increased, TAC levels were decreased in serum. EA pretreatment prevented this increase in MDA. In kidney tissue, EA pretreatment ameliorated the oxidative damage by decreasing TOS levels and OSI index and supporting TAC levels.

Free radicals and ROS are important factors that contribute to I/R injury [25,26] and their deleterious effects on various cell functions are well known, including consequences such as alterations in the level of mitochondrial oxidative phosphorylation, ATP depletion, increases in the intracellular calcium, and activation of protein kinases, phosphatases, proteases, lipases and nucleases, which lead to a loss of cellular function and integrity [26]. Reoxygenation of ischemic tissue may promote the generation of ROS [27], which can then react with lipids, proteins, and nucleic acids and lead to lipid peroxidation of biological membranes [28]. Renal I/R injury has been associated with oxidative stress in kidney tissue, together with a decrease in antioxidant defense [29,30].

Hosseini *et al*.[31] showed increased oxidative stress and decreased TAC after renal I/R injury, supporting our results. Antioxidants, antioxidant enzyme mimetics, nitric oxide,

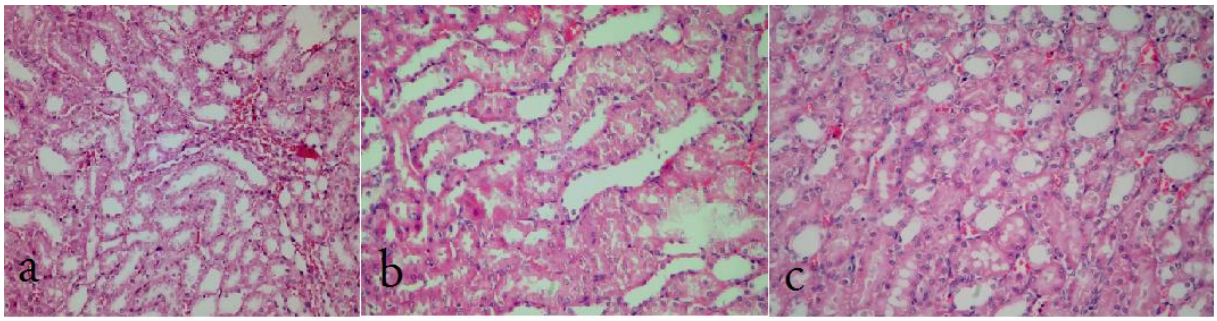

Fig 1. a- Sham group. Normal histomorphological features of the renal tubules. Mild tubular cell swelling is seen in a few tubular structures (H&E stain, x200), **b-** Ischemia reperfusion group. Note tubular cell swelling, brush border loss, and nuclear condensation with nuclear losses (H&E stain, x200), **c-** Ischemia reperfusion+Ellagic acid group. Note tubular cell swelling, slight brush border loss, and a few instances of nuclear condensation with some nuclear losses (H&E stain, x200)

Şekil 1. a- Sham grubu. Böbrek tübülünün normal histomorfolojik özellikleri. Birkaç tübülde hafir tübüler hücre şişmesi görülüyor (H&E boyası, x200), **b-** İskemi reperfüzyon grubu. Tübüler hücre şişmesi, fırça kenar kaybı ve nükleer kayıpları ile nükleer yoğuşma görülmektedir (H&E boyası, x200), **c-** İskemi reperfüzyon+Elajik asit grubu. Tübüler hücre şişmesi, hafif fırça kenar kaybı ve bir kaç örnekte nükleer kayıplarla birlikte nükleer yoğuşma görülmektedir (H&E boyası, x200)

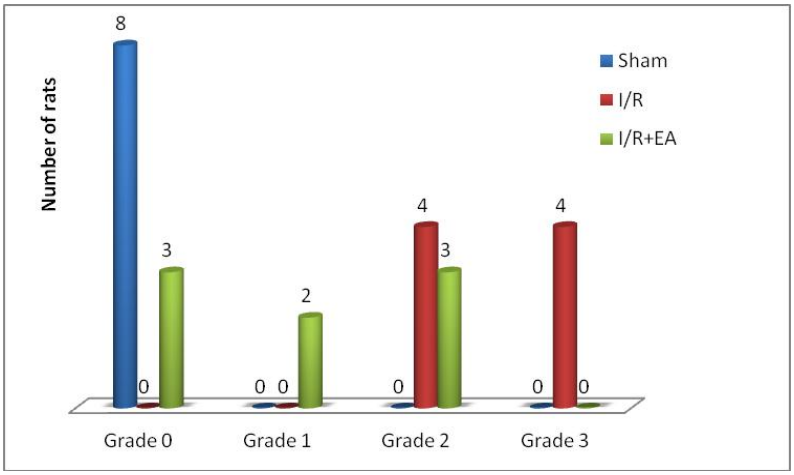

Fig 2. Histopathological evaluation of renal tissue for each group. Scores were significantly lower in IR+EA group than in the I/R group (Pearson Chi-Square test, P<0.027), **I/R:** Ischemia/reperfusion, **EA:** Ellagic acid

Şekil 2. Her grubun böbrek dokusunun histopatolojik değerlendirilmesi. Skorlar IR+EA grubunda, I/R grubuna göre anlamlı derecede düşüktü (Pearson Chi-Square test, P<0.027), **I/R:** İskemi/reperfüzyon, **EA:** Elajik asit

and nitric oxide synthase inhibitors, erythropoietin, statins, beta carotene and ascorbic acid have all been reported to have potential beneficial effects for reducing renal I/R injury and helping to maintain kidney function [31-33].

EA is a phenolic compound and its antioxidant, anti-inflammatory, anticarcinogenic, and antihyperlipidemic activities have been shown both *in vitro* and *in vivo* [34]. EA has also been reported to have protective effects on hepatic tissue via decreasing the Cu/Zn ratio in an experimental biliary obstruction model [35]. Ateşşahin *et al*.[36] reported that EA markedly reduced oxidative stress markers and also ameliorated cisplatin-induced pathological changes including tubular necrosis, degeneration, and tubular dilatation. They concluded that EA might have protective effects against cisplatin-induced nephrotoxicity and oxidative stress in rats. Similarly, in the present study, EA significantly decreased oxidative stress markers and especially ameliorated histopathological changes such as tubular cell damage in the kidney.

As a result, our data suggest that EA may be an effective chemoprotective agent against renal I/R injury. It may provide protection by reducing the concentration of oxidant products by scavenging free radicals and supporting the antioxidant system**.** However, further research is needed to understand the possible mechanisms by which EA is able to prevent renal I/R injury.

REFERENCES

1. Donnahoo KK, Meldrum DR, Shenkar R, Chung CS, Abraham E, Harken AH: Early renal ischemia, with or without reperfusion, activates NFkappaB and increases TNF-alpha bioactivity in the kidney. *J Urol*, 163, 1328-1332, 2000.

2. Sivarajah A, Chatterjee PK, Patel NS, Todorovic Z, Hattori Y, Brown PA, Stewart KN, Mota-Filipe H, Cuzzocrea S, Thiemermann C: Agonists of peroxisome-proliferator activated receptor-gamma reduce renal ischemia/reperfusion injury. *Am J Nephrol,* 23, 267-276, 2003.

3. Parks DA and Granger DN: Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol* 250, 749-753, 1986.

4. El-kossi A, Abdellah M, Rashad A, Hamed S: The effectiveness of evening primrose oil and alpha lipoic acid in recovery of nerve function in diabetic rats. *J Clin Exp Invest*, 2, 245-253, 2011.

5. Mejia-Meza EI, Yanez JA, Remsberg CM, Takemoto JK, Davies NM, Rasco B, Clary C: Effect of dehydration on raspberries: polyphenol and anthocyanin retention, antioxidant capacity, and antiadipogenic activity. *J Food Sci,* 75, H5-12, 2010.

6. Hagiwara Y, Kasukabe T, Kaneko Y, Niitsu N, Okabe-Kado J: Ellagic acid, a natural polyphenolic compound, induces apoptosis and potentiates retinoic acid-induced differentiation of human leukemia HL-60 cells. *Int J Hematol,* 92, 136-143, 2010.

7. Edderkaoui M, Odinokova I, Ohno I, Gukovsky I, Go VL, Pandol SJ, Gukovskaya AS: Ellagic acid induces apoptosis through inhibition of nuclear factor kappa B in pancreatic cancer cells. *World J Gastroenterol,* 14, 3672-3680, 2008.

8. Whitley AC, Stoner GD, Darby MV, Walle T: Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acid-extensive binding to protein and DNA. *Biochem Pharmacol,* 66, 907-915, 2003.

9. Rogerio AP, Fontanari C, Melo MC, Ambrosio SR, de Souza GE, Pereira PS, Franca SC, da Costa FB, Albuquerque DA, Faccioli LH: Anti-inflammatory, analgesic and anti-oedematous effects of Lafoensia pacari extract and ellagic acid. *J Pharm Pharmacol,* 58, 1265-1273, 2006.

10. Falsaperla M, Morgia G, Tartarone A, Ardito R, Romano G: Support ellagic acid therapy in patients with hormone refractory prostate cancer (HRPC) on standard chemotherapy using vinorelbine and estramustine phosphate. *Eur Urol,* 47, 449-454, 2005.

11. Alp H, Aytekin I, Esen H, Basarili K, Kul S: Effects of caffeic acid phenethyl ester, ellagic acid, sulforaphane and curcumin on diazinon induced damage to the lungs, liver and kidneys in an acute toxicity rat model. *Kafkas Univ Vet Fak Derg,* 17 (6): 927-933, 2011.

12. Iino T, Tashima K, Umeda M, Ogawa Y, Takeeda M, Takata K, Takeuchi K: Effect of ellagic acid on gastric damage induced in ischemic rat stomachs following ammonia or reperfusion. *Life Sci,* 70, 1139-1150, 2002.

13. Tasaki M, Umemura T, Maeda M, Ishii Y, Okamura T, Inoue T, Kuroiwa Y, Hirose M and Nishikawa A: Safety assessment of ellagic acid, a food additive, in a subchronic toxicity study using F344 rats. *Food Chem Toxicol,* 46, 1119-1124, 2008.

14. Yu YM, Wang ZH, Liu CH, Chen CS: Ellagic acid inhibits IL-1beta-induced cell adhesion molecule expression in human umbilical vein endothelial cells. *Br J Nutr,* 97, 692-698, 2007.

15. Lei F, Xing DM, Xiang L, Zhao YN, Wang W, Zhang LJ, Du LJ: Pharmacokinetic study of ellagic acid in rat after oral administration of pomegranate leaf extract. *J Chromatogr B Analyt Technol Biomed Life Sci,* 796, 189-194, 2003.

16. Teel RW, Martin RM: Disposition of the plant phenol ellagic acid in the mouse following oral administration by gavage. *Xenobiotica*, 18, 397-405, 1988

17. Böyük A, Önder A, Kapan M, Gümüş M, Fırat U, Başaralı MK, Alp H: Ellagic acid ameliorates lung injury after intestinal ischemia-reperfusion. *Pharmacognosy Magazine*, 7, 224-228, 2011.

18. Uzar E, Alp H, Cevik MU, Fırat U, Evliyaoglu O, Tufek A, Altun Y: Ellagic acid attenuates oxidative stress on brain and sciatic nerve and improves histopathology of brain in streptozotocin-induced diabetic rats. *Neurol Sci,* [Epub ahead of print] PubMed PMID: 21922312.

19. Alp H, Aytekin I, Atakisi O, Hatipoglu NK, Basarali K, Ogun M, Buyukbas S, Altintas L, Ekici H, Alp A: The effects of caffeic acid phenethyl ester and ellagic acid on the levels of malondialdehyde, reduced glutathione and nitric oxide in the lung, liver and kidney tissues in acute diazinon toxicity in rats. *J Anim Vet Adv* 10, 1488-1494, 2011.

20. Draper HH, Csallany AS, Hadley M: Urinary aldehydes as indicators of lipid peroxidation in vivo. *Free Radic Biol Med,* 29, 1071-1077, 2000.

21. Erel O: A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem,* 37, 112-119, 2004.

22. Erel O: A new automated colorimetric method for measuring total oxidant status. *Clin Biochem,* 38, 1103-1111, 2005.

23. Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O: Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. *BMC Infect Dis,* 5, 95, 2005.

24. Solez K, Morel-Maroger L, Sraer JD: The morphology of "acute tubular necrosis" in man: analysis of 57 renal biopsies and a comparison with the glycerol model. *Medicine (Baltimore),* 58, 362-376, 1979.

25. Chander V, Chopra K: Renal protective effect of molsidomine and L-arginine in ischemia-reperfusion induced injury in rats. *J Surg Res,* 128, 132-139, 2005.

26. Sekhon CS, Sekhon BK, Singh I, Orak JK, Singh AK: Attenuation of renal ischemia/reperfusion injury by a triple drug combination therapy. *J Nephrol,* 16, 63-74, 2003.

27. Bulkley GB: Free radical-mediated reperfusion injury: A selective review. *Br J Cancer Suppl,* 8, 66-73, 1987.

28. Karaman A, Turkmen E, Gursul C, Tas E, Fadillioglu E: Prevention of renal ischemia/reperfusion-induced injury in rats by leflunomide. *Int J Urol,* 13, 1434-1441, 2006.

29. Tugtepe H, Sener G, Biyikli NK, Yuksel M, Cetinel S, Gedik N, Yegen BC: The protective effect of oxytocin on renal ischemia/reperfusion injury in rats. *Regul Pept,* 140, 101-108, 2007.

30. Sener G, Sehirli O, Velioglu-Ogunc A, Cetinel S, Gedik N, Caner M, Sakarcan A, Yegen BC: Montelukast protects against renal ischemia/reperfusion injury in rats. *Pharmacol Res,* 54, 65-71, 2006.

31. Hosseini F, Naseri MK, Badavi M, Ghaffari MA, Shahbazian H, Rashidi I: Effect of beta carotene on lipid peroxidation and antioxidant status following renal ischemia/reperfusion injury in rat. *Scand J Clin Lab Invest,* 70, 259-263, 2010.

32. Chatterjee PK: Novel pharmacological approaches to the treatment of renal ischemia-reperfusion injury: A comprehensive review. *Naunyn Schmiedebergs Arch Pharmacol,* 376, 1-43, 2007.

33. Korkmaz A, Kolankaya D: The protective effects of ascorbic acid against renal ischemia-reperfusion injury in male rats. *Ren Fail,* 31, 36-43, 2009.

34. Devipriya N, Sudheer AR, Menon VP: Dose-response effect of ellagic acid on circulatory antioxidants and lipids during alcohol-induced toxicity in experimental rats. *Fundam Clin Pharmacol,* 21, 621-630, 2007.

35. Gumus M, Yuksel H, Evliyaoglu O, Kapan M, Boyuk A, Onder A, Aldemir M: Effects of ellagic acid on copper, zinc, and biochemical values in serum and liver of experimental cholestatic rats. *Biol Trace Elem Res,* 143, 386-393, 2010.

36. Ateşşahin A, Ceribasi AO, Yuce A, Bulmus O, Cikim G: Role of ellagic acid against cisplatin-induced nephrotoxicity and oxidative stress in rats. *Basic Clin Pharmacol Toxicol,* 100, 121-126, 2007.