

Effect of gallic acid on liver oxidative stress markers in renal ischemia-reperfusion injury in rats

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Abstract

Introduction: Renal ischemia-reperfusion (RIR) is one of the reasons of acute renal failure (ARF). Furthermore, RIR induces oxidative stress in the liver.

Objectives: Considering the role of gallic acid (GA) as an antioxidant, we used GA to investigate its effect on liver oxidative stress markers in RIR injury in rats.

Materials and Methods: Twenty-two adult male Sprague-Dawley rats were randomly divided into 3 groups; group I (control; non-ischemic animals, n = 8), group II (renal I/R injury, n = 7) and group III (renal I/R injury + GA 100 mg/kg i.p daily, n = 7). Pedicles were occluded for 45 minutes (ischemia) and subjected to 24 hours of reperfusion. Daily pretreatment began 15 days before the induction of RIR. Hepatic malondialdehyde (MDA) and glutathione (GSH) were determined, and catalase (CAT) and glutathione peroxidase (GPX) activities in the liver were assessed.

Results: The level of MDA in the liver significantly decreased in treated RIR rats compared with the untreated group. The level of GSH in the liver increased in treated RIR rats compared with the untreated group. CAT and GPX activities in the liver were significantly less in the untreated group than the control one. GA significantly increased CAT and GPX activities in the liver of the treated group.

Conclusion: Our study showed that the pretreatment with GA as an antioxidant agent has protective effects on liver oxidative stress markers in RIR injury in rats.

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Introduction

Renal ischemia-reperfusion (RIR) is one of the reasons of acute renal failure (ARF) (1). It has been demonstrated that renal ischemia is a major incident that usually happens due to renal transplantation, heminephrectomy, repair of suprarenal aneurysm, cardiac arrest, hemorrhagic shock and heart failure (1,2). Ischemia reperfusion injury (IRI) is a pathophysiological process that induces cell damage, cell death, tissue necrosis and multi-organ dysfunction. I/R also causes oxidative stress (3). The main factors which cause kidney damage during I/R are the production of reactive oxygen (ROS) or nitrogen species (RNS) (4). These free radicals have cytotoxic effects such as protein oxidation, lipid peroxidation (LPO), and DNA damage and apoptosis creation (5). Malondialdehyde (MDA) is produced by the oxidation of polyunsaturated fatty acids and provides a good marker of oxidative stress-

Core tip

Our study shows GA reduced the level of MDA and increased level of GSH and CAT and GPX activity in renal ischemia-reperfusion treated group. The authors hope, the results of the present study help to improve liver function in patients with renal injuries.

mediated LPO (6).

The endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) are involved in the defense against free radicals and oxidative stress. In the pathogenesis of many disorders such as renal I/R injury, an imbalance between oxidant and antioxidant system can be observed (5). Hence, using exogenous antioxidants for the treatment of such diseases has attracted special attention (7).

Gallic acid (GA) is also called 3,4,5-trihydroxybenzoic acid. We can find it

in gallnuts, sumac, witch hazel, oak bark, green tea, pineapple, grapes and other plants (8). Tea is the major source of GA (8). GA is known as a strong antioxidant and has antimutagenic and anticarcinogenic activities (8,9). Regarding the biochemical structure of GA, it has three hydroxyl groups bonded to the aromatic ring in the an ortho position, which shows the strongest free radical, scavenging activity (9). This property is good for the antioxidant activity of phenolic acids (8,9).

Renal ischemia-reperfusion (RIR) may cause the insufficiency of other organs such as the lung, brain and liver (10,11). RIR makes hepatotoxicity in rats (10) and induces oxidative stress in the liver, which causes its dysfunction (10,11). Previous studies have shown that GA has a protective effect on RIR injury and hepatic damage (10,11).

Objectives

Considering the role of RIR in the induction of liver oxidative stress, we used GA as an antioxidant agent to investigate its effect on liver oxidative stress markers in RIR injury in rats. So far, no detailed research has been carried out on the effect of GA on liver oxidative stress markers in RIR injury in rats.

Materials and Methods

Twenty-two adult male Sprague-Dawley rats (weighting 180-200 g) were taken from Pasteur Institute of Iran. They were housed in a room with the controlled temperature of 22°C and humidity of 50 ± 10% with the 12 hours light/dark cycle in the animal lab of Razi Herbal Medicines Research Center. These animals were fed on laboratory pellet chows and given water *ad libitum*. They were randomly divided into 3 groups; group I (control; nonischemic animals); group II (renal I/R injury) and group III (renal I/R injury + GA 100 mg/kg i.p daily). Daily pretreatment began 15 days before the induction of RIR and GA was administrated intraperitoneally to the rats.

Surgery method

The rats were anesthetized intraperitoneally by the injection of chloral hydrate (400 mg/kg). Then, their abdominal regions were shaved and sterilized with the povidone-iodine solution. To perform ischemia, the abdominal cavity was opened by a midline incision and the renal pedicles were carefully isolated. The right and left pedicles were occluded by using no traumatic vascular clamps for 45 minutes. Occlusion of renal artery was confirmed by observing color change in the entire kidney surface and some increase in the kidney size. After removing the renal clamps, the kidneys were observed for 5 minutes to ensure the return of the renal blood circulation. Then, 1 mL of sterile saline (37°C) was injected intraperitoneally and the incision was closed in 2 layers with a 4-0 silk suture. During 45 minutes of ischemia, both intestines and kidneys were covered with a humid, hot and sterilized gauze (12). At the end

of the reperfusion period, the animals were euthanized with Nesdonal (50 mg/kg, i.p). The livers were removed immediately and used fresh or kept frozen for further analyses.

Biochemical analysis

Determination of LPO

The liver content of MDA, as a product of LPO, was measured by the thiobarbituric acid (TBA) assay. The liver content of MDA was also analyzed using a Shimadzu spectrophotometer (Tokyo, Japan) (13).

Assay of CAT activity

CAT activity was estimated using the method of Sinha. The reaction was started by the addition of 20 µL of the sample to 2 mL of 30 mmol/L hydrogen peroxide (H₂O₂) in 50 mmol/L potassium phosphate buffer with pH 7.0. Enzyme units were expressed as mmol/L of the consumed H₂O₂ per mg-protein (14).

Assay of GPX activity

GPX activity was measured according to our previous study (14).

Assay of GSH

The liver content of GSH was assayed spectrophotometrically at 412 nm according to the method of Ellman using a Shimadzu spectrophotometer (Tokyo, Japan). The content of GSH was expressed in nmol/mg protein (14).

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The research was approved by ethical committee of Lorestan University of Medical Sciences. Prior to the experiment, the protocols were confirmed to be in accordance with the Guidelines of Animal Ethics Committee of Lorestan University of Medical Sciences. We also followed the guidelines of National Health and Medical Research Council.

Statistical analysis

All the values were expressed as mean ± standard deviation (SD). The data were compared between the groups by one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical analyses were done in SPSS 13 for Windows. *P* values < 0.05 were considered statistically significant.

Results

Effect of GA on the liver MDA level of RIR rats

The level of MDA in the liver is demonstrated in Figure 1. The level of liver MDA significantly increased in the untreated compared with control groups (111.71 ± 7.02 vs. 47.21 ± 9.64 nmol/mg protein). The level of the liver MDA significantly decreased in the treated group and was close to the liver MDA level in the control group (64.83 ± 7.51 vs. 47.21 ± 9.64 nmol/mg protein). These numbers

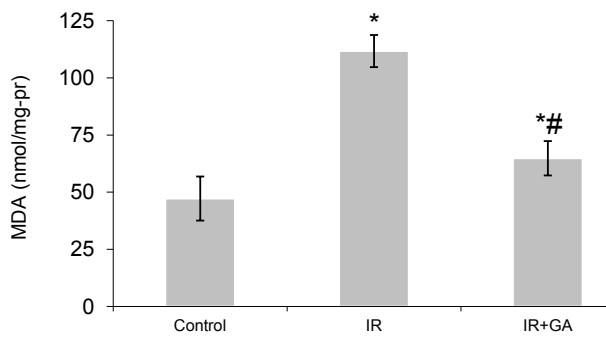


Figure 1. Effect of GA on MDA level in liver homogenate in control, untreated and treated RIR rats.

Values represented as mean \pm SD. *Indicates significance of data comparing with control group ($P < 0.001$). # Indicates significance of data comparing with IR group ($P < 0.001$)

showed that the pretreatment of GA in the RIR rats could inhibit the increase of MDA in comparison with the untreated animals.

Effect of GA on the liver GSH level of RIR rats:

The level of GSH in the liver is demonstrated in Figure 2. The level of the liver GSH significantly decreased in the untreated compared with control groups (16.96 ± 2.22 vs. 37.06 ± 7.21 nmol/mg protein). The level of the liver GSH significantly increased in the treated group and was close to the liver GSH level in the control group (27.94 ± 10.11 vs. 37.06 ± 7.21 nmol/mg protein). These numbers showed that the pretreatment of GA in the RIR rats could inhibit the decrease of GSH in comparison with the untreated animals.

Effect of GA on liver CAT activity in RIR rats

The liver CAT activity is demonstrated in Figure 3. The liver CAT activity in the untreated group was significantly less than the control group (11.54 ± 4.02 vs. 54.73 ± 15.97 U/mg protein). The liver CAT activity in the treated group was significantly more than that in the untreated group (29.18 ± 13.56 vs. 11.54 ± 4.02 U/mg protein). The CAT activity of the liver of the treated group was less than that of the control group, while it was more than that of the untreated group.

Effect of GA on liver GPX activity in RIR rats

The liver GPX activity is demonstrated in Figure 4. The liver GPX activity in the untreated group was significantly less than that of the control group (85.35 ± 7.51 vs. 108.23 ± 12.35 U/mg protein). The liver GPX activity in the treated group was significantly more than that of the untreated group (145.73 ± 16.59 vs. 85.35 ± 7.51 U/mg protein). The GPX activity in the liver of the treated group was more than that of the control group.

Discussion

The results of this study showed that GA could reduce the liver LPO and increase GSH in RIR rats. It could also increase the reduced liver CAT and GPX in treated animals. It has been proved that oxidative stress

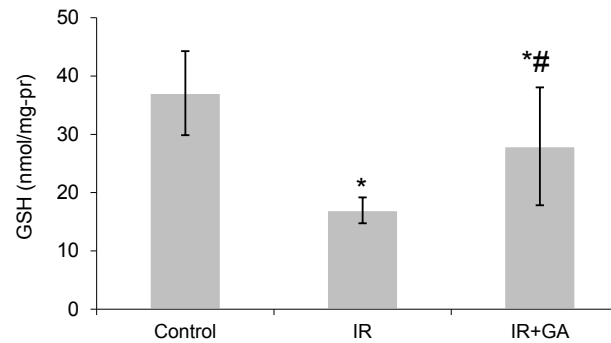


Figure 2. Effect of GA on GSH level in liver homogenate in control, untreated and treated RIR rats.

Values represented as mean \pm SD. *Indicates significance of data comparing with control group ($P < 0.001$). # Indicates significance of data comparing with IR group ($P < 0.001$)

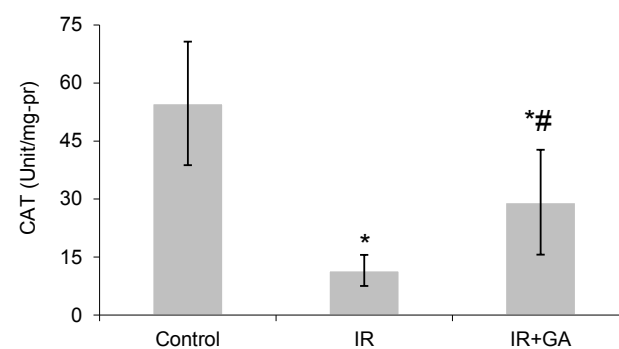


Figure 3. Effect of GA on CAT activity in liver homogenate in control, untreated and treated RIR rats.

Values represented as mean \pm SD. *Indicates significance of data comparing with control group ($P < 0.001$). # Indicates significance of data comparing with IR group ($P < 0.001$)

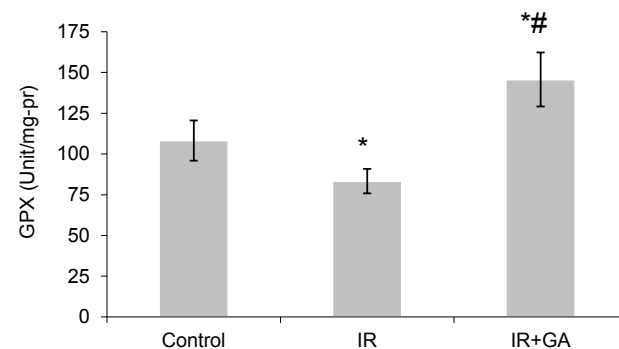


Figure 4. Effect of GA on GPX activity in liver homogenate in control, untreated and treated RIR rats.

Values represented as mean \pm SD. *Indicates significance of data comparing with control group ($P < 0.001$). # Indicates significance of data comparing with IR group ($P < 0.001$)

contributes to the pathogenesis of many disorders such as renal I/R injury (5). It also happens in the liver during renal I/R (11). Free radicals cause LPO, DNA damage and apoptosis creation (5) and have a major role in the development of many diseases including atherosclerosis, inflammatory injury, neurodegenerative diseases, cancer and accelerated ageing of organisms (5). It has also been

reported that ROS and RNS are related to renal injury during I/R (4). A previous study showed that GA as an antioxidant agent had protective effects on the liver and kidney LPO, GSH contents, GPX, SOD, GST and CAT activities in lindane-induced toxicity in experimental rats (15). Another study demonstrated that GA had beneficial effects on LPO, total thiol and GPX contents in oxidative stress induced by 6-hydroxydopamine in rats (16). Antioxidants such as tannic acid, GA, ellagic acid and propyl gallate can protect cells from oxidative stress (17). Thus, using antioxidants is useful for the treatment of diseases caused by oxidative stress. In this study, the liver LPO was significantly increased in RIR animals compared with the control group. Pretreatment of I/R group with GA significantly inhibited the elevation of MDA compared with the untreated group. MDA is a good marker of LPO caused by oxidative stress (6). The liver GSH level was significantly decreased in the untreated animals compared with the control group, while GA pretreatment significantly increased GSH level in the treated compared with untreated groups. GPX and CAT activities were significantly less in the untreated rats than the control group. The antioxidant enzymes GPX and CAT are indicators of antioxidant status (18). Results of this study have been confirmed by similar studies on different antioxidants (19).

A previous study demonstrated that GA improved antioxidant defenses against toxicity caused by lead in the blood, liver and kidney of rats (20). GA reduced LPO and ameliorated oxidative stress in the liver from hepatic ischemia and reperfusion rats (21). Furthermore another research has also shown that both GA and tannic acid decrease LPO and increase the biomarkers of oxidative stress including GSH, SOD, CAT and GPX in cisplatin-induced nephrotoxicity in rats (22). Results of our study corresponded with other research showing that GA can reduce LPO and increase GSH level, GPX and CAT activities. Hence, using GA as a natural antioxidant, has protective effects on LPO, GSH level, GPX and CAT activities, and might be helpful in inhibiting the bad effects of RIR on other organs such as the liver. Moreover, another research has shown the protective effect of free radical scavengers and antioxidants on IRI (18,22). Thus, antioxidant therapy is an important strategy for reducing the bad effects of renal IRI. However, the detailed protective mechanisms of GA against the liver oxidative stress-induced RIR cannot be fully explained by our results. It has been suggested that antioxidant preservation applied by GA is directly related to its direct function as a free radical scavenger (23). Hence, GA as an effective antioxidant could be suggested as a therapeutic agent for decreasing complications of RIR such as liver injury.

Conclusion

Our results showed that GA had a good antioxidant protection and also beneficial effects on decreasing elevated liver LPO and increasing reduced liver GSH

level and antioxidant enzymes in RIR rats. Therefore, the reduction of LPO and elevation of antioxidant enzyme activities can inhibit the bad effects of RIR on other organs such as the liver.

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Authors' contribution

All authors wrote the manuscript equally.

Conflicts of interest

The authors declared no competing interests.

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References

- Hu L, Yang C, Zhao T, Xu M, Tang Q, Yang B, et al. Erythropoietin ameliorates renal ischemia and reperfusion injury via inhibiting tubulointerstitial inflammation. *J Surg Res.* 2012;176:260-6.
- Dorai T, Fishman AI, Ding C, Batinic-Haberle I, Goldfarb DS, Grasso M. Amelioration of renal ischemia-reperfusion injury with a novel protective cocktail. *J Urol.* 2011;186:2448-54.
- Salehipour M, Monabbati A, Salehi H, Nikeghbalian S, Bahador A, Marvasti VE, et al. Protective effect of parenteral vitamin E on ischemia-reperfusion injury of rabbit kidney. *Urology.* 2010;75:858-61.
- Devarajan P. Update on mechanisms of ischemic acute kidney injury. *J Am Soc Nephrol.* 2006;17:1503-20.
- Vajdovich P. Free radicals and antioxidants in inflammatory processes and ischemia-reperfusion injury. *Vet Clin North Am Small Anim Pract.* 2008;38:31-123.
- Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R, et al. Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester (CAPE) on mobile phone-induced renal impairment in rat. *Mol Cell Biochem.* 2005;276:31-7.
- Murphy MP. Antioxidants as therapies: can we improve on nature? *Free Radic Biol Med.* 2014;66:20-3.
- Yoon CH, Chung SJ, Lee SW, Park YB, Lee SK, Park MC. Gallic acid, a natural polyphenolic acid, induces apoptosis and inhibits proinflammatory gene expressions in rheumatoid arthritis fibroblast-like synoviocytes. *Joint Bone Spine.* 2013;80:274-9.
- Yigitturk G, Acara AC, Erbas O, Oltulu F, Yavasoglu NU, Uysal A, et al. The antioxidant role of agomelatine and gallic acid on oxidative stress in STZ induced type I diabetic rat testes. *Biomed Pharmacother.* 2017;87:240-246.
- Golab F, Kadkhodae M, Zahmatkesh M, Hedayati M, Arab H, Schuster R, et al. Ischemic and non-ischemic acute kidney injury cause hepatic damage. *Kidney Int.* 2009;75:783-92.
- Kadkhodae M, Golab F, Zahmatkesh M, Ghaznavi R, Hedayati M, Arab HA, et al. Effects of different periods of renal ischemia on liver as a remote organ. *World J Gastroenterol.* 2009;15:1113-8.
- Rajan D, Wu R, Shah KG, Jacob A, Coppa GF, Wang P. Human ghrelin protects animals from renal ischemia-reperfusion injury through the vagus nerve. *Surgery.* 2012;151:37-47.
- Ahmadvand H, Tavafi M, Khosrowbeygi A. Amelioration of altered antioxidant enzymes activity and glomerulosclerosis by coenzyme Q10 in alloxan-induced diabetic rats. *J Diabetes Complications.* 2012;26:476-82.
- Ahmadvand H, Ghasemi Dehnoo M, Cheraghi R, Rasouljan B, Ezatpour B, Azadpour M, et al. Amelioration of altered serum,

- liver, and kidney antioxidant enzymes activities by sodium selenite in alloxan-induced diabetic rats. *Rep Biochem Mol Biol.* 2014;3:14-20.
15. Vijaya Padma V, Sowmya P, Arun Felix T, Baskaran R, Poornima P. Protective effect of gallic acid against lindane induced toxicity in experimental rats. *Food Chem Toxicol.* 2011;49:991-8.
 16. Mansouri MT, Farbood Y, Sameri MJ, Sarkaki A, Naghizadeh B, Rafeirad M. Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. *Food Chem.* 2013;138:1028-33.
 17. Chen CH, Liu TZ, Chen CH, Wong CH, Chen CH, Lu FJ, et al. The efficacy of protective effects of tannic acid, gallic acid, ellagic acid, and propyl gallate against hydrogen peroxide-induced oxidative stress and DNA damages in IMR-90 cells. *Mol Nutr Food Res.* 2007;51:962-8.
 18. Flores-Mateo G, Carrillo-Santistevan P, Elosua R, Guallar E, Marrugat J, Bleyers J, et al. Antioxidant enzyme activity and coronary heart disease: meta-analyses of observational studies. *Am J Epidemiol.* 2009;170:135-47.
 19. Khastar H. Protective effects of vitamin E against liver damage caused by renal ischemia reperfusion. *Ren Fail.* 2015;37:494-6.
 20. Reckziegel P, Dias VT, Benvegnú DM, Bouffleur N, Barcelos RC, Segat HJ, et al. Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. *Toxicol Rep.* 2016;3:351-6.
 21. Bayramoglu G, Kurt H, Bayramoglu A, Gunes HV, Degirmenci i, Colak S. Preventive role of gallic acid on hepatic ischemia and reperfusion injury in rats. *Cytotechnology.* 2015;67:845-9.
 22. Akomolafe SF, Akinyemi AJ, Anadozie SO. Phenolic Acids (gallic and tannic acids) modulate antioxidant status and cisplatin induced nephrotoxicity in rats. *Int Sch Res Notices.* 2014;2014:984709.
 23. Sohi KK, Mittal N, Hundal MK, Khanduja KL. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: A Bcl-2 independent mechanism. *J Nutr Sci Vitaminol (Tokyo).* 2003;49:221-7.