

# Protective Effect of Gallic Acid on Mercuric Chloride-Nephrotoxicity in Experimental Rats

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## Abstract:

**Background:** Mercuric chloride (HgCl<sub>2</sub>) causes renal injury partly through oxidative stress and inflammation. This study investigated the protective effects of gallic acid (GA) against HgCl<sub>2</sub>-induced nephrotoxicity in rats.

**Methods:** Thirty-five Wistar rats were randomly assigned to five groups (n = 7): control, HgCl<sub>2</sub> (0.4 mg/kg/day), HgCl<sub>2</sub> plus GA (50 or 200 mg/kg/day), and GA alone (200 mg/kg/day). Treatments were administered orally for 28 days, with GA given 1 h before HgCl<sub>2</sub>. Renal function was assessed using serum blood urea nitrogen (BUN), creatinine (Cr), kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). Renal tissue was analyzed for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 beta (IL-1 $\beta$ ) and renal histopathology.

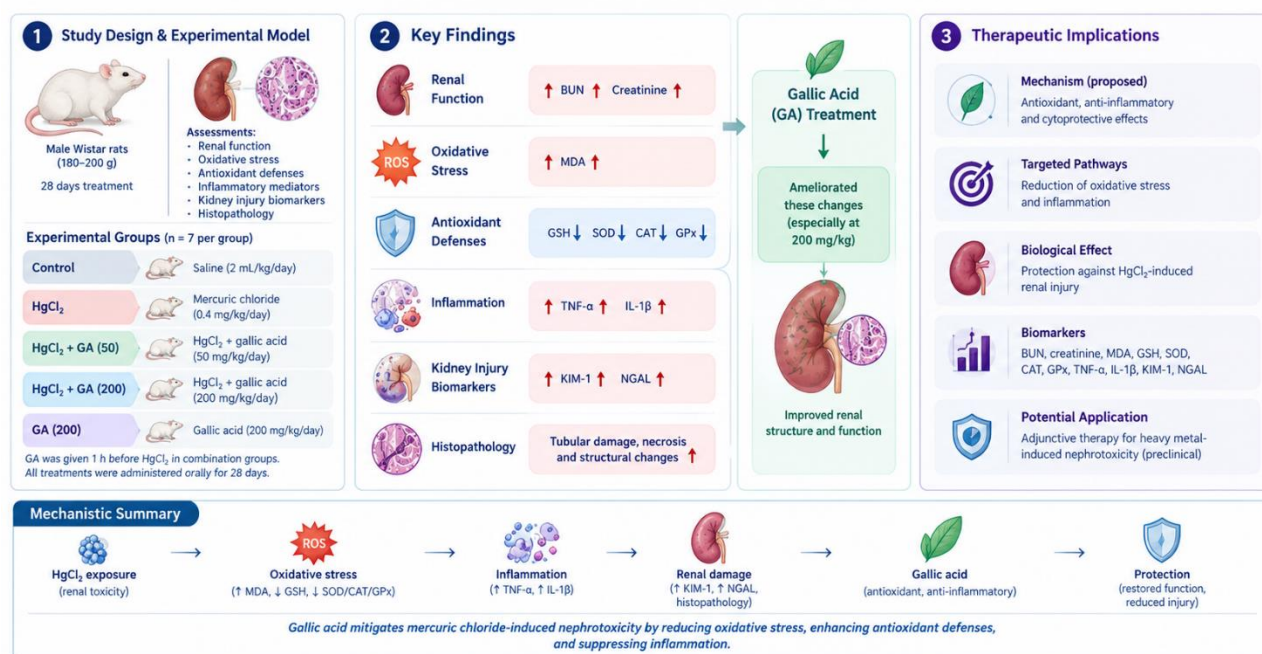
**Results:** HgCl<sub>2</sub> significantly increased serum BUN, Cr, KIM-1 and NGAL, renal MDA, TNF- $\alpha$ , IL-1 $\beta$ , while reducing GSH content and SOD, CAT, and GPx activities. GA at both doses improved several of these changes. The 200 mg/kg GA dose reduced both KIM-1 and NGAL and produced a greater reduction in histopathological injury than the 50 mg/kg dose. However, several inflammatory and injury markers remained above control levels, indicating incomplete protection.

**Conclusion:** GA attenuated multiple biochemical and histological changes associated with HgCl<sub>2</sub>-induced renal toxicity in rats. Its effects were associated with improved antioxidant status and lower inflammatory and kidney injury markers. Further studies are needed to establish the underlying mechanisms and clinical relevance.

**Mechanistic and Translational Relevance:** Gallic acid may attenuate HgCl<sub>2</sub>-induced renal injury by enhancing antioxidant defenses and modulating inflammation, alongside reductions in renal function markers, MDA, TNF- $\alpha$ , IL-1 $\beta$ , KIM-1, and NGAL. The stronger effects observed at the higher dose warrant investigation of dose dependence, although underlying mechanisms remain unclear. Further studies should assess whether these protective effects translate into clinical benefits, as efficacy and safety in humans remain unestablished.

**Keywords:** Gallic Acid; Mercuric Chloride; Nephrotoxicity; Oxidative Stress; Renal Injury

### Gallic Acid Attenuates Mercuric Chloride-Induced Nephrotoxicity in Rats



**Graphical Abstract: Gallic Acid Attenuates Mercuric Chloride-Induced Nephrotoxicity in Rats.** This study investigated the effects of gallic acid (GA) on renal injury in rats exposed to mercuric chloride (HgCl<sub>2</sub>) for 28 days. GA improved renal function markers, oxidative stress, antioxidant defenses, inflammatory mediators, kidney injury biomarkers, and histopathological outcomes. The 200 mg/kg dose generally showed greater improvements than 50 mg/kg. These findings suggest potential renoprotective effects in rats, although the underlying mechanisms and clinical relevance remain to be established.

## Introduction

Mercury (Hg) is a naturally occurring heavy metal that has been used in various industrial and medical applications (1). Its release into the environment can result in human and animal exposure through contaminated air, water, and food (1). Mercury exposure is a public health concern because of its potential toxicity across multiple organ systems (1). Mercury can exert hematotoxic, hepatotoxic, neurotoxic, nephrotoxic, and genotoxic effects, with the kidneys being an important target of inorganic mercury toxicity (2). Inorganic mercury occurs in different chemical forms, including mercuric salts such as mercuric chloride (HgCl<sub>2</sub>) (2). Mercuric chloride (HgCl<sub>2</sub>) can induce renal injury through several interrelated processes, including oxidative stress and disruption of cellular antioxidant defenses

(3). Excessive ROS generation is associated with increased lipid peroxidation and cellular injury, potentially compromising cellular structural integrity (3). Mercuric chloride exposure has been associated with depletion of reduced glutathione (GSH) and altered activities of antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (4). The liver and kidneys are important target organs of mercuric chloride toxicity (5). Despite the recognized role of oxidative stress, mercuric chloride-induced renal injury involves multiple processes that warrant further investigation. Gallic acid (3,4,5-trihydroxybenzoic acid; GA) is a phenolic compound with reported antioxidant activity, partly attributable to its free-radical-scavenging properties (6). Moreover, GA exhibits

potent antioxidant activity by neutralizing reactive free radicals and has also been reported to promote apoptosis in tumor cells (6). Previous studies have investigated the potential protective effects of GA against oxidative tissue injury, including renal injury (7).

Oxidative stress and inflammation may contribute to mercuric chloride-induced renal injury (8). Evaluating inflammatory signaling, renal function indices, and biochemical markers of oxidative injury may provide a broader assessment of renal damage and the potential protective effects of GA (7). Their assessment, together with oxidative stress parameters and inflammatory mediators, may help provide a more comprehensive characterization of mercuric chloride-induced renal injury (8) and the effects associated with GA treatment. The present study investigated the protective effects of gallic acid against mercuric chloride-induced nephrotoxicity in rats by assessing renal function, oxidative stress and antioxidant defenses, inflammatory mediators, kidney injury-associated biomarkers, and renal histopathology.

## **Materials and Methods**

### **Animals and Experimental Design**

Adult male Wistar rats weighing 180–200 g were obtained from the animal research facility at Ahvaz Jundishapur University of Medical Sciences. The animals were housed under controlled laboratory conditions at  $20 \pm 2^\circ\text{C}$  with a 12-h light/dark cycle and had unrestricted access to standard laboratory chow and drinking water. All animal procedures were conducted in accordance with the relevant institutional guidelines for the care and use of laboratory animals and were approved by the institutional ethics committee (Ethics Code: IR.AJUMS.ABHC.REC.1394.626)

The rats were randomly allocated to five groups ( $n = 7$  per group): (1) control, receiving normal saline

(2 mL/kg/day, orally); (2) mercuric chloride ( $\text{HgCl}_2$ , 0.4 mg/kg/day, orally); (3)  $\text{HgCl}_2$  plus gallic acid (GA, 50 mg/kg/day, orally); (4)  $\text{HgCl}_2$  plus GA (200 mg/kg/day, orally); and (5) GA alone (200 mg/kg/day, orally). In the combination groups, GA was administered 1 h before  $\text{HgCl}_2$ . Treatments were administered by oral gavage for 28 consecutive days. The selected doses were based on previously published studies.

### **Sample Collection and Biochemical Assessment**

Blood was drawn from rats anesthetized with diethyl ether. The samples were then centrifuged at 3,000 rpm for 10 min to obtain serum, which was stored at  $-20^\circ\text{C}$  until biochemical analyses were performed. Following blood collection, the animals were sacrificed by decapitation, and the kidneys were removed and washed. The left kidney was weighed and homogenized at a 1:10 (w/v) ratio in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The right kidney was reserved for histopathological assessment. Total protein levels in tissue homogenates were quantified using the Bradford assay, with bovine serum albumin serving as the reference standard. Serum creatinine and blood urea nitrogen (BUN) concentrations were determined using an RA-XT automated analyzer in conjunction with commercially available diagnostic kits (Technicon, Bayer S.A. Diagnostic).

### **Oxidative Stress and Antioxidant Parameters**

Renal tissue malondialdehyde (MDA) and reduced glutathione (GSH) levels, as well as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, were assessed to evaluate oxidative stress and antioxidant status (9, 10).

MDA concentrations were assessed using a modified thiobarbituric acid (TBA) assay (11). Briefly, tissue homogenate was mixed with 10%

trichloroacetic acid (TCA) and centrifuged at  $1,500 \times g$  for 10 min. The supernatant was reacted with 0.67% TBA in a boiling water bath for 10 min. Absorbance was measured at 532 nm, and MDA concentrations were calculated using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

GSH levels were measured using Ellman's method (12). Tissue homogenates were treated with 25% TCA and centrifuged. The resulting supernatant was reacted with 0.5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.2 M phosphate buffer (pH 8.0), and absorbance was recorded at 412 nm. GSH levels were expressed as nmol/mg protein.

CAT activity was determined by monitoring the decrease in absorbance at 240 nm over 60 s following the addition of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to the tissue supernatant, using the method of Aebi (13). SOD activity was assessed based on inhibition of hematoxylin auto-oxidation, according to the method of Martin et al (14). GPx activity was measured using a commercial kit (Randox Laboratories, Crumlin, UK). Enzyme activities were normalized to protein content where applicable.

### Inflammatory and Kidney Injury Markers

Serum concentrations of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 beta (IL-1 $\beta$ ), kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL) were quantified by enzyme-linked immunosorbent assay (ELISA) (15). These markers were assessed to characterize inflammatory responses and renal injury across the experimental groups.

### Histological Examination

Renal specimens were immersed in 10% neutral-buffered formalin for fixation, processed for paraffin embedding, and cut into sections measuring 5  $\mu\text{m}$  in thickness. The sections were stained with

hematoxylin and eosin (H&E) and examined under a light microscope at magnifications of  $40\times$ – $400\times$ . Histopathological evaluation was performed by an experienced pathologist blinded to the experimental group assignments. Renal injury was assessed semi-quantitatively based on tubular epithelial cell swelling, vacuolar degeneration, epithelial desquamation, and necrosis. The severity of tubular injury was graded on a scale of 0–4 according to the percentage of the affected tubular profile, as follows: 0, normal tubules without detectable histological alterations; 1, injury involving less than 25% of the tubular profile; 2, injury involving 25–50% of the tubular profile; 3, injury involving 50–75% of the tubular profile; and 4, injury involving more than 75% of the tubular profile. Higher scores indicated more severe renal tissue damage. For each section, approximately 100 tubules were evaluated, and the mean injury score was calculated by dividing the sum of the individual tubular scores by the number of tubules examined.

### Statistical Analysis

Results are expressed as mean  $\pm$  standard deviation (SD). Differences among the five groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. A blinded statistician performed the analyses using coded group identities. Laboratory personnel were also blinded to group assignments during sample processing and data collection. Statistical significance was set at  $p < 0.05$ .

### Result

#### Effects of GA on Serum Biochemical Markers of Renal Function in $\text{HgCl}_2$ -Induced Renal Toxicity

Mercuric chloride ( $\text{HgCl}_2$ ) significantly increased serum BUN and creatinine levels compared with the control group ( $P < 0.05$ ). GA treatment at 50 mg/kg reduced both parameters compared with the  $\text{HgCl}_2$

group although this decrease was not significant ( $P > 0.05$ ). At 200 mg/kg, GA further reduced BUN levels, with a significant difference from both the control and HgCl<sub>2</sub> groups ( $P < 0.05$ ). Serum

creatinine levels in the 200 mg/kg GA groups were also significantly lower than those in the HgCl<sub>2</sub> group ( $P < 0.05$ , Figure 1).

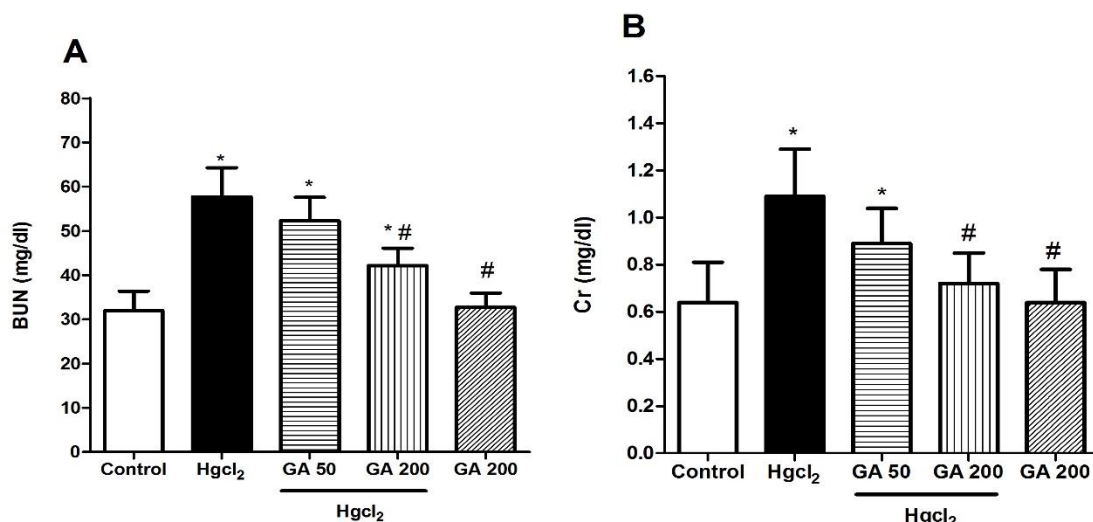


**Figure 1.** Effects of GA on serum BUN and creatinine levels in rats with HgCl<sub>2</sub>-induced renal toxicity. Data are presented as mean  $\pm$  SD ( $n = 7$  per group). Serum BUN (A) and creatinine (B) levels were measured in control rats, HgCl<sub>2</sub>-treated rats, and rats receiving HgCl<sub>2</sub> plus GA (50 or 200 mg/kg). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. \* $P < 0.05$  versus the control group; # $P < 0.05$  versus the HgCl<sub>2</sub> group.

### Effects of GA on Renal Oxidative Stress Markers in HgCl<sub>2</sub>-Induced Renal Toxicity

Mercuric chloride (HgCl<sub>2</sub>) significantly increased renal MDA levels and decreased GSH content compared with the control group ( $P < 0.05$ ). GA treatment at 50 mg/kg significantly reduced MDA levels compared with the HgCl<sub>2</sub> group ( $P < 0.05$ ), although MDA remained significantly higher than in the control group ( $P < 0.05$ ). At 200 mg/kg, GA further reduced MDA levels, with both treatment

groups showing significant differences from the HgCl<sub>2</sub> group ( $P < 0.05$ ). Regarding GSH content, GA treatment at 50 mg/kg significantly increased renal GSH levels compared with both the control and HgCl<sub>2</sub> groups ( $P < 0.05$ ). The two groups receiving GA at 200 mg/kg also showed significantly higher GSH levels than the HgCl<sub>2</sub> group ( $P < 0.05$ , Figure 2).

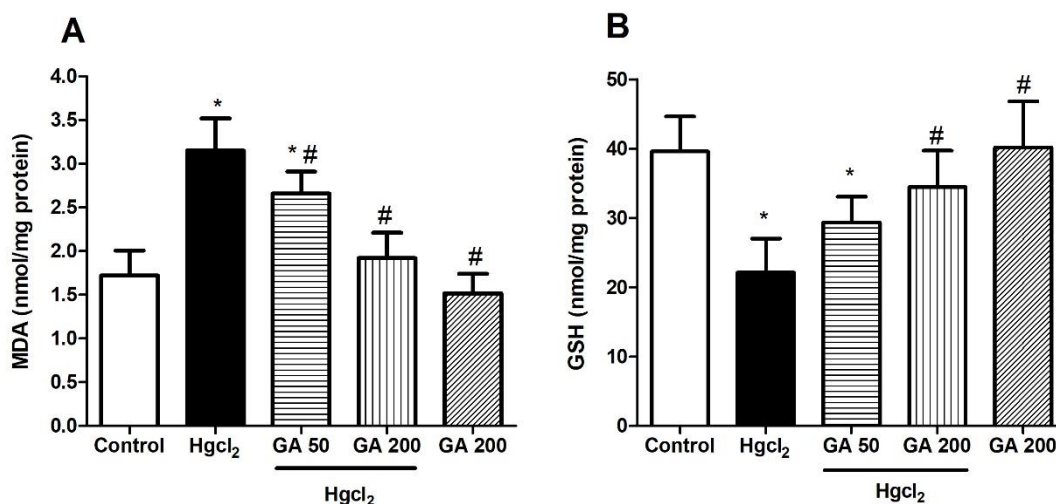


**Figure 2. Effects of GA on Renal MDA Levels and GSH Content in HgCl<sub>2</sub>-Induced Renal Toxicity.** Data are presented as mean  $\pm$  SD (n = 7 per group). Renal tissue MDA levels (A) and GSH content (B) were measured in control rats, HgCl<sub>2</sub>-treated rats, and rats receiving HgCl<sub>2</sub> plus GA (50 or 200 mg/kg). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. \*P < 0.05 versus the control group; #P < 0.05 versus the HgCl<sub>2</sub> group.

#### Effects of GA on Renal Antioxidant Enzyme Activities

HgCl<sub>2</sub> significantly reduced renal SOD, CAT, and GPx activities compared with the control group ( $P < 0.05$ ). GA treatment at 50 mg/kg significantly increased all three antioxidant enzyme activities compared with the HgCl<sub>2</sub> group ( $P < 0.05$ ), although the values remained significantly lower than those of the control group ( $P < 0.05$ ). At 200 mg/kg, GA significantly increased SOD and GPx activities compared with both the control and HgCl<sub>2</sub> groups ( $P < 0.05$ ). In the GA-only group, CAT, SOD and GPx values were significantly higher than those in the HgCl<sub>2</sub> group ( $P < 0.05$ , Table 1).

#### Effects of GA on Renal Inflammatory Markers

As shown in Figure 3, mercuric chloride (HgCl<sub>2</sub>) significantly increased renal TNF- $\alpha$  and IL-1 $\beta$  levels compared with the control group ( $P < 0.05$ ). GA treatment at 50 and 200 mg/kg significantly reduced both inflammatory markers compared with the HgCl<sub>2</sub> group ( $P < 0.05$ ). However, TNF- $\alpha$  and IL-1 $\beta$  levels remained significantly higher than those in the control group at both doses ( $P < 0.05$ ). Treatment with GA at 200 mg/kg alone also significantly reduced TNF- $\alpha$  and IL-1 $\beta$  levels compared with the HgCl<sub>2</sub> group ( $P < 0.05$ ). These findings indicate that GA treatment was associated with lower renal inflammatory marker levels in HgCl<sub>2</sub>-treated rats.

| Groups                             | SOD<br>(U/mg protein)       | CAT<br>(U/mg protein)        | GPx<br>(U/mg protein)       |
|------------------------------------|-----------------------------|------------------------------|-----------------------------|
| control                            | 2.77 ± 0.29                 | 47.64 ± 3.50                 | 7.04 ± 0.11                 |
| HgCl <sub>2</sub>                  | 1.35 ± 0.18                 | 25.16 ± 3.30                 | 3.03 ± 0.22                 |
| HgCl <sub>2</sub> + GA (50 mg/kg)  | 1.84 ± 0.18 <sup>a, b</sup> | 40.36 ± 4.52 <sup>a, b</sup> | 3.92 ± 0.39 <sup>a, b</sup> |
| HgCl <sub>2</sub> + GA (200 mg/kg) | 2.37 ± 0.20 <sup>a, b</sup> | 49.76 ± 2.76 <sup>b</sup>    | 5.35 ± 0.42 <sup>a, b</sup> |
| GA (200 mg/kg)                     | 2.58 ± 0.25 <sup>b</sup>    | 51.26 ± 4.39 <sup>b</sup>    | 7.10 ± 0.45 <sup>b</sup>    |

**Table 1. Effects of GA on Renal Antioxidant Enzyme Activities in HgCl<sub>2</sub>-Induced Renal Toxicity.** Values are presented as mean ± SD (n = 7 per group). SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase. Superscript a indicates a significant difference compared with the control group (P < 0.05), and superscript b indicates a significant difference compared with the HgCl<sub>2</sub> group (P < 0.05).

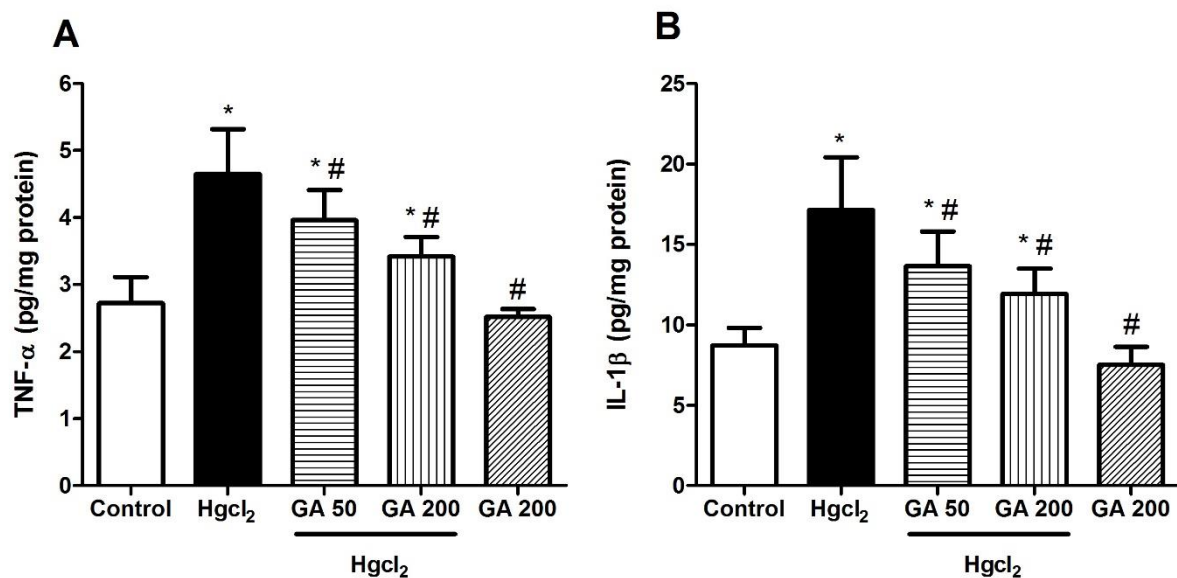


**Figure 3. Effects of GA on Renal TNF-α and IL-1β Levels in HgCl<sub>2</sub>-Induced Renal Toxicity.** Renal tissue levels of TNF-α (A) and IL-1β (B) in control rats, HgCl<sub>2</sub>-treated rats, and rats receiving GA at 50 or 200 mg/kg, as well as rats receiving GA alone at 200 mg/kg. Data are presented as mean ± SD (n = 7 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. \*P < 0.05 versus the control group; #P < 0.05 versus the HgCl<sub>2</sub> group.

### Effects of GA on Renal Injury Markers

As shown in Figure 4, mercuric chloride (HgCl<sub>2</sub>) significantly increased serum KIM-1 and NGAL

levels compared with the control group (P < 0.05). GA treatment at 50 mg/kg significantly reduced NGAL levels compared with the HgCl<sub>2</sub> group (P <

0.05), whereas KIM-1 levels remained higher than those in the control group. At 200 mg/kg, GA significantly reduced both KIM-1 and NGAL levels compared with the HgCl<sub>2</sub> group ( $P < 0.05$ ). In the

group receiving GA alone at 200 mg/kg, both KIM-1 and NGAL levels were significantly lower than those in the HgCl<sub>2</sub> group ( $P < 0.05$ ).

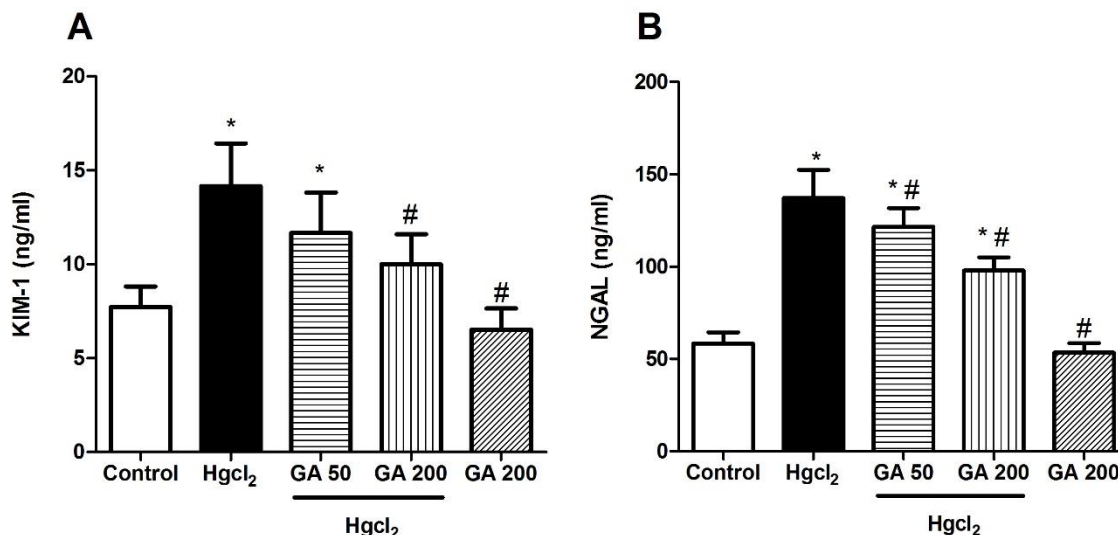


**Figure 4. Effects of GA on Renal KIM-1 and NGAL Levels in HgCl<sub>2</sub>-Induced Renal Toxicity.** Renal levels of kidney injury molecule-1 (KIM-1) (A) and neutrophil gelatinase-associated lipocalin (NGAL) (B) in control rats, HgCl<sub>2</sub>-treated rats, rats receiving HgCl<sub>2</sub> plus GA (50 or 200 mg/kg), and rats receiving GA alone (200 mg/kg). Data are presented as mean  $\pm$  SD ( $n = 7$  per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. \* $P < 0.05$  versus the control group; # $P < 0.05$  versus the HgCl<sub>2</sub> group.

### Effects of GA on Renal Histopathological Changes

As shown in Figure 5, HgCl<sub>2</sub> exposure markedly increased the renal histopathological score compared with the control group ( $P < 0.05$ ). GA treatment at 50 and 200 mg/kg/day reduced the histopathological score compared with the HgCl<sub>2</sub> group, although the scores remained significantly higher than in the control group ( $P < 0.05$ ). The reduction was greater at 200 mg/kg/day than at 50

mg/kg/day. GA alone at 200 mg/kg/day showed a low histopathological score, which was significantly lower than that of the HgCl<sub>2</sub> group ( $P < 0.05$ ).

### Discussion

The present study demonstrated that GA treatment attenuated HgCl<sub>2</sub>-induced renal dysfunction, as evidenced by reductions in serum BUN and creatinine levels. Renal injury caused by mercury exposure is associated with impaired glomerular filtration and the accumulation of nitrogenous waste products in the circulation. The observed improvements in these biochemical parameters suggest that GA may help preserve renal function following HgCl<sub>2</sub> exposure. This effect was accompanied by changes in renal oxidative stress markers, antioxidant enzyme activities, inflammatory mediators, and kidney injury biomarkers, indicating that the protective effects of GA may involve multiple interconnected mechanisms. Notably, the effects observed at the higher dose suggest a potential dose-related

response, although further studies are needed to establish this relationship.

Oxidative stress is an important contributor to mercury-induced renal injury (16). Mercury exposure can induce oxidative stress, as reflected by increased lipid peroxidation and depletion of cellular antioxidant defenses, potentially contributing to cellular injury (16). In this study,

HgCl<sub>2</sub> increased renal MDA levels and reduced GSH content, whereas GA treatment attenuated these changes, suggesting improved redox balance. MDA is a commonly used indicator of lipid peroxidation, reflecting oxidative damage to membrane lipids and complementing the assessment of antioxidant defenses provided by GSH and antioxidant enzyme activities (17).

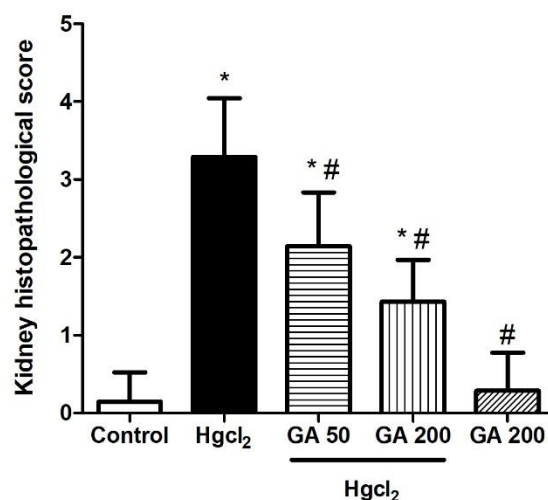


**Figure 5. Effects of GA on Renal Histopathological Scores in HgCl<sub>2</sub>-Induced Renal Toxicity.** Histopathological scores of renal necrosis in control rats, HgCl<sub>2</sub>-treated rats, rats receiving HgCl<sub>2</sub> plus GA (50 or 200 mg/kg/day), and rats receiving GA alone (200 mg/kg/day). Necrosis was scored as follows: 0, absent; 1, single-cell necrosis; 2, involvement of 0–30% of the tissue; 3, involvement of 30–60%; and 4, involvement of more than 60% of the tissue. Data are presented as mean ± SD (n = 7 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. \*P < 0.05 versus the control group; #P < 0.05 versus the HgCl<sub>2</sub> group.

Changes in antioxidant enzyme activities provide further insight into the renal response to GA. HgCl<sub>2</sub> reduced SOD, CAT, and GPx activities, suggesting impairment of enzymatic antioxidant defenses. SOD converts superoxide radicals into hydrogen peroxide, while CAT and GPx contribute to its removal (18). Reduced activity of these enzymes may therefore increase the susceptibility of renal tissue to oxidative damage (18). GA treatment increased the activities of all three enzymes compared with the HgCl<sub>2</sub> group. At 200 mg/kg, CAT activity was not significantly different from the control group, whereas SOD and GPx activities remained significantly lower. This difference

suggests that the antioxidant response was not uniform across enzymes. The combined changes in MDA, GSH, and antioxidant enzyme activities support an association between GA treatment and improved redox balance. GSH is an important component of the cellular antioxidant defense system and contributes to the maintenance of intracellular redox balance, including through its role as a substrate for GPx-mediated peroxide reduction (19). Whether these effects involve changes in antioxidant gene expression or direct regulation of enzyme activity remains to be determined. The chemical structure of GA, which contains three phenolic hydroxyl groups, provides a

plausible basis for its radical-scavenging activity reported in previous studies (20). However, the contribution of this property to the renal effects observed in the present study cannot be established from the measured endpoints alone.

Inflammation represents another component of HgCl<sub>2</sub>-induced renal injury (21). TNF- $\alpha$  and IL-1 $\beta$  levels increased following HgCl<sub>2</sub> exposure, consistent with an inflammatory response in different body tissues (22). GA treatment reduced both mediators compared with the HgCl<sub>2</sub> group. These findings suggest that GA may attenuate renal inflammation alongside its effects on oxidative stress. Oxidative stress and inflammation can interact and amplify tissue injury, although the relationship between these processes was not directly examined in this study. More broadly, inflammatory mediators are best understood as components of interconnected signaling networks rather than isolated drivers of tissue injury (23).

Both KIM-1 and NGAL levels increased following HgCl<sub>2</sub> exposure, consistent with renal injury, particularly tubular damage. The changes in these biomarkers following GA treatment further support an effect of GA on markers associated with renal injury (24). GA at 50 mg/kg significantly reduced NGAL but did not produce a statistically significant reduction in KIM-1 relative to the HgCl<sub>2</sub> group. At 200 mg/kg, both markers were lower than in the HgCl<sub>2</sub> group. This difference between biomarkers highlights the importance of interpreting each outcome separately rather than assuming that all indicators of renal injury respond in the same way. The changes in KIM-1 and NGAL complement the improvements in serum BUN and creatinine, although these biochemical findings alone cannot establish the extent of structural recovery. If supported by the histopathological findings, the observed improvement in renal tissue architecture would provide complementary structural evidence

alongside the changes in renal function and injury-associated biomarkers.

Taken together, the findings suggest that the effects of GA in HgCl<sub>2</sub>-exposed rats involve several aspects of renal injury, including renal function, oxidative stress, antioxidant defenses, inflammation, and injury-associated biomarkers. However, the findings do not establish a specific mechanism underlying these effects. Moreover, differences in the responses of individual biomarkers suggest that the effects of GA may vary across biological processes and may not represent complete protection. The GA-only group provides information on the effects of GA in the absence of HgCl<sub>2</sub> exposure; however, the measured parameters are insufficient to establish its overall renal safety.

### Limitations

Several limitations should be considered. First, the study was conducted in rats, and the findings cannot be directly extrapolated to humans. Second, the relatively small sample size (seven animals per group) limits the precision and generalizability of the findings. In addition, the study duration may not capture longer-term effects. Although several biochemical markers were assessed, ROS generation and specific molecular signaling pathways were not directly measured. Further studies incorporating renal histopathology, direct assessment of relevant oxidative and inflammatory signaling pathways, and longer follow-up are warranted. Dose-response studies would also help characterize the relationship between GA dose and its observed effects.

### Conclusions

In conclusion, GA attenuated several biochemical alterations associated with HgCl<sub>2</sub>-induced renal toxicity in rats. These findings suggest that the observed effects may be associated with improved antioxidant status and reduced inflammatory

responses, together with changes in markers of renal injury. Further studies are needed to clarify the underlying mechanisms and determine whether these biochemical changes translate into sustained structural and functional renal protection.

### **Mechanistic and Translational Relevance**

The present findings suggest that gallic acid may protect against HgCl<sub>2</sub>-induced renal injury through effects on oxidative stress, antioxidant defenses, and inflammatory responses. Improvements in renal function markers were accompanied by lower MDA, TNF- $\alpha$ , IL-1 $\beta$ , KIM-1, and NGAL levels, together with changes in GSH content and antioxidant enzyme activities. These findings suggest that the renal effects of gallic acid extend beyond changes in serum biochemical markers and involve several interconnected aspects of kidney injury. The greater improvement observed with the higher dose across several outcomes also supports further investigation of its dose-dependent effects. From a translational perspective, gallic acid may represent a candidate for further investigation as a protective agent against toxicant-induced renal injury. However, these findings are limited to an experimental rat model and do not establish clinical efficacy or safety in humans. Further studies should clarify the underlying molecular pathways, characterize dose-response relationships, and determine whether the observed biochemical and histological improvements translate into sustained renal protection.

### **Conflict of Interest**

The authors declare that they have no conflict of interest.

### **Author Contributions**

M.G. conducted the experimental procedures and data collection. H.K. contributed to the experimental work and performed the statistical

analysis. M.F. contributed to the study and drafted the initial version of the manuscript. M.K. conceived the study, provided scientific guidance, and coordinated and supervised the research. All authors reviewed and approved the final manuscript.

### **Ethics Statement**

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

### **Data Availability Statement**

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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### **Artificial Intelligence (AI) Disclosure**

Artificial intelligence tools were used exclusively for final language editing and grammatical refinement of the manuscript, with no other AI-assisted activities involved in the research or manuscript preparation.

### **Publisher's Note**

The graphical abstract was prepared by the journal's editorial team using artificial intelligence-assisted design tools and was reviewed and approved by the authors prior to publication.

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