

Full Length Research Paper

The effect of gallic acid on kidney and liver after experimental renal ischemia/reperfusion injury in the rats

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To investigate the effect of gallic acid (GA) on kidney and liver against oxidative stress during renal ischemia-reperfusion (I/R) injury, by determining biochemical parameters and evaluating histological examinations.

Key words: Gallic acid, ischemia-reperfusion, kidney, liver, antioxidant enzyme.

INTRODUCTION

Many urological surgeries such as partial nephrectomy, traumatic kidney repair, renal vessels repair, and renal transplantation use clamping of the renal arteries as a common procedure. Ischemia and reperfusion (I/R) induce oxidative stress and free radical formation. I/R injury is a complex pathophysiological process, which can eventually lead to cell damage, cell death, increased vascular permeability, tissue necrosis, and multi organ dysfunction (Salehipour et al., 2010). Restoration of blood flow to ischemic tissues can result in recovery of cells if the injury is not permanent. However, depending on the intensity and duration of the ischemia, the variable number of cells may still die after blood flow began (Yurdakul et al., 2010). Oxidative stress is a relative excess of oxidants caused by increased free radical production and/or decreased antioxidant defense systems that impairs cellular function and contributes to the pathophysiology of many diseases (Zhao, 2005). The protective effects of antioxidants on kidney tissue, including

renal I/R injury, have yielded a reduction of lipid peroxidation and positive effects on the antioxidant system status (Aktöz et al., 2007). Reactive oxygen species (ROS) produced in oxidative stress are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). It has been demonstrated in numerous studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues. Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation (LPO) (Serel et al., 2004; Ozguner et al., 2005). The most widely recognized properties of tea polyphenols are their antioxidative properties due to their ability to sequester metal ions and to scavenge reactive oxygen species. One of the polyphenols identified in tea is gallic acid (GA, 3, 4, 5-trihydroxybenzoic acid). Yang et al. (2001) reported that antioxidant activity of tea polyphenols including gallic acid has protection of carcinogenesis. GA is a polyhydroxyphenolic compound which is widely used in the traditional medicine, found in various natural products

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such as gallnuts, tea leaves, bark, green tea, apple-peels, grapes, strawberries, pineapples, bananas, lemons, and in red and white wine. It has been shown to be an antiallergic, antimutagenic, anti-inflammatory and anticarcinogenic agent and strong natural antioxidant. GA inhibits melanogenesis which may be related to its ability to scavenge reactive oxygen species (Jadon et al., 2007; Niho et al., 2001). Multiple organ dysfunctions caused by hepatic (Yoshidome et al., 1999) mesenteric (Aytekin et al., 2005) or lower limb I/R have been reported previously (Yassin et al., 2002). Wang et al. (2009) showed that renal I/R can cause hepatotoxicity in rats. Another study revealed in kidney transplantation model that, the liver was affected during I/R process as evidenced by antioxidant enzymes in pigs (Gulec et al., 2008). In this study, we aimed to investigate the effect of GA on kidney and liver against oxidative stress during I/R injury of the kidney.

MATERIALS and METHODS

Animals

The experimental protocols were approved by the institutional animal ethics committee. Twenty-eight adult male Sprague-Dawley rats weighting 250 to 280 g were obtained from Medical and Surgical Experimental Research Center (Eskisehir-Turkey) and housed in polycarbonate cages in a room with controlled temperature ($22\pm 2^\circ\text{C}$), humidity ($50\pm 5\%$), and a 12 h cycle of light and dark (07:00 AM to 07:00 PM). Rats were fed laboratory pellet chows and given water *ad libitum*. The animals were divided into four groups (7 animals each); Group I (Control; non-ischemic animals); Group II (renal I/R injury+Saline), Group III (renal I/R injury+ GA 50 mg per kg), Group IV (renal I/R injury+ GA 100 mg per kg).

Experimental protocol

Under anesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine), a right nephrectomy was performed and the rats were allowed to recover for 15 days before they were subjected to I/R injury. On the 15th day following nephrectomy, rats were fasted overnight. The renal pedicle was occluded for 45 min to induce ischemia and then subjected for 24 h of reperfusion (I/R groups). GA (Matheson Coleman and Bell Manufacturing Chemists, USA) was dissolved in saline as 2 cc/kg and was given as 50 and 100 mg/kg; I/R+ GA groups. GA or saline (I/R group) was administered intraperitoneally 15 min before ischemia and 12 h after reperfusion. The animals were decapitated after 24 h of reperfusion period. After induction of I/R injury, on left kidney and liver histopathological examinations were performed.

Histopathological evaluation

Left nephrectomy specimens and livers were processed routinely in 10% formalin solution, and embedded in paraffin. Tissue sections of 5 μm were obtained, and stained with hematoxylin and eosin (H&E). All histopathological examinations were performed under a light microscope (NIKON, Japan) by the histologist of the institute, who was blinded to all tissue specimens regarding their group. A minimum of 10 fields for each kidney and liver slides with minimum $\times 50$ magnification were examined to assign the severity of the

morphological changes; tubular structure, glomerulus, cortex and medulla were evaluated in renal histological examination. For the evaluation of tubular structure; epithelial desquamation and degeneration, loss of cilia, epithelial cell swelling, picnosis, vacuolization, cast accumulation in the lumen and focal necrotic areas were assessed. Glomerular congestion and necrosis were examined. Congestion, polymorphonuclear leukocytes (PMNL) and mononuclear leucocytes (MNL) infiltration were evaluated in cortex and medulla. In addition, fibrosis in cortex was examined.

In examination of hepatic damage; congestion in central vein, portal area and sinusoids, severity and location of hepatic bubbling degeneration, parenchymal focal necrotic areas, PMNL and MNL infiltration, karyolysis in hepatic nucleus, picnosis, loss of intercellular border, damage in hepatic cords were evaluated using scores.

Biochemical analysis

Homogenate preparation

After sacrificing the animals, kidneys and liver were quickly removed and perfused immediately with ice-cold normal saline, and homogenized in chilled potassium chloride (1.17%). The homogenate was centrifuged at $800 \times g$ for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at $10,500 \times g$ for 20 min at 4°C to get the homogenate which was used to assay MDA, CAT, and SOD activity.

Determination of lipid peroxidation

MDA production was an end product of lipid peroxidation reacts with thiobarbituric acid to form a red colored complex. 0.1 ml of homogenate, 3 ml of 1% phosphoric acid, 0.5 ml of distilled water and 1.0 ml of 0.6% 2-thiobarbituric acid were added. The mixture was boiled in water bath for 45 min, followed by cooling in an ice, and addition of 4.0 ml of n-butanol to extract the cold thiobarbituric acid reactants. The optical density of the n-butanol layer was determined at 532 nm after centrifugation at 1,000 g for five minutes and expressed as nmol MDA/g of wet tissue (Mihara and Uchiyama, 1978).

Assay of SOD activity

SOD activity was spectrophotometrically assayed with commercial kits (Fluka SOD kit USA). It is an indirect assay method based on xanthine oxidase and a novel color reagent. SOD activity in the homogenate was determined by inhibition of Formosan dye (450 nm) employing the xanthin-xanthin oxidase enzymatic method to generate superoxide radicals and calculated the active SOD concentration according to inhibition curve graphic expressed as U/g of wet tissue.

Assay of CAT activity

One unit (1U) of CAT equals the enzyme activity that recognized 1 μmol of hydrogen peroxide in 60 s at 37°C . CAT activity was measured with determination of absorbance of three blank samples at 405 nm. CAT activity (KU/L) was calculated as = $[(\text{Abs}_{\text{blank1}} - \text{Abs}_{\text{blanksample}}) / (\text{Abs}_{\text{blank 2}} - \text{Abs}_{\text{blank 3}})] \times 271$ (Goth, 1991).

Statistical analysis

All statistical analysis was performed with the computer program

“SPSS for Windows” (SPSS Inc; Release 11.5; Sep 6, 2002). All of the data were expressed as means \pm SD. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The significance was tested at $p > 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

In our study, MDA levels of group II in renal tissue were significantly higher than control group, group III and IV. GA administration had significantly decreased MDA levels in groups III and IV when compared to group II. But, there was no significant difference between the administered drug doses (groups III and IV). The SOD levels significantly increased in group II when compared with control group. But there was no significant difference in groups III and IV when compared with control group. This may reflect that, different doses of GA may not be beneficial in decreasing oxidative injury. The CAT levels were increased significantly in group II when compared with control group, but after GA administration CAT levels decreased and there were no difference between groups III and IV. Also these results suggested that GA may be effective in preventing oxidative injury. The enzymatic activity changes in kidney were demonstrated in Table 1a. Similarly, in liver MDA levels of group II were significantly higher than control group, groups III and IV. GA administration had significantly decreased MDA levels in groups III and IV when compared to group II. When we compared the SOD levels it significantly decreased in group II when compared with control group. SOD levels in groups III and IV decreased significantly when compared with control group. There was a significant difference between groups III and IV. The CAT levels were increased significantly in group II when compared with control group, but after GA administration CAT levels increased and in groups III and IV. Also these results suggested that the other organs such as liver may be affected in renal ischemia/reperfusion injury. GA may be effective in preventing oxidative injury for other organs and tissues. The enzymatic activity changes in liver were demonstrated in Table 1b.

Histopathologic findings of kidney

Group I, regular morphology of kidney was observed. Group II showed severe damage in all tubular structures. Epithelial desquamation and degeneration, loss of cilia, epithelial cell swelling, and picnosis were observed in very severe degree. Vacuolization was scored as mild. Cast accumulation in lumen was scored as severe, but focal necrotic areas were not seen. When glomerular structure was examined; there was not any necrosis. But there was mild congestion. In cortex, there was not any fibrosis. In cortex and medulla, congestion and MNL infiltration were scored as severe although PMNL

infiltration was not seen. Tubular vacuolization was mild in group III. Epithelial desquamation and degeneration, loss of cilia were scored as severe, while epithelial cell swelling, picnosis and cast accumulation in lumen scored as mild. But focal necrotic areas were not observed. There was not any congestion and necrosis in glomerular examination. In cortex, there was not any fibrosis. In cortex and medulla, congestion and MNL infiltration were scored as severe although PMNL infiltration was not seen. Group IV: When tubular structure was examined, epithelial desquamation and degeneration, loss of cilia and cast accumulation in lumen were observed as severe degree, swelling and picnosis as moderate, vacuolization as mild. There were not any focal necrotic areas. Glomerular congestion was in mild degree. Necrosis was not observed. There was not any cortical fibrosis, PMNL infiltration in cortex. But congestion and MNL infiltration were observed in moderate degree. In medulla, congestion was scored as severe, MNL infiltration as moderate. PMNL infiltration was not observed. Histopathologic findings of liver and kidney are presented in Table 2.

DISCUSSION

Acute renal ischemia and oxidative stress contribute to the pathophysiology of renal damage. Previous research showed that a large amount of free radicals are generated immediately after the onset of reperfusion (Bi et al., 2009). It is generally believed that reactive oxygen and nitrogen species (ROS, RNS) related to oxidative stress are key mediators of I/R induced damage to the kidney. Restoration of blood flow to ischemic tissues can result in recovery of cells if the injury is not permanent. I/R injury damages depend on the intensity and duration of ischemia (Yurdakul et al., 2010).

In our study, GA treatments in renal ischemia-reperfusion decreased MDA in kidney and liver. But SOD activity was decreased with low and high doses of GA treatment in kidney while it was increased in liver with low dose GA treatment. On the other hand, high dose GA showed decreased SOD level in liver. GA administration decreased CAT levels in kidney and there were no difference between GA doses. But CAT was increased in liver. These results suggested that different doses of GA may not be beneficial in decreasing oxidative injury. There are a lot of studies examining the antioxidant effects of different substances (such as beta carotene and *Nigella sativa*) in I/R injury in renal tissue. Two of them revealed an increase in CAT levels compared to I/R (Hosseini et al., 2010; Yildiz et al., 2010). Yurdakul et al. (2010) showed that the treatment with erdosteine and α -tocopherol as an antioxidant in renal I/R significantly decreased level of MDA and increased SOD activity. Bi et al. (2009) also showed that renal I/R in rats are attenuated by a synthetic glycine derivative and decrease

Table 1a. Histopathological findings in the kidney.

Group	Tubular structures						Glomerulus			Cortex			Medulla			
	Epithelial desquamation and degeneration	Loss of brush border	Epithelial cell swelling	Epithelial cell picnosis	Epithelial cell vacuolization	Cast cumulation in the lumen	Focal necrotic areas	Congestion	Necrosis	Congestion	MNL infiltr.	PMNL infiltr.	Fibrosis	Congestion	MNL infiltr	PMNL infiltr
Group II	+++	+++	+++	+++	+	+++	-	+	-	+++	+++	-	-	+++	+++	-
Group III	+++	+++	++	++	+	++	-	-	-	+++	+++	-	-	+++	+++	-
Group IV	+++	+++	++	++	+	+++	-	+	-	++	++	-	-	+++	++	-
Interpretation	n.s	n.s	Decrease	Decrease	n.s	n.s	-	n.s	-	Decrease	Decrease	-	-	n.s	Decrease	-

- : none, ++: moderate, n.s: not significant, +: mild, +++: severe.

Table 1b. Histopathological findings in the liver.

Group	Congestion in			Hepatic bubbling degeneration	Focal necrosis	PMNL infiltration in			MNL infiltration in			Karyolysis and picnosis	Loss of intercellular border	Damage in hepatic cords
	Central vein	Portal area	Sinusoid			Central vein	Portal area	Sinusoid	Central vein	Portal area	Sinusoid			
Group II	++	+++	++	-	-	-	+	+	+	+++	++	++	++	++
Group III	+	++	++	MZ,Z2 ++	-	-	+	-	+	++	++	+	+	+
Group IV	+	++	++	MZ,Z2 +	-	-	+	+	+	+	+	+	+	+
Interpretation	Decrease	Decrease	n.s	Decrease	-	-	n.s	n.s	n.s	Decrease	Decrease	Decrease	Decrease	Decrease

-: none, ++: moderate, Z2: Midzonal area (Zone-2), n.s: no difference, +: mild, +++: severe, MZ: Microvesicular bubbling degeneration.

in MDA levels administration after I/R injury. MDA levels in our study were similar to Yurdakul et al. (2010) and Bi et al. (2009) studies. GA being an antioxidant reduces the stress to a considerable extent thereby reducing the demand of excess sugar and thus recoups the glycogen content in kidney and liver (Jadon et al., 2007).

Hepatic and renal cells participate in a variety of metabolic activities and contain a host of enzymes (Jadon et al., 2007). Niho et al. (2001) investigated the toxicity of GA in different doses on liver and kidney. They revealed some weak pathological changes. Li et al. (2005) studied

antioxidant activities of GA in senescence accelerated mice and they observed increase in CAT levels compared to controls similar to our study. Liao et al. (2010) mentioned that augments liver regeneration (ALR), which is expressed in both the liver and kidney, and recently, was also found to be an important intracellular survival factor for hepatocytes, (Thirunavukkarasu et al., 2008) effectively reduces tubular injury and ameliorates the impairment of renal function. The protective effect of ALR is associated with enhancement of renal tubular cell regeneration (Liao et al., 2010). Ozer

et al. (2009) observed severe vascular congestion, degeneration of the tubular epithelium, glomerular and Bowman's space structures, and severe inflammatory cell infiltration in the IR group. These degenerations were clearly improved when the animals were treated by Pycnogenol. Rahman et al. (2009) indicated histopathologically lower damage in Manganese Superoxide Dismutase (MnSOD) administered group compared with the I/R group. The studies examine the effects of different antioxidants on I/R injury revealed different results for enzymatic activities and severe histopathological findings. In

Table 2. The MDA levels, SOD and CAT enzyme activities in kidney and liver.

Group	SOD kidney	SOD liver	MDA kidney	MDA liver	CAT kidney	CAT liver
Group I (G1)	55.68±6.48	54.12±5.01	1.79±0.19	2.52±0.24	308.30±62.81	149.13±41.56
Group II (G2)	67.53±5.77	38.71±1.50	3.18±0.31	7.3139±0.76	755.55±336.14	297.18±168.83
Group III (G3)	53.83±2.17	43.28±9.62	1.88±0.31	5.7627±1.21	602.85±380.68	461.72±108.43
Group IV (G4)	52.86±6.77	21.58±4.80	1.86±0.35	5.3560±1.28	715.93±63.23	709.63±174.84
<i>p</i> -values and multiple comparison of the groups	0.001	0.001	0.000	0.000	0.017	0.000
	0.902	0.023	0.885	0.000	0.170	0.000
	0.756	0.000	0.915	0.000	0.032	0.000
	0.000	0.492	0.000	0.031	0.688	0.031
	0.000	0.000	0.000	0.005	0.991	0.005
	0.990	0.000	0.936	0.863	0.844	0.863

The significance was tested at n.s $p > 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$. n.s: not significant.

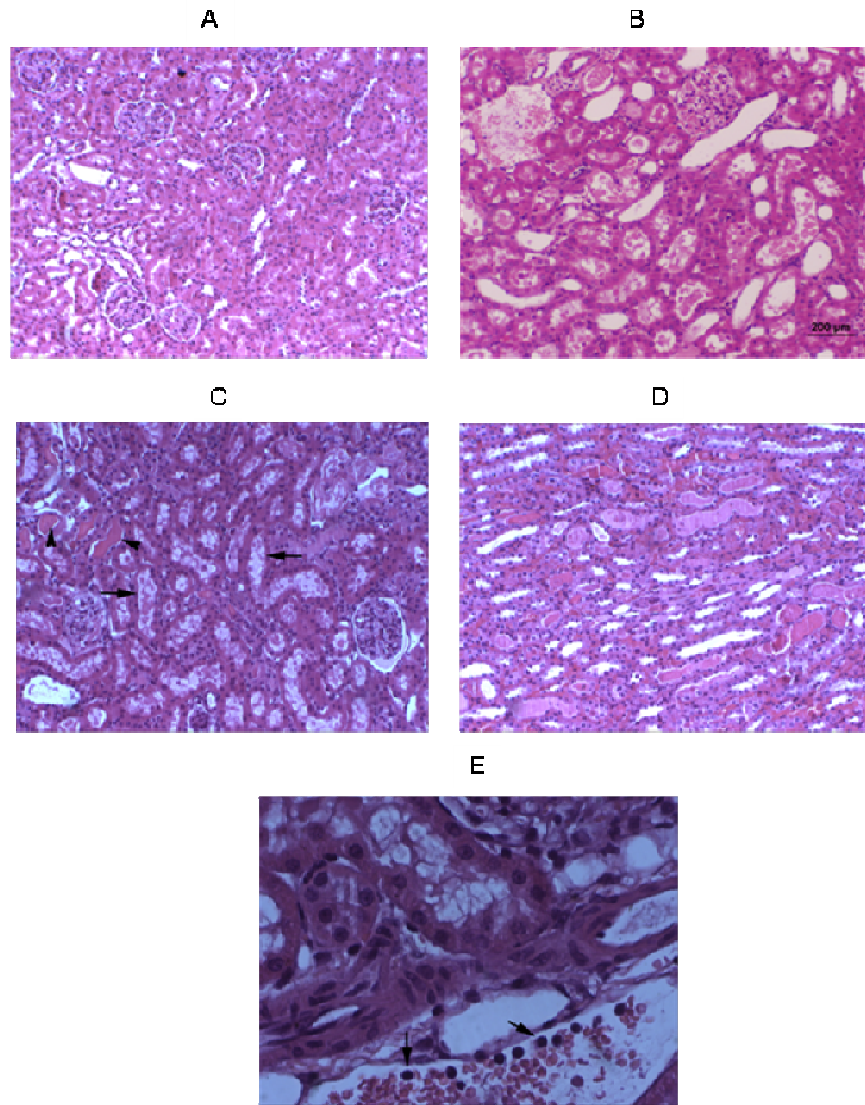


Figure 1. Light micrographs of kidney sections. (A) Kidney of an untreated control. (B) Degeneration in tubular structures, (C) epithelial desquamation and degeneration, (D) congestion, (E) leukocyte infiltration in kidney. Scale bar 200 and 100 μm .

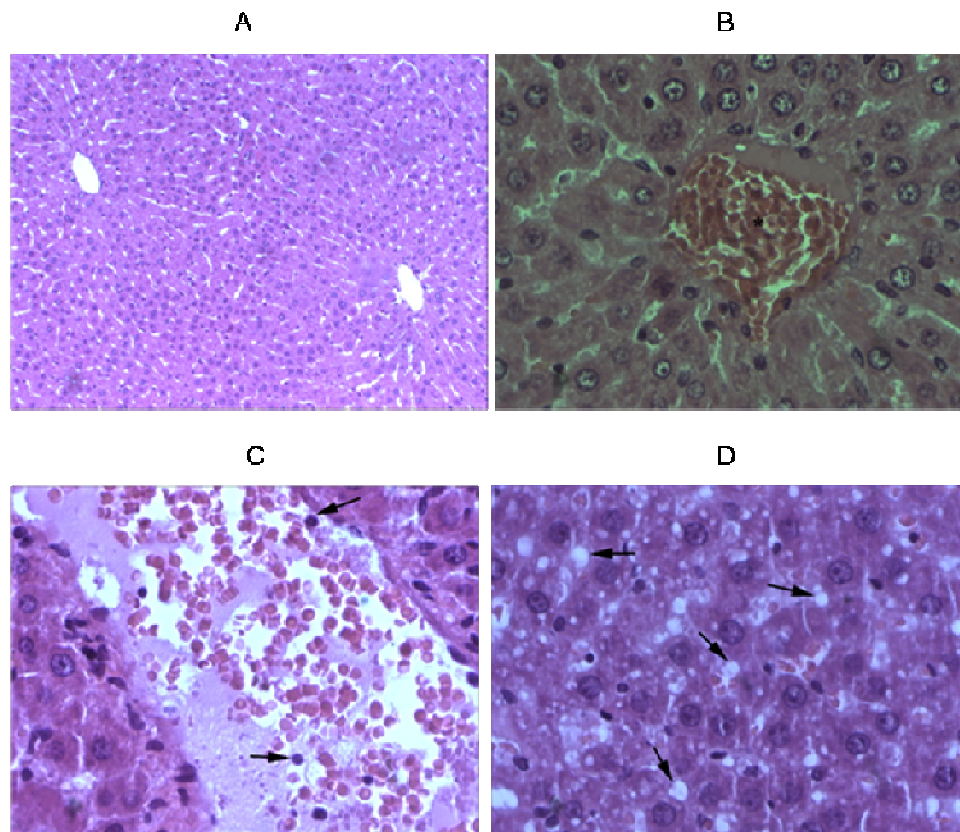


Figure 2. Light micrographs of liver sections. (A) Liver of an untreated control. (B) Congestion in central vein, portal area and sinusoids, damage in hepatic cords, (C) PMNL and MNL infiltration in sinusoids, (D) midzonal (Zone-2) microvesicular hepatic bubbling degeneration in liver. Scale bar 100 and 50 μm .

addition, antioxidants improved most of the damages.

Conclusions

The results of this study have demonstrated that GA may have protective effect on renal I/R injury-induced renal tubular and hepatic damages in the rat. GA in 50 mg/kg was observed to be enough to significantly prevent renal injury. According to these findings, it is necessary to make more experimental studies for the evaluation of GA effects.

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