Cytotechnology (2016) 68:1763–1770
DOI 10.1007/s10616-015-9928-z

ORIGINAL ARTICLE

Protective effects of ellagic acid in D-galactosamine-induced kidney damage in rats

Adnan Ayhanci · Mustafa Cengiz · H. Mehtap Kutlu · Djanan Vejselova

Received: 12 August 2015 / Accepted: 26 October 2015 / Published online: 12 December 2015

Abstract D-Galactosamine (D-GalN), which is an established experimental toxin, primarily causes liver injury by the generation of free radicals and depletion of UTP nucleotides. D-GalN intoxication also induces renal dysfunction thus, renal failure is often associated with the end-stage of the liver damage. We have investigated both preventive and curative effects of ellagic acid (EA) in this study. EA treatment at a gavage dose of 20 mg/kg body weight was administered before and after intraperitoneal (i.p.) injection of D-GalN at a dose of 750 mg/kg. Tissue and blood samples of animals were collected for morphological and biochemical evaluations. Our study results suggest that EA treatment both prior to and after the toxin administration successfully altered the toxic effects on the rats. Moreover, pre-treatment of EA was more protective than post-treatment indicated by histopathological and biochemical values. In conclusion, EA treatment both before and after D-GalN intoxication could protect kidney tissues against D-GalN induced oxidative stress.

Keywords D-GalN · Ellagic acid · Apoptotic markers · Renal failure

A. Ayhanci
Department of Biology, Faculty of Arts and Science, Eskisehir Osmangazi University, Eskisehir, Turkey

M. Cengiz (✉)
Department of Elementary Education, Faculty of Education, Siirt University, Siirt, Turkey
e-mail: mustafacengizogu@gmail.com

H. Mehtap Kutlu · D. Vejselova
Department of Biology, Faculty of Science, Anadolu University, Eskisehir, Turkey

Introduction

Acute liver diseases are of a global concern and treatments have limited efficacy. Toxic agents cause an acute damage on liver and kidney where the toxic substances are metabolized and excreted respectively. There is a need for clinical applications of therapeutically effective natural agents with reduced toxicity (Tolstikov et al. 2007). Acute liver failure due to GalN administration is well-documented (Keppler et al. 1968; Makin et al. 1997; EI-Mofty et al. 1975). GalN is a toxic substance whose toxic effect is associated with the depletion of UTP nucleotides followed by the formation of UDP hexosamines (Kmiec et al. 2000). This results in the inhibition of transcription (Keppler et al. 1974). GalN-induced liver injury has also been linked to renal failure (Javle et al. 1998). Anand et al. (2002) showed that treatment of D-GalN results in renal failure manifested by the decrease in renal blood flow, creatinine clearance in renal cortex. It was also reported that a patient suffering from liver disease showed a decrease in the renal blood flow, indicating renal vasoconstriction (Guarner et al. 1987). Sire et al.

(1983) reported that GalN damaged the enzymes involved in the transport of substrates into the mitochondria by raising caspase-3 and reactive oxygen radical levels (Quintero et al. 2002) and, as a result, phospholipids composition of the membrane is modified (Sire et al. 1983). Flavonoids, which are also a group of polyphenolic antioxidants, occur naturally in vegetables and fruits. Ellagic acid [EA (2,3,7,8-tetrahydroxy[1]-benzopyrano [5,4,3-cde][1]benzopyran-5,10-dione)] is also a naturally occurring phenolic constituent in certain fruits and nuts, like raspberries, strawberries, walnuts, longan seed, mango kernel pomegranate (Soong and Barlow 2004). EA is known to possess a variety of biological activities including potent antioxidant anticancer and antimutagen properties (Hassoun et al. 1997). Even though the exact molecular mechanism of EA remains unknown, its potent scavenging action on OH and (O_2^-) might be responsible for these effects (Han et al. 2006). All these taken into account, the present study aims to investigate the therapeutic and protective effects of EA on D-GalN-induced kidney damage in rats.

Materials and methods

Materials

EA and D-GalN were supplied by Sigma Chemicals Company (St. Louis, MO, USA) with the remaining chemicals and biochemicals obtained from local firms.

Animals experimental design

Sprague–Dawley strain male rats weighing 180–240 g were used in the present study (n = 35). Rats were kept in controlled laboratory conditions where they were fed with pellet and tap water. They were categorized into five groups randomly, each of them containing seven rats. Animals were kept daylight and dark cycle for 12 h at a temperature of 22 ± 2 °C, along with humidity of 45–50 % in automated controlled rooms. All animal procedures complied with the approval obtained from the Animal Welfare Committee of Anadolu University.

EA was suspended in DMSO and administered to the animals by gavage at a single dose of 20 mg/kg. D-GalN was suspended in saline and injected intraperitoneally (i.p.) to the animals at a single dose of 750 mg/kg. On the first day (24 h after applications of the agents to the animals at day 0) animals in groups 1, 2 and 3 were sacrificed (Table 1). On the second day (24 h after the D-GalN (750 mg/kg) and EA (20 mg/kg) administration to the animals at first day) groups 4 and 5 were sacrificed (Table 1). 24 h after last administration the rats were anaesthetized with ether and the blood was drawn by cardiac puncture. The kidneys were removed and washed with ice-cold 0.9 % NaCl. The dose and administration period have been selected corresponding to the previous studies (Rosillo et al. 2011; Shi et al. 2008; Zhou et al. 2008).

Light Microscopic Evaluation

Kidneys were cut into small pieces and fixed in Bouin's solution. Following dehydration in ascending series of ethanol (70, 90, 96, 100 %), tissue samples were cleared in xylene, and then embedded in paraffin and 5–6 μm thick sections were made. Sections were stained with Haematoxylin–Eosin (H–E) and Masson's trichrome (Masson).

Immunohistochemistry

Sections were deparaffinized and rehydrated. Antigen retrieval by citrate buffer (pH 6.0) was done with heating the sections in a microwave at 700 W for 10 min. After blocking with 3 mL/L H_2O_2 and swine serum, sections were exposed to incubation with the primary antibodies, directed against Bcl-2 (Abcam, Cambridge, UK), Bax (Abcam) and caspase-3 (Thermo, Waltham, MA, USA) at dilutions of Ultra vision quanta detection system (Thermo Scientific), respectively.

Biochemistry assays

Sera were obtained from the blood samples by centrifugating for 10 min at 3000 rpm. Serum lactate dehydrogenize (LDH), blood urea nitrogen (BUN) and creatinine (Cre) levels were analyzed with an automated biochemical auto-analyzer (HITACHI-917).

Statistical analysis

A package software version of SPSS 12.0 for windows was used in assessing the data obtained in the present

Table 1 Description of the experimental groups and the doses of applied chemicals

Days Group	0	1	2
1	Saline (0.5 mL)	Sacrifice	
2	DMSO (0.2 %, 0.5 mL)	Sacrifice	
3	D-GalN (750 mg/kg)	Sacrifice	
4	EA (20 mg/kg)	D-GalN (750 mg/kg)	Sacrifice
5	D-GalN (750 mg/kg)	EA (20 mg/kg)	Sacrifice

study. The difference observed for serum LDH, BUN and Cre levels in the study groups were assessed via one-way ANOVA. The numerical value (p) for the difference was determined as significant when $p < 0.05$.

Results

EA prevents histopathological changes caused by D-GalN

Hematoxylin-eosin stained samples were observed under light microscope (Leica DM6000 B). Structure of kidney tissues of the control group (Group 1) were normal. Kidney tissues of 0.2 % DMSO-administrated rats (Group 2) were almost negligibly impaired. However, kidney structures of the rats given 750 mg/kg D-GalN (Group 3) were severely impaired. Inflammation, tubular dilatation, narrowing in Bowman's space, vascular congestion in the interstitial region, and tubular damages were detected in the experimentally damaged kidney tissues (Fig. 1 and Table 2). In Group 4, there was a near-normal kidney cortex along with a reduced tubular damage. Furthermore, both vascular congestion and narrowing in Bowman's space of Malpighian corpuscles were determined to be far less than those in Group 3. As for the last group, there was reduced tubular damage and a near-normal kidney cortex, though the damage was of a far less extent in comparison with Group 4. Also, there was partial vacuolization in some of the tubules and fractional glomerular damage in Malpighian corpuscles. Kidney samples of Group 4 in which EA was injected 24 h before D-GalN treatment were better protected against toxicity than those in Group 5, in which EA was given 24 h after the D-GalN administration. Besides, the results of Group 5 were remarkably similar to those of Control Group (Fig. 1).

EA decreased apoptosis

Kidney specimens cultivated from all study groups were immunohistochemically stained to determine concentration and intensity of Bcl-2, Bax and caspase-3 antigens. In Group 3, the number of Bcl-2 positive kidney cells were decreased (Fig. 2) while the number of caspase-3 and Bax positive kidney cells were significantly increased (Fig. 2) when compared to that of the control and DMSO groups, which was of statistical significance ($p < 0.05$). Furthermore, Bcl-2 positive kidney cells increased in Groups 4 and 5, while the number of caspase-3 and Bax positive kidney cells showed a significant decrease compared to Group 3, which was also of statistical significance ($p < 0.05$) (Fig. 3). Immunohistochemical findings showed that EA pre-treatment seems to provide a better protection than EA post-treatment against toxicity.

Biochemical results

D-GalN (Group 3) administration increased serum LDH, BUN and Cre levels. Both pre and post treatment of EA to the GalN intoxication decreased serum LDH, BUN and Cre level significantly ($p < 0.05$) (Table 3).

Discussion

D-GalN-induced acute liver failure in rats is the result of high free radical production that leads to oxidative stress (Catal and Bolkent 2008). Experimental liver injury is reported to be associated with the development of renal failure (Gezginci-Oktayoglu et al. 2008). Moore et al. (1991) reported severe hepatocyte injury or massive hepatic necrosis is accompanied by renal failure in approximately 50 % of patients (Moore et al.

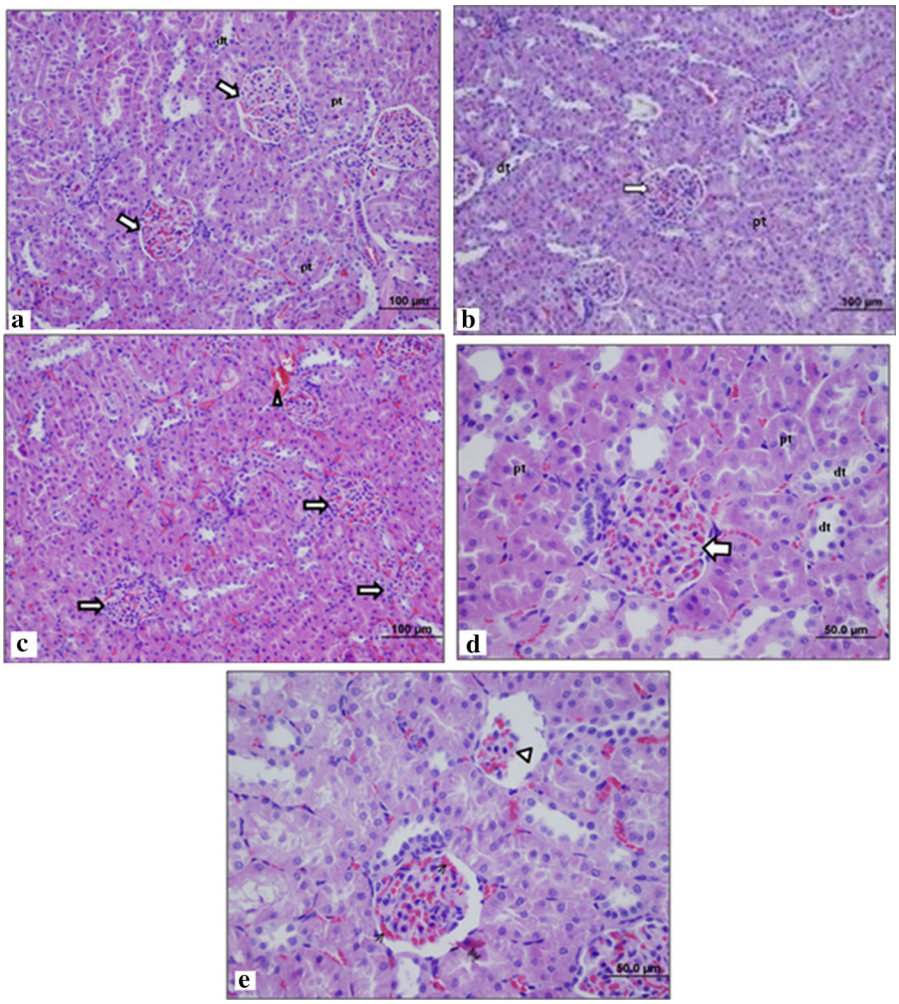

Fig. 1 Kidney with normal histological structures (group 1) (**a**) and (group 2) (**b**) (Glomerulus (*Open arrow*), proximal tubule (PT), distal tubule (DT), kidney with damaged histological structures by D-GaIN (group 3) (**c**) (Tubular damage, narrowing, Bowman's space (*right arrow*) and vacuolization (*triangle*). A near-normal kidney cortex, along with tubular damage, is observed in group 4 (**d**) (vascular congestion and narrowing observed in the Bowman space of the Malpighian corpuscles were reduced compared to the damaged group). A near-normal kidney cortex, along with tubular damage, is observed in group 5 (**e**), though the damage did not completely disappear compared to group 4. Partial vacuolization in some tubules (*right arrow*) (**b**) and partial glomerular damage in the Malpighian corpuscles (*triangle*)

Table 2 Histological scores of the study groups

Groups	Tubular damage	Glomerular damage	Congestion	Narrow in Bowman space	Inflammation	Total Score
1	0	0	0	0	0	0
2	0	0	0	0	1	1
3	3	3	3	3	2	14*
4	0	1	0	0	1	2
5	1	1	1	1	0	3

* Statistical significance $p < 0.05$ compared to control group (Group 1)

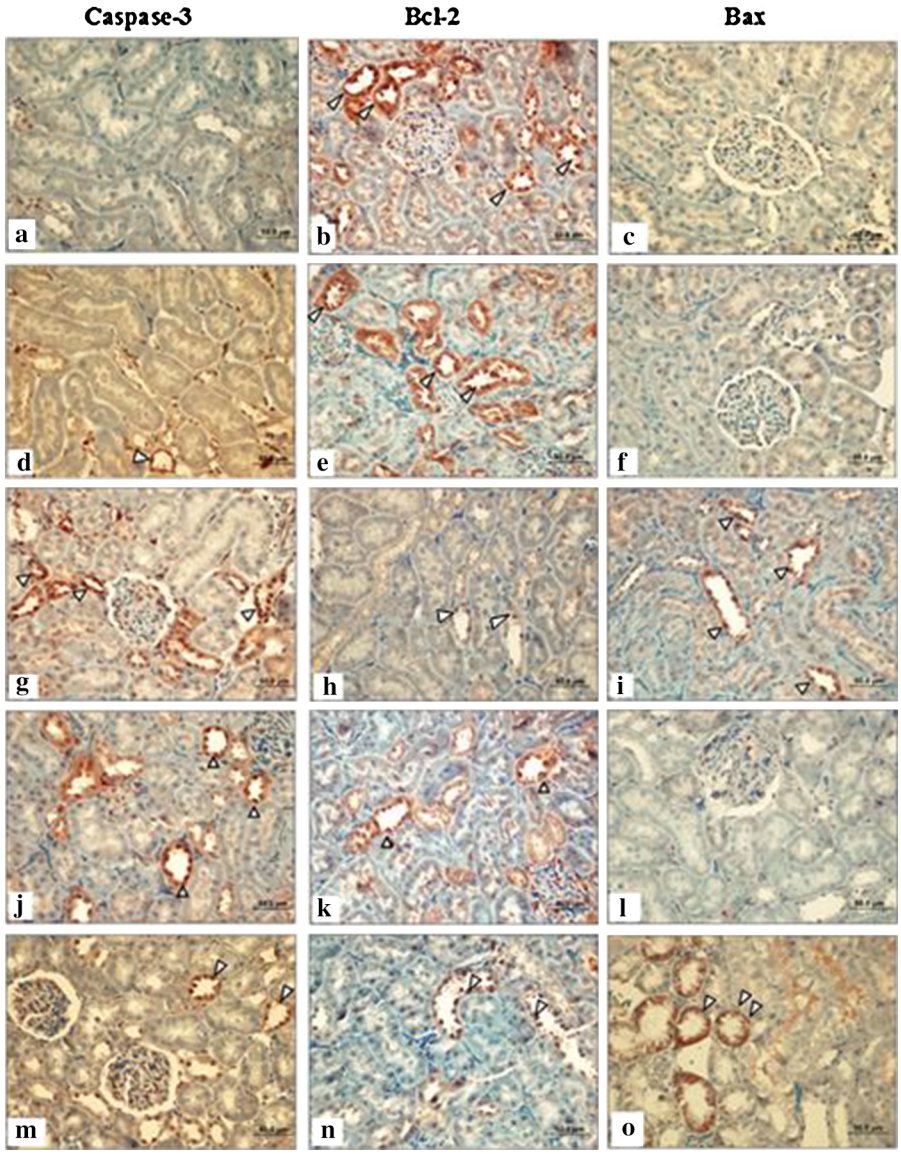

Fig. 2 The immunohistochemical staining of kidney specimens of different study groups. Activated caspase-3 and Bax in control group (1) and 0.2 % DMSO-treatment group (2) show no staining in normal kidney cell (**a**, **c**, **d**, **f**) while Bcl-2 protein expression in control group shows a less-intense staining on normal cells as shown by the *arrow heads* (**b**, **e**). Caspase-3 and Bax protein expression in the D-GalN-treated group (3) show a more intense staining of kidney cells and a diffused staining as shown by the *arrow heads* (**g**, **i**) while activated Bcl-2 staining was decreased in D-GalN-treated group compared to the normal cell as shown by the *arrow heads* (**h**). Activated Caspase-3 and Bax immunostaining of kidney treated with EA before D-GalN (group 4) and D-GalN before EA (group 5) show a less intense staining of kidney cells as shown by the *arrow heads* (**j**, **l**, **m**, **o**) while Bcl-2 protein expression in EA before and after D-GalN groups show an intense staining of kidney cells as shown by the *arrows* (**k**, **n**) (*bar* 50 μm)

1991). It has also been determined that a patient with a liver disease had a decline in renal blood flow that indicated renal vasoconstriction (Guarner et al. 1987). Anand et al. (2002) also showed that treatment of D-GalN results in renal failure that manifests itself through the decrease in the renal blood flow, creatinine clearance, along with a rise in plasma endothelin-1 (ET-1) concentration, and also upregulation of ET-1 receptors in renal cortex. Catal and Bolkent (2008) reported that animals administered 500 mg/kg D-GalN

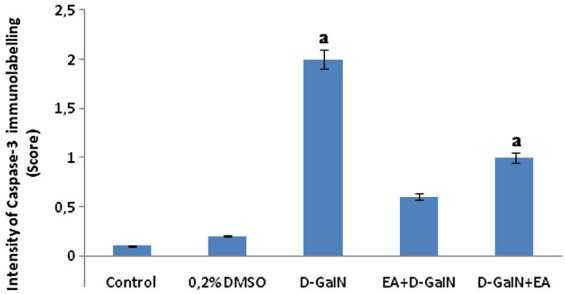

Fig. 3 Intensity of immunolabelling score of activated caspase-3 positive cells in the groups. [a] $p < 0.05$ compared to control (Group 1)

Table 3 Serum BUN, Cre and LDH levels of the blood samples taken from rats

Group	BUN (mg/dL) Mean ± SD	Cre (mg/dL) Mean ± SD	LDH (U/L) Mean ± SD
1	14.86 ± 1.21	0.20 ± 00	576.71 ± 49.40
2	16.19 ± 1.84	0.20 ± 00	592.29 ± 42.41
3	19.42 ± 0.98[a,b]	0.23 ± 0.008[a,b]	956.29 ± 83.04[a,b]
4	14.85 ± 1.32[c]	0.20 ± 00[c]	664.14 ± 47.73[c]
5	16.39 ± 1.52[c]	0.20 ± 00[c]	640.86 ± 141.52[c]
p	$p < 0.001$	$p < 0.001$	$p < 0.001$

All values are the mean ± SD (n = 7)

[a] $p < 0.001$ compared to control

[b] $p < 0.001$ compared to Group 2

[c] $p < 0.001$ compared to Group 3

had distinct degenerative changes, particularly in the proximal tubular cells and glomerulus when compared to the kidney tissues of control groups. In addition, in the group administered D-GalN, serum urea, creatinine and LDH levels were found to be significantly higher than those of the control groups. The renal dysfunction due to 800 mg/kg GalN treatment was also manifested by the increase in serum creatinine and BUN levels as compared to the normal group of mice (Sinha et al. 2007). Ghosh et al. (2012) showed that D-GalN significantly increased the protein expression of Bax, cytochrome c, caspase 3/9. The same exposure, on the other hand, reduced the protein expression of Bcl-2 in the cytosol. Our study results suggest that the injection of 750 mg/kg D-GalN causes degenerative changes in the kidney tissue, a significant rise in the number of caspase-3-positive (Fig. 3) kidney cells while decreasing the number of Bcl-2 (Fig. 4) protein expression along with a rise in the levels of BUN, Cre, and LDH activities (Table 3). Also the number of activated Bax

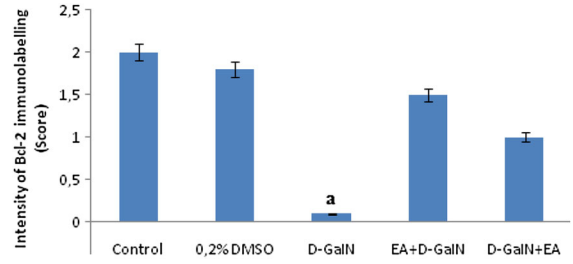

Fig. 4 Intensity of immunolabelling score of activated Bcl-2 positive cells in the groups. [a] $p < 0.05$ compared to control (Group 1)

positive kidney cells was significantly increased (Fig. 5).

Various antioxidants such as melatonin (Rezzani et al. 2005), lycopene (Atessahin et al. 2006), lipoic acid (Amudha et al. 2006) and green tea (Mohamadin et al. 2005) have been investigated for their potential effects to prevent the side effects of toxic substances. There is another type of antioxidants, flavonoids, which are richly found in fruits and vegetables. Flavonoids have many functions as antioxidants, scavengers of free radicals, chelating agents, and modifiers of various enzymatic and biological reactions (Khanduja et al. 1999). EA is a naturally occurring plant polyphenol (Soong and Barlow 2004) that exhibits antioxidative properties both in vivo (Hassoun et al. 1997) and in vitro (Seeram et al. 2005). EA has wide array of biological properties, ranging from radical scavenging, to chemopreventive (Atessahin et al. 2006; Turk et al. 2008) and antiapoptotic properties (Turk et al. 2010). The antioxidant effects of EA on cytotoxicity have been evaluated by different studies. For example, it was reported that EA (2 mg/kg) can improve cyclophosphamide (CP)-induced testicular and liver damage by

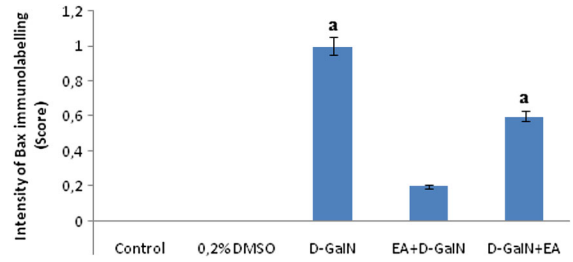

Fig. 5 Intensity of immunolabelling score of activated Bax positive cells in the groups. [a] $p < 0.05$ compared to control (Group 1)

preventing lipid peroxidation and reducing apoptosis through scavenging ROS (Turk et al. 2011). It was also reported that EA can regulate Cre and BUN concentrations, elevate the levels of glutathione (GSH), and reduce the necrotic lesions in renal tubular tissues (Atessahin et al. 2007). Plasma Cre and BUN levels were found to be increased more than four- and tenfold, respectively, following either CP treatment or CP combined with 10 mg/kg EA. However, Cre and BUN concentrations decreased by at least half (from 3.3 to 1.4 mg/dL and from 31 to 16 mg/dL, respectively) when 30 mg/kg EA was administered prior to and post CP treatment (Al-kharusi et al. 2013). Our study showed similar results to the above-mentioned studies, we have found that kidney damage induced by 750 mg/kg D-GalN, was decreased in groups that were given EA, there was also reduction in the serum levels of LDH, BUN, Cre. EA also decreased the number of activated Bax and caspase-3 kidney cells and also increased the number of Bcl-2 positive cells. Histopathological observations further confirm the membrane stabilizing effect of EA in D-GalN challenged rats. EA was found to be more effective in curing kidney damage in pretreated groups than the post-treated groups. In conclusion, kidney tissue necrosis induced by D-GalN was prevented by the treatment of EA. Thus, EA treatment resulted in significant renoprotection against D-GalN-induced kidney injury in rats.

Acknowledgments This work was supported by Anadolu University Scientific Research Project Unit (Project No: 1501F028, Ethical Committee No: 2014-22).

References

Al-Kharusi N, Babiker HA, Al-Salam S, Walys MI, Nemmar A, Allawati I, Yasin J, Beegam S, Ali BH (2013) Ellagic acid protects against cisplatin-induced nephrotoxicity in rats: a dose-dependent study. Eur Rev Med Pharmacol Sci 17:299–310

Amudha G, Josephine A, Varalakshmi P (2006) Role of lipoic acid in reducing the oxidative stress induced by cyclosporine A. Clin Chim Acta 372:134–139

Anand R, Harry D, Holt S, Milner P, Dashwood M, Goodier D, Jarmulowicz M, Moore K (2002) Endothelin is an important determinant of renal function in a rat model of acute liver and renal failure. Gut 50:111–117

Atessahin A, Karahan I, Turk G, Gur S, Yılmaz S, Ceribası AO (2006) Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Reprod Toxicol 21:42–47

Atessahin A, Ceribası AO, Yılmaz S (2007) Lycopene, a carotenoid, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rats. Basic Clin Pharmacol Toxicol 100:372–376

Catal T, Bolkent S (2008) Combination of selenium and three naturally occurring antioxidants administration protects D-galactosamine-induced liver injury in rats. Biol Trace Elem Res 122:127–136

EI-Mofty SK, Scrutton MC, Serroni A, Nicolini C, Farbar JL (1975) Early reversible plasma membrane injury in galactosamine induced liver cell death. Am J Pathol 79:579–595

Gezginci-Oktayoglu S, Tunali S, Yanardag R, Bolkent S (2008) Effects of Z-FA.FMK on D-galactosamine/tumor necrosis factor-alpha-induced kidney injury and oxidative stress in mice. Mol Cell Biochem 309:9–20

Ghosh M, Das J, Parames C (2012) D(+) galactosamine induced oxidative and nitrosative stress-mediated renal damage in rats via NF-κB and inducible nitric oxide synthase (iNOS) pathways is ameliorated by a polyphenol xanthone, mangiferin. Free Radic Res 46:116–132

Guarner F, Hughes RD, Gimson AE, Williams R (1987) Renal function in fulminant hepatic failure: haemodynamics and renal prostaglandins. Gut 28:1643–1647

Han KH, Hashimoto N, Shimada K, Sekikawa M, Noda T, Yamauchi H, Hashimoto M, Chiji H, Topping D, Fukushima M (2006) Hepatoprotective effect of purple potato extract against D galactosamine induced liver injury in rats. Biosci Biotechnol Biochem 70:1432–1437

Hassoun EA, Walter AC, Alsharif NZ, Stohs SJ (1997) Modulation of TCDD-induced fetotoxicity and oxidative stress in embriyonic and placental tissues of C57BL/6J mice by vitamin E succinate and ellagic acid. Toxicology 124:27–37

Javle P, Yates J, Kynaston HG (1998) Hepatoplanchnic hemodynamics and renal blood flow and function in rats with liver failure. Gut 43:272–279

Keppler D, Lesch R, Reutter W (1968) Experimental hepatitis induced by D-galactosamine. Exp Mol Pathol 9:279–290

Keppler D, Pausch J, Decker K (1974) Selective uridine triphosphate deficiency induced by D-galactosamine in liver and reversed by pyrimidine nucleotide precursors. Effect on ribonucleic acid synthesis. J Biol Chem 249:211–216

Khanduja KL, Gandhi RK, Pathania V, Syal N (1999) Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in mice. Food Chem Toxicol 37:313–318

Kmiec Z, Smolenski TR, Zych M, Mysliwski A (2000) The effects of galactosamine on UTP levels in the livers of young, adult and old rats. Acta Biochim Pol 47:349–353

Makin AJ, Hughes RD, Williams R, Mysliwski A (1997) Systemic and hepatic hemodynamic changes in acute liver injury. Am J Physiol 272:617–625

Mohamadin AM, El-Beshbishy HA, El-Mahdy MA (2005) Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. Pharmacol Res 51:51–57

Moore K, Taylor G, Ward P (1991) Aetiology and management of renal failure in acute liver failure. In: Williams R, Hughes RD (eds) Acute liver failure: improved understanding and better therapy. Miter Press, London, pp 47–53

Quintero A, Pedraza CA, Siendones E, El-Said AMK, Colell A, Ruiz C, Montero JL, De la Mata M, Fernández-Checa JC, Miño G, Muntané J (2002) PGE1 protection against apoptosis induced by D-galactosamine is not related to the modulation of intracellular free radical production in primary culture of rat hepatocytes. Free Radic Res 36:345–355

Rezzani R, Buffoli B, Rodella L, Stacchiotti A, Bianchi R (2005) Protective role of melatonin in cyclosporine A-induced oxidative stress in rat liver. Int Immunopharmacol 5:1397–1405

Rosillo MA, Sanchez-Hidalgo M, Cardeno A, Alarcon de la Lastra C (2011) Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. Biochem Pharmacol 82:737–776

Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D (2005) In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem 16:360–367

Shi Y, Sun J, He H, Guo H, Zhang S (2008) Hepatoprotective effects of Ganoderma lucidumpeptides against D-galactosamine-induced liver injury in mice. J Ethnopharmacol 117:415–419

Sinha M, Manna P, Parames C (2007) Amelioration of galactosamine-induced nephrotoxicity by a protein isolated from the leaves of the herb, Cajanus indicus L. BMC Complement Altern Med 7:11

Sire O, Mangenev M, Montagne J, Nordmann R, Nordmann J (1983) Carnitine palmitoyltransferase I. Inhibition by D-galactosamine and role of phospholipids. Eur J Biochem 136:371–375

Soong YY, Barlow PJ (2004) Antioxidant activity and phenolic content of selected fruit seeds. Food Chem 88:411–417

Tolstikov GA, Baltina LA, Grankina VP, Kondratenko RM, Tolstikova TG (2007) Biodiversity, chemistry and application in medicine. Academic Publishing House, 'Geo' Novosibirsk

Turk G, Atessahin A, Sönmez M, Ceribası AO, Yüce A (2008) Improvement of cisplatin-induced injuries to sperm quality, the oxidant–antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil Steril 89(5S):1474–1481

Turk G, Eribas I, Sakin AO, Sönmez F, Atessahin A (2010) Antiperoxidative and anti-apoptotic effects of lycopene and ellagic acid on cyclophosphamide induced testicular lipid peroxidation and apoptosis. Reprod Fertil Dev 22:587–596

Turk G, Ceribasi AO, Sahna E, Atessahin A (2011) Lycopene and ellagic acid prevent testicular apoptosis induced by cisplatin. Phytomedicine 18:356–361

Zhou Y, Park C-M, Cho C-W, Song Y-S (2008) Protective effect of pinitol against D-galactosamine-induced hepatotoxicity in rats fed on a high-fat diet. Biosci Biotechnol Biochem 72(7):1657–1666