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Elucidating the Molecular Mechanism by Which Gallic Acid Alleviates Oxidative Stress and Inflammatory Response of Acrylamide-Induced Renal Injury in a Rat Model

Bir Rat Modelinde Gallik Asidin Oksidatif Stresi ve Akrilamid Kaynaklı Böbrek Hasarının İnflamatuar Yanıtını Hafiflettiği Moleküler Mekanizmanın Aydınlatılması

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ABSTRACT

This study investigates the molecular effects of Acrylamide (ACR)-induced kidney damage and the potential protective role of Gallic acid (GA). Forty male rats were divided into five groups: Control, ACR, ACR+GA50, ACR+GA100, and GA100. The ACR groups received a daily oral dose of 50 mg/kg, while GA groups received 50 or 100 mg/kg oral doses for 14 consecutive days. On the 15th day, the animals were euthanized, and kidney samples were collected. The MDA, GSH, SOD, GPx, and CAT oxidative stress parameters were measured. The renal inflammatory response was evaluated by measuring the level of TNF-α, IL-1β, IL-6, NF-κB, COX-2, and IL-10. The downstream pro-apoptotic signaling pathway was resolved by measuring the levels of p38 MAPK and p53. The ACR induced renal oxidative stress with aggravated lipid peroxidation as revealed by the reduction in the levels GSH, SOD, GPx, and CAT of antioxidants while over-increase in the level of MDA, respectively. The levels of IL-1β, IL-6, NF-kB, COX-2 pro-inflammatory mediators as well as the p38 MAPK and p53 pro-apoptotic intermediates were further elevated. This increase in inflammatory response was met with marked decrease in anti-inflammatory IL-10 level. However, GA treatments- in dose dependent manner- had been demonstrated to effectively mitigate oxidative stress and reduce inflammatory responses, while also enhancing the cellular anti-inflammatory defense mechanisms. The GA can be considered as a novel protective antioxidant, anti-apoptotic drug against ACR-induced nephrotoxic insult. Further study should be performed to estimate the exact effective dose.

Keywords: Acrylamide, apoptosis, gallic acid, inflammation, nephrotoxicity

ÖZ

Bu çalışma, Akrilamid (ACR) kaynaklı böbrek hasarının moleküler etkilerini ve Galik asidin (GA) potansiyel koruyucu rolünü araştırmaktadır. Kırk erkek sıçan, Kontrol, ACR, ACR+GA50, ACR+GA100 ve GA100 olmak üzere beş gruba ayrılmıştır. ACR grupları günlük oral 50 mg/kg dozda alırken, GA grupları ise 14 ardışık gün boyunca 50 veya 100 mg/kg oral dozda almıştır. 15. gününde hayvanlar uyuşturularak böbrek örnekleri alınmıştır. MDA, GSH, SOD, GPx ve CAT oksidatif stres parametreleri ölçülmüştür. Böbrek iltihabi yanıtı, TNF-α, IL-1β, IL-6, NF-κB, COX-2 ve IL-10 seviyeleri ölçülerek değerlendirilmiştir. Aşağı akım pro-apoptotik sinyal yolakları, p38 MAPK ve p53 seviyeleri ölçülerek çözümlenmiştir. ACR, antioksidanların GSH, SOD, GPx ve CAT seviyelerinde azalma ve MDA seviyesinde artış ile belirginleşen lipid peroksidasyonunu şiddetlendirerek renal oksidatif stresi tetiklemiştir. IL-1β, IL-6, NF-kB, COX-2 pro-iltihabi mediatörler ve ayrıca p38 MAPK ve p53 pro-apoptotik ara maddelerin seviyeleri artmıştır. Bu artan iltihabi yanıt, anti-iltihabi IL-10 seviyesinde belirgin bir azalma ile karşılanmıştır. Bununla birlikte, doza bağlı olarak GA tedavilerinin oksidatif stresi etkin bir şekilde azalttığı, iltihabi yanıtları düşürdüğü ve hücresel anti-iltihabi savunma mekanizmalarını artırdığı gösterilmiştir. GA, ACR kaynaklı böbrek hasarına karşı yeni bir koruyucu antioksidan ve anti-apoptotik ilaç olarak değerlendirilebilir. Kesin etkili dozu belirlemek için ileri çalışmalar yapılmalıdır.

Anahtar Kelimeler: Akrilamid, apoptoz, galik asit, iltihap, nefrotoksisite

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INTRODUCTION

Acrylamide (ACR) is a compound containing highly reactive α-β unsaturated carbonyl groups that widely used in the synthesis of many occupational and dietary industrial polymerized products including polyacrylamides. Moreover, its frequent daily use (as water management and treatment and/or gel chromatography in molecular laboratories[1]) increases its risk of undesirable toxic consequences to humans.[2-4] Furthermore, ACR can be instantly produced during high temperature processing of carbohydrate-rich foods.[5,6] Many studies have been conducted on ACR so far. They cause damage to many tissues and organs, such as the liver[7], kidney[8], brain[9], heart[10], and testis[11].

Because of its wide range of solubility, as in water and other solvents, it fairly reaches the liver and kidney after ingestion[12-14] with marked damage to cellular genome after the binding of its metabolite Glicamide to cellular DNA.[15,16] It triggers cellular oxidative stress, while increasing ROS production with parallel drain of cellular antioxidants. This scenario consequently disrupts the antioxidant defense mechanisms involving enzymes such as SOD, GPx and CAT as well as GSH, leading to accelerated lipid peroxidation as indicated by an increase in MDA levels. Oxidative stress is closely linked to inflammation. When cells are exposed to ACR, pro-inflammatory markers increase, while anti-inflammatory markers decrease. This dual impact of oxidative stress and inflammation induced by ACR exposure in cells can trigger apoptosis.[17,18]

GA is a plant-derived polyphenol compound abundantly found in many fruits and beverages.[19] GA protects against oxidative damage and inflammation on cellular and tissue levels.[20-23] Besides it has a powerful antioxidant with free radicals scavenger properties[24], it also demonstrated antibacterial, anti-viral, and anti-cancer effects.[25]

Our study investigated its potential anti-oxidant, anti-inflammatory and anti-apoptotic roles against ACR-induced kidney damage.

MATERIALS AND METHODS

Reagents and Chemicals

Acrylamide (≥ 99%) (Cas No: 10236-47-2) and Gallic acid (Cas No: 149-91-7) were purchased by Sigma Chemical Co (St. Louis, MO). Commercially available rat ELISA kits were used to determine the level of Malondialdehyde (MDA) (Cat. No:201-11-0157) and Superoxide Dismutase (SOD) (Cat. No:201-11-0169) in renal tissue and to determine Glutathione Peroxidase (GPx) (Cat. No: 201-11-5104), Catalase (CAT) (Cat. No: 201-11-5106) and Glutathione (GSH) activities (Cat. No: 201-11-7122; SunRedBio, Shanghai-China). Interleukin-6 (IL-6) (Cat. No: 201-11-0136), Nuclear factor kappa-B (NF-κB) (Cat. No: 201-11-0288), Tumor necrosis factor-α (TNF-α) (Cat. No: 201-11-0765), cyclooxygenase-2 (COX-2) (Cat. No: 201-11-0297), interleukin-1β (IL-1β) (Cat. No: 201-11-0288), and interleukin-10 levels (IL-10) (Cat. No:201-11-0109 were analyzed in the renal tissue of rats using Commercial ELISA kits (SunRedBio, Shanghai, China). Analysis of p38 mitogen-activated protein kinases (P-38MAPK) (Cat.No: 201-11-5464) and tumor suppressor protein p53 (TP53) (Cat. No:201-11-0072) as pro-apoptotic parameters in the kidney tissue was performed with the help of commercially available ELISA kits (SunRedBio, Shanghai, China).

Experimental Animals

Sprague Dawley male rats were obtained from Atatürk University Experimental Research and Application Center. A total number of 40 rats with an average weight of 200-250 g were used. The rats were housed in ventilated rooms with ambient temperature of 25oC and humidity of 60-65% using 12-hour light/dark cycle. Animals had water and feed ad libitum during the whole experimental period. The study protocol was approved by Atatürk University Animal Experiments Local Ethics Committee (Decision No: 2021/166).

Experimental Design

The rats were divided into five equal groups. Experimental groups were formed as follows.

Control: 1 ml of saline was given intragastric (ig) for 14 days.

ACR: ACR was given at a dose of 50 mg/kg ig for 14 days.

ACR+GA50: ACR at a dose of 50 mg/kg and GA at a dose of 50 mg/kg were given ig for 14 days.

ACR+GA100: ACR at a dose of 50 mg/kg and GA at 100 mg/kg were given ig for 14 days.

GA100: GA was given at 100 mg/kg ig for 14 days.

On the 15th day, all rats were weighed and euthanized under sevoflurane anesthesia. The kidneys of all rats were washed with cold phosphate buffer and then placed in a -20 freezer until analysis.

Homogenization of Kidney Tissue

Kidney tissue samples were cut into equal small pieces and homogenized in ice-cold phosphate buffer saline adjusted to pH 7.4 in MagNA Lyser device. Then the homogenate was centrifuged at 4000 rpm at 4 ºC for 10 minutes and the supernatant was recovered and stored at – 40 ºC for further analysis.

Analysis of Oxidant and Antioxidant Enzymes

MDA and GSH levels and SOD, GPx, and CAT activities were measured in the supernatant using commercial ELISA kits.

Analysis of Inflammation Markers in Kidney Tissue

The IL-1β, IL-6, NF-κB, COX-2, IL-10, and TNF-α levels were determined after the protocol of commercial ELISA kits in the supernatant of homogenized kidney samples.

Analysis of Apoptosis Markers in Kidney Tissue

Similarly, the apoptotic biomarkers p38 MAPK and p53 levels were determined using their available ELISA kits following their manufacturer's instructions.

Statistical Analyzes

The data were analyzed using one-way ANOVA with more than two independent groups in the SPSS 20.00 statistical programming software. The obtained values were evaluated by the Tukey test. The values were expressed as mean ± standard error (± SEM), and $P < .05$ and $P < .01$ were considered significant.

RESULTS

Oxidative Stress in ACR-induced Kidney Damage

The oxidative stress parameters in ACR-induced kidney damage and the effects of GA on these parameters are shown in Figure 1 and Figure 2.

The level of MDA in the kidney of the ACR group recorded a significant rise compared with the control ($P < .01$). While the ACR+GA(50) group demonstrated a slight non-significant decrease in MDA level ($P > .05$), the ACR+GA(100) and GA(100) groups, however, showed a normalization of the lipid peroxidation level with non-significant MDA value compared with control ($P > .05$, Figure 1).

The activity of antioxidant SOD and the reserve of GSH level were both significantly ($P < .01$) decreased in the ACR group compared to the control. Despite observed ($P < .05$) fluctuation of SOD and GSH levels in ACR+GA(50) group than that of control groups, their measured levels in the ACR+GA(100) and GA(100) groups were not differed than that of control ($p>0.05$, Figure 1).

Both GPx and CAT levels showed marked decrease ($p<0.01$) in the ACR group than their level in control. There were slight increase in their levels in the ACR+GA(50), but significantly not differed than the ACR group. The GPx and CAT levels, however, recorded a non-significance difference in the ACR+GA(100) and GA(100) groups when compared with control ($P > .05$, Figure 2).

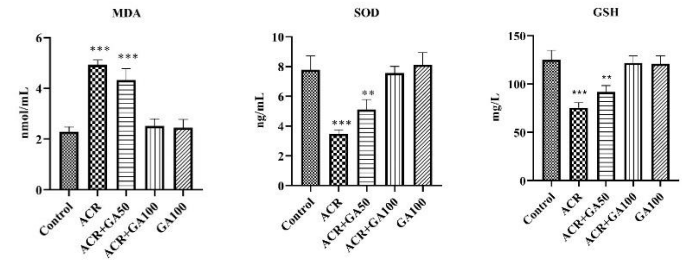

Figure 1. The effects of ACR and GA administration on MDA (A), SOD (B), and GSH (C) levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group. *** $P < .01$, ** $P < .05$; n =8)

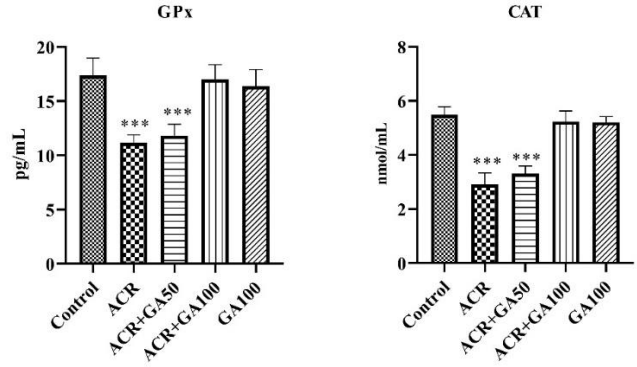

Figure 2. The effects of ACR and GA administration on GPx ve CAT levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group. *** $P < .01$, n =8)

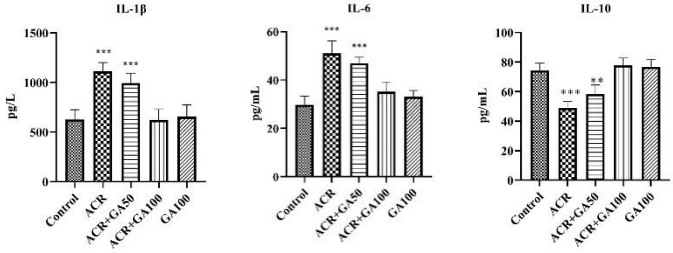

Figure 3. The effects of ACR and GA administration on IL-1β, IL-6, and IL-10 levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group. ***$P < .01$, **$P < .05$, n =8)

Inflammation in ACR-induced Kidney Injury

The Some parameters related to inflammation due to ACR-induced renal insult as well as the protective role after GA treatments are shown in Figure 3 and Figure 4.

The ACR-treatment induced significant rise ($P < .01$) in IL-1β and IL-6 levels compared with control. These levels showed non-significant decrease ($P > .05$) in the ACR+GA(50) group than their level in the ACR group. The IL-1β and IL-6 levels of the ACR+GA(100) and GA(100) groups were not statistically differed than control ($P < .05$, Figure 3).

The IL-10 levels, however, not differed significantly in the ACR+GA(100) and GA(100) groups when compared with the control ($P > .01$, Figure 3). While the IL-10 level in the ACR group recorded a significant decrease ($P > .01$) in its level in comparison with the control, the ACR+GA(50) group showed a significant increase ($P < .05$) in its level than the ACR groups.

The levels of pro-apoptotic mediators NF-κB and COX-2 were significantly elevated ($P < .01$) in ACR treated group compared to the control. This elevation was not recorded in the ACR+GA(50) group, where the levels slightly decreased to be not significantly differed than that of the control level ($P < .01$). Also their levels in the ACR+GA(100) and GA(100) groups showed no statistical difference to that of control ($P > .05$, Figure 4).

Similarly, the inflammatory mediator TNF-α levels in the ACR+GA(100) and GA(100) groups were not differed significantly than the control level ($P > .05$, Figure 4). But the TNF-α level in the ACR group recorded a significant difference to the control value ($P > .01$). In the ACR+GA(50) group, however, a slight- but significant- decrease in TNF-α level that reported a significant difference ($P < .05$) to the TNF-α level of ACR group.

Apoptosis in ACR-induced Kidney Injury

The changes in the apoptotic markers were reported in Figure 5. The levels of apoptotic markers p38-MAPK and p53 increased significantly ($P < .01$) in ACR treated group compared to control. Unlike the significant change ($P < .05$) that was observed in the level of p38-MAPK between the ACR+GA (50) group and the ACR group, there was no statistical difference ($P > .05$) in the p53 level between the ACR+GA(50) group and the ACR group. The p38-MAPK and p53 levels of the ACR+GA(100) and GA(100) groups were close to the control level with no statistical difference between them ($P < .05$, Figure 5).

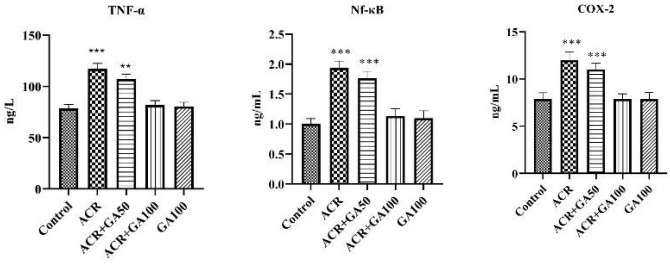

Figure 4. The effects of ACR and GA administration on TNF-α, NF-κB, and COX-2 levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group. ***$P < .,01$, ** $P < .05$, n =8)

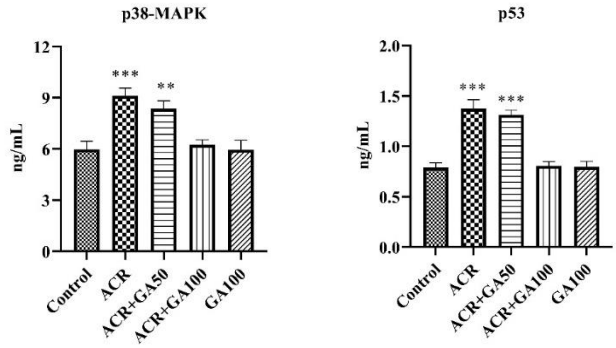

Figure 5. The effects of ACR and GA administration on TNF-α, NF-κB, and COX-2 levels in the experimental groups (There are statistically significant differences between the values expressed with different symbols between the control group. ***$P < .01$, **$P < .05$, n =8)

DISCUSSION

The study tested the potential protective role of GA in a dose dependent manner against ACR renal insult. Here the levels of oxidative stress parameters (SOD, GSH, GPx, and CAT), lipid peroxidation (MDA), inflammatory response (IL-1β, IL-6, IL-10, TNF-α ve NF-κB), and pro-apoptotic (p38-MAPK and p53) markers were determined to evaluate the possible role of GA to alleviate the oxidative stress, inflammatory response and accelerated apoptosis in the kidney tissue following ACR insult. The results confirmed that the GA- in high dose- had demonstrated a considerable mitigation of the ACR-induced renal oxidative and inflammatory insults.

Imbalances in the typical cellular redox state result in disruptions to biological components like lipids, proteins, and DNA. The extent of ROS generation determines the extent of cell membrane damage, leading to the creation of lipid peroxidation through the oxidative modification of polyunsaturated fatty acids within the membrane's composition.[10] MDA, one of the lipid peroxidation indicators, increased in our study, and GA application significantly decreased the MDA level. Oxidative stress can be defined as an imbalance between the oxidant and antioxidant defense systems.

The MDA is a convenient lipid peroxidation biomarker. Its observed elevation after ACR renal insult was significantly mitigated with oral dosing of GA. Since oxidative stress can be characterized as a disturbance in the equilibrium between the mechanisms that generate oxidants and those that provide antioxidant protection. The generation of reactive oxygen species (ROS) efficiently counteracts both enzymatic (such as SOD, GSH-Px, and CAT) and non-enzymatic (like GSH) antioxidant defenses.[25-30] While ACR

increases MDA levels, it significantly decreases SOD, CAT, GPx, and GSH levels.[31] Ghaznavi et al.[32] investigated the antioxidant role of GA in gentamicin-induced nephrotoxicity as well as the same role in methotrexate-induced hepatotoxicity.[33] Gallic had been demonstrated to modulate cellular redox hemostasis.[34] Consistently, our study proved the gallic acid role in this modulation after ACR-induced renal insult.

TNF-α, IL-1β, and IL-6 are proinflammatory cytokines, and they cause acute inflammation by stimulating the expression of adhesion molecules in endothelial cells and inflammatory cells.[35] It has been shown in a study that ACR application increases TNF-α and IL-6 levels.[36] In another study, it causes an increase in TNF-α and IL-1β levels in acrylamide-induced brain damage.[37] IL-10 is an anti-inflammatory cytokine. Acrylamide administration causes a decrease in IL-10 levels.[38] NF-κB is one of the critical transcription factors activated by oxidative stress and plays a role in inflammation. NF-κB increases the levels of IL-1β, TNF-α, and IL-6, which are involved in inflammation, and accordingly, it increases the inflammatory response. Many studies have shown that NF-κB stimulates inflammation.[39,40] NF-κB is a transcription factor involved in signal transduction between the cytoplasm and the nucleus in various cell types. COX-2 acts as an enzyme that catalyzes the oxidation of arachidonic acid. NF-κB regulates the expression of the COX-2 enzyme.[41] ACR increases COX-2 expression.[10] Gallic acid is a phenolic compound that regulates inflammation in various tissues.[6] In our study, we found that gallic acid, which has protective effects against the inflammation caused by ACR, has also a protective role against ACR-induced renal insult.

Against the stimuli outside the cell, signal pathways such as MAPK are activated inside the cell. MAPK consists of 4 subgroups. These are Extracellular Signal-Regulated Kinases (ERKs), c-Jun N-Terminal and Stress-Activated Protein Kinases (JNK/SAPK), ERK/ Big MAPK 1 (BMK1), and p38 protein kinase. To date, it is well known that p38 plays a role in apoptosis. Caspases are the best markers of apoptosis. However, caspase inhibitors inhibit p38, which means that p38 has a role in caspase activation.[42] Expression of the p53 gene is increased in DNA damage and apoptosis.[43,44] Li et al. observed that p38-MAPK and p53 levels increased in neural damage induced by hydrogen peroxide.[45] In another study, Khan et al. showed increased p38-MAPK and p53 levels in cisplatin-induced colon toxicity.[8] Gallic acid has a suppressive role on free radicals that trigger the apoptosis pathway and enzymes involved in their production.[25] Our study found that the kidney damage induced by Acrylamide increased p38-MAPK and p53, and GA had protective effects against their increase.

In conclusion, the study revealed that ACR induced renal toxicity in rats as revealed by increased oxidative stress parameters with aggravated lipid peroxidation confirmed by the rise in the levels of MDA biomarker as well as inflammatory mediators IL-1β, IL-6, TNF-α, and NF-κB. These finding were concomitant with marked drain of the cellular antioxidant activity reflected by the marked decrease in SOD, GSH, GPx, and CAT levels with a rise in the levels of pro-apoptotic markers p38-MAPK and p53. Interestingly, the use of GA that well known phenolic compound with potent antioxidant, anti-inflammatory, and anti-apoptotic properties, had been demonstrated to mitigate the adverse nephrotoxic effects induced by ACR treatment. This mitigation, however, was in a dose dependent manner. The study warrants about the possible use GA as novel pharmaceutical intervention to alleviate renal toxicity induced by ACR application.

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