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Glycyrrhizic acid attenuates cisplatin-induced nephrotoxicity: anti-inflammatory mechanisms and renoprotective effects in Wistar rats

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This study explored the renoprotective effect of glycyrrhizic acid (GA) in a Wistar rat model of cisplatin (CDDP)-induced nephrotoxicity, focusing on its anti-inflammatory mechanisms. Thirty-five male rats were divided into five groups: saline control, ethanol vehicle, GA (100 mg/kg/day, i.p., for 7 days), CDDP (10 mg/kg, i.p., single dose), and GA + CDDP. GA was administered once daily, and CDDP was injected on the second day. Renal function was assessed by measuring serum creatinine, BUN, and urinary KIM-1, while inflammatory markers including IL-1β, IL-18, TNF-α, NF-κB, TLR4, and TLR9 were evaluated using ELISA. Histopathological assessment was performed using H&E staining. CDDP significantly elevated BUN, creatinine, and KIM-1 levels, accompanied by increased expression of IL-1β, TNF-α, NF-κB, and TLR4. GA administration attenuated these alterations, indicating reduced renal damage and inflammation. No significant effects were observed on IL-18 or TLR9 levels. Histological examination revealed that GA treatment helped preserve tubular structure and reduce tissue injury. These results indicate that the protective effects of GA in this model may be linked to its ability to downregulate inflammatory mediators, particularly those involving NF-κB and TLR4. Based on these findings, GA holds promise as a supportive intervention to lessen cisplatin-associated renal toxicity during chemotherapy.

Keywords: acute kidney injury, chemoprotection, cisplatin, glycyrrhizic acid, inflammation, nephrotoxicity

Cisplatin (*cis-diamminedichloroplatinum*, CDDP) is a widely used and highly effective chemotherapeutic agent in the treatment of various cancers. However, its clinical use is significantly limited by nephrotoxicity, a major adverse effect. Studies have reported that acute kidney injury occurs in a substantial proportion of patients receiving CDDP, with estimates ranging from 30%[1] to 45.2%[2]. Moreover, follow-up studies in pediatric cancer survivors have shown that renal dysfunction may persist long after cisplatin therapy ends. Findings such as decreased glomerular filtration rate and hypomagnesemia have been documented in up to 30% of this population, indicating that the nephrotoxic effects of CDDP may extend well beyond the treatment period[3].

The nephrotoxic effects of CDDP are primarily due to direct injury to renal tubular epithelial cells, the stimulation of pro-inflammatory signaling cascades, and alterations in renal hemodynamics, including vasoconstriction. These interconnected processes lead to impaired glomerular filtration and progressive renal dysfunction[4]. As a clinical consequence, patients may develop acute kidney injury, which not only impairs their overall well-being but also requires dose reductions or early discontinuation of CDDP, thereby limiting its oncologic effectiveness.

Inflammation plays a critical role in the pathogenesis of CDDP-induced nephrotoxicity[5]. Exposure to CDDP has been shown to elevate the expression of pro-inflammatory cytokines, thereby initiating inflammatory responses and promoting apoptotic pathways within renal tissues[6]. The inflammatory cascade intensifies further through the activation of NF-κB, a central transcription factor in immune regulation, which subsequently enhances the expression of pro-inflammatory cytokines and perpetuates a harmful positive feedback mechanism[6]. Toll-like receptors (TLRs), with TLR4 being especially prominent, are also implicated in this process by amplifying NF-κB signaling, thereby aggravating cisplatin-induced renal injury[7]. Although multiple interconnected mechanisms contribute to cisplatin-induced nephrotoxicity, the present study specifically emphasizes the inflammatory response as a principal factor in mediating renal injury.

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Considering the pivotal contribution of inflammation to cisplatin-induced nephrotoxicity, numerous studies have sought to identify therapeutic agents with the potential to counteract these harmful effects. Glycyrrhizic acid (GA), a bioactive constituent extracted from licorice root, has garnered attention as a potential therapeutic agent owing to its strong anti-inflammatory activity. Studies have demonstrated the efficacy of GA and its derivatives in reducing inflammatory responses in various conditions, including myocardial injury[8], spinal cord injury[9], and adjuvant-induced arthritis[10]. In addition, GA has been reported to exert renoprotective effects in models of kidney injury induced by sepsis[11] and gentamicin[12], primarily through its modulation of inflammatory pathways. Collectively, these observations support the hypothesis that GA may alleviate cisplatin-induced renal injury by suppressing key inflammatory signaling pathways.

Several studies have demonstrated the renoprotective effects of GA, underscoring its capacity to preserve renal architecture, sustain kidney function, and attenuate molecular indicators of injury in experimental models of cisplatin-induced nephrotoxicity[13–15]. Despite the insights provided by previous studies into the nephroprotective properties of GA, a more comprehensive understanding of its underlying mechanisms remains to be established. The anti-inflammatory actions of GA are diverse in nature, encompassing the regulation of immune responses[16], modulation of monocyte-derived macrophage activity[17], and downregulation of pro-inflammatory cytokine expression[8–10]. By modulating key inflammatory mediators, GA may help interrupt the ongoing interplay between inflammation and tissue damage observed in cisplatin-induced nephrotoxicity.

The present study was designed to evaluate the renoprotective properties of glycyrrhizic acid in a rat model of cisplatin-induced nephrotoxicity, with particular attention to its anti-inflammatory effects and its role in maintaining renal function. To evaluate renal injury, we measured plasma levels of blood urea nitrogen (BUN) and creatinine, as well as urinary concentrations of Kidney Injury Molecule-1 (KIM-1), and performed histopathological analysis of kidney tissue. Furthermore, we explored the molecular mechanisms underlying protective effects of GA, including its impact on inflammatory signaling pathways such as NF-κB and TLRs.

Materials and Methods

Animals and Experimental Protocol

Male Wistar rats (200-250 g) were obtained from Canakkale Onsekiz Mart University Experimental Research Application and Research Center and acclimatized for one week under controlled environmental conditions (12-hour light/dark cycle). All experimental protocols were approved by Canakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (approval no. 2022/07-03, dated 29 July 2022). Rats were randomly divided into five groups (n= 7 rats/group): 1) Saline Control: Received intraperitoneal (i.p.) injections of saline. 2) Ethanol Vehicle: Received i.p. injections of the ethanol vehicle used for GA administration. 3) GA: Received GA (100 mg/kg/day, i.p.) for 7 days. GA was dissolved in 20% ethanol[18]. 4) CDDP: Received a single i.p. injection of CDDP (10 mg/kg). 5) GA + CDDP: Received GA (100 mg/kg/day, i.p.) for 7 days. On day 2, a single i.p. injection of CDDP (10 mg/kg) was administered.

On day 6, rats were placed in metabolic cages for 24-hour urine collection. Urine was collected on day 7, immediately before sacrifice. On day 7, rats were anesthetized with xylazine (5 mg/kg) and ketamine (80 mg/kg) and then sacrificed. Blood samples were collected for serum analysis. Kidney samples were also collected. Serum samples were analyzed for BUN (*OttoBC157; Otto Scientific*), creatinine (*OttoBC139; Otto Scientific*), and urine samples for KIM-1 (*E0549Ra; BT-lab*). Kidney tissues were processed for histopathological examination. Kidney tissue levels of IL-1β (*E0119Ra, BT-lab*), IL-18 (*E-EL-R0567, Elabscience*), TNF-α (*E0764Ra, BT-lab*), NF-κB (*E-EL-R0673, Elabscience*), TLR4 (*E0080Ra, BT-lab*), and TLR9 (*E0082Ra, BT-lab*) were measured using ELISA.

Histopathological Examination

Kidney tissues were fixed in 10% buffered formaldehyde and processed using an automatic tissue processor (TP 1020, Leica, Wetzlar, Germany). Paraffin-embedded tissues were sectioned (4 μm) using a sliding microtome (SM 2010 R, Leica, Wetzlar, Germany) and deparaffinized. Sections were stained with hematoxylin and eosin, mounted with entellan, and examined under a light microscope (Olympus BX43, DP22 digital camera). Histopathological scoring was performed as described previously[19] using a semi-quantitative scale of 0 (not detected), 1 (mild), 2 (widespread), and 3 (severe) in 10 random fields at 400x magnification. Interstitial mononuclear cell infiltration,

congestion, tubular epithelial degeneration/necrosis, Bowman's space expansion, and hyperemia were evaluated. ImageJ software (Version 1.53J, NIH, USA) was used for image acquisition and evaluation.

Chemicals

CDDP was obtained from Koçak Pharmaceuticals. GA (CAS number: 53596-04-0) was purchased from Sigma-Aldrich.

Statistical Analysis

Data was analyzed using SPSS version 19.0. The Shapiro-Wilk test was used to assess normality. As the data were not normally distributed, non-parametric tests were used for all analyses. The Kruskal-Wallis test, followed by Dunn's post-hoc test, was used for multiple comparisons. Histopathological data were also analyzed using the Kruskal-Wallis test. Differences were considered statistically significant if the *P*-value was <0.05.

Results

Over the one-week treatment period, rat body weights were monitored daily. No significant weight changes were observed between groups. Similarly, no significant differences in left kidney weights were found at euthanasia.

CDDP administration significantly increased both serum BUN and creatinine levels, indicating renal dysfunction ($P<0.05$). GA treatment significantly attenuated this dysfunction, reducing both BUN (Fig. 1a) and creatinine (Fig. 1b) levels compared to the CDDP-only group ($P<0.05$).

CDDP also induced a significant pro-inflammatory response in kidney tissues. Compared with the saline group, renal IL-1β levels were significantly increased ($P<0.05$) (Fig. 2a), whereas IL-18 showed no significant difference between groups (Fig. 2b). TNF-α (Fig. 2c), NF-κB (Fig. 2d), and TLR4 (Fig. 2e) were

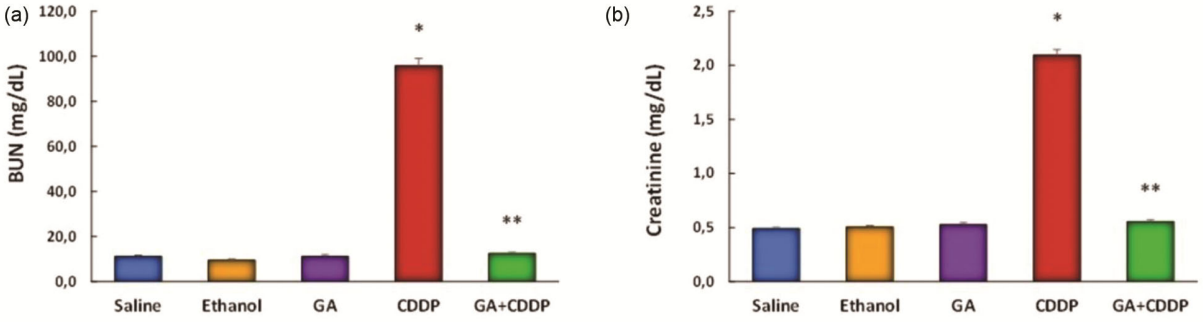

Fig. 1 — Effects of glycyrrhizic acid on cisplatin-induced changes in (A) blood urea nitrogen and (B) plasma creatinine levels in Wistar rats. Rats were treated with saline (control), ethanol (vehicle for GA), GA alone, CDDP alone, or GA + CDDP. Data are presented as mean ± S.E.M. * $P < 0.05$ compared to control; ** $P < 0.05$ compared to CDDP.

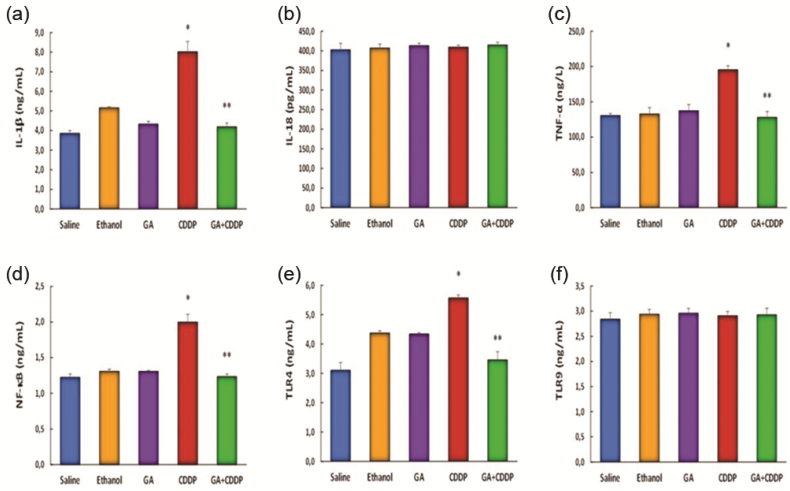

Fig. 2 — Effects of glycyrrhizic acid on cisplatin-induced changes in inflammatory markers in kidney tissue of Wistar rats. Levels of (A) interleukin-1β (IL-1β), (B) interleukin-18, (C) tumor necrosis factor-α, (D) nuclear factor-κB, (E) toll-like receptor 4, and (F) toll-like receptor 9 were measured. Rats were treated with saline (control), ethanol (vehicle for GA), GA alone, CDDP alone, or GA + CDDP. Data are presented as mean ± S.E.M. * $P < 0.05$ compared to control; ** $P < 0.05$ compared to CDDP.

significantly increased (P<0.05), while TLR9 expression remained unchanged (Fig. 2f). GA treatment significantly reduced the elevated pro-inflammatory markers compared to the CDDP-only group (P<0.05).

Urinary KIM-1, a sensitive biomarker of renal injury, was significantly elevated on day 5 in the CDDP group. This elevation was significantly attenuated by GA treatment (Fig. 3).

Histopathological Results

Histopathological examination of H & E-stained kidney sections revealed clear differences among the experimental groups (Fig. 4 and Table 1). The saline control (Fig. 4a) and GA-alone (Fig. 4b) groups showed preserved renal architecture with low histopathological scores. In contrast, the ethanol (vehicle) group (Fig. 4c) exhibited significantly increased scores for interstitial mononuclear cell infiltration, interstitial congestion, tubular epithelial degeneration/necrosis, and Bowman's space enlargement/hyperemia compared with the saline group (P<0.05, Table 1).

As expected, the CDDP group (Fig. 4d) demonstrated the most severe histopathological injury, characterized by marked interstitial congestion/hyperemia, focal mononuclear cell infiltration, prominent tubular degeneration with areas of tubular cell necrosis, and Bowman's space enlargement (P<0.05 vs. saline), (Table 1). Importantly, co-treatment with glycyrrhizic acid significantly attenuated these CDDP-induced lesions. The GA + CDDP group (Fig. 4e) showed reduced tubular degeneration/necrosis, less interstitial

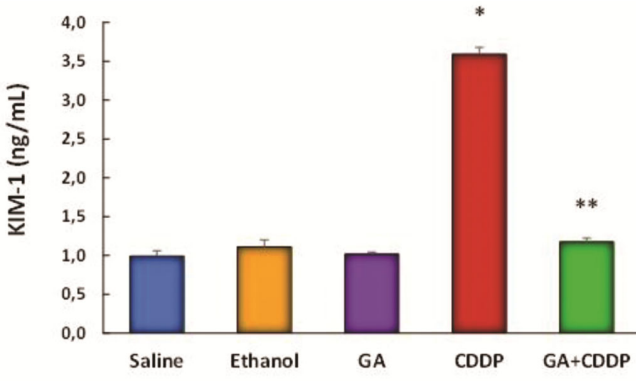

Fig. 3 — Effect of glycyrrhizic acid on cisplatin-induced changes in urinary kidney injury molecule-1 levels in Wistar rats. Rats were treated with saline (control), ethanol (vehicle for GA), GA alone, CDDP alone, or GA + CDDP. Data are presented as mean ± S.E.M. * P < 0.05 compared to control; ** P < 0.05 compared to CDDP.

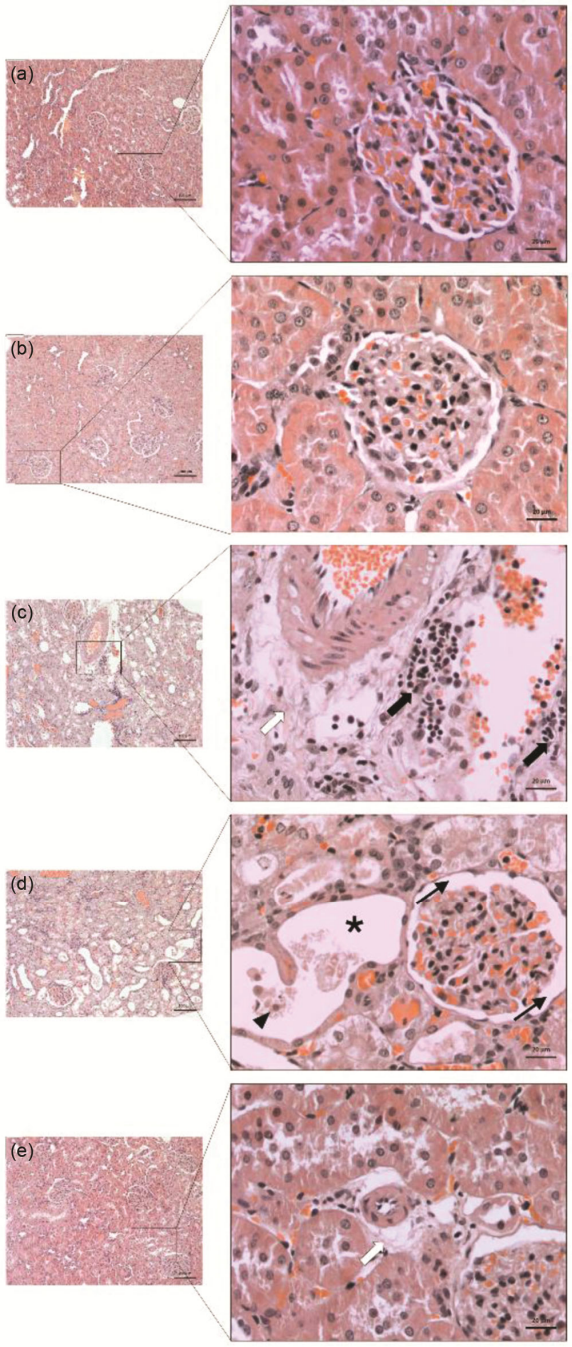

Fig. 4 — Representative photomicrographs of kidney sections stained with hematoxylin and eosin showing the effects of glycyrrhizic acid on cisplatin-induced histopathological changes. (A) Saline control group; (B) GA alone group; (C) Ethanol (vehicle for GA) group; (D) CDDP alone group; (E) GA + CDDP group. Note the marked interstitial congestion/hyperemia (thick white arrows), focal mononuclear cell infiltration (thick black arrows), tubular degeneration (asterisk) and areas of tubular cell necrosis (arrow head) and Bowman's space enlargement (thin arrows) in the CDDP alone group (D). GA treatment (E) visibly reduced these pathological changes compared to the CDDP group. Scale bar = 20 μm, magnification: 100x for small images, and scale bar = 100 μm, magnification: 400x for large images.

Table 1 — Histopathological scoring of kidney sections

Histopathological Parameters	Saline	GA	Ethanol	CDDP	GA+CDDP
Focal mononuclear cell infiltration in the interstitial region	0,25±0,16	0,43±0,20	1,86±0,14*	2,50±0,19*	1,28±0,18**
Congestion in the interstitial region	0,25±0,16	0,57±0,20	2,00±0,22*	2,88±0,12*	1,57±0,20**
Degenerative changes and focal necrosis in tubular epithelial cells	0,12±0,12	0,43±0,20	2,28±0,18*	2,50±0,19*	1,14±0,14**
Bowman's space enlargement and hyperemia	0,12±0,12	0,43±0,20	2,00±0,22*	2,88±0,12*	1,43±0,20**

Mean scores ± SEM for four renal histopathological parameters in each experimental group. Cisplatin (CDDP) administration significantly increased the scores for all parameters compared to the saline and GA groups, indicating pronounced kidney damage. Co-treatment with glycyrrhizic acid (GA) attenuated the CDDP-induced histopathological alterations, particularly in tubular epithelial cell degeneration and necrosis. *P<0.05 vs. Saline; **P<0.05 vs. CDDP.

congestion/hyperemia, and decreased inflammatory cell infiltration compared with the CDDP-only group (P<0.05 vs. CDDP), (Table 1), indicating a protective effect of GA against cisplatin-induced renal injury. Representative low- (100×) and high-magnification (400×) photomicrographs further illustrate these histopathological changes and the improvement observed with GA treatment (Fig. 4).

Discussion

This study provides compelling evidence for the renoprotective potential of GA against CDDP-induced nephrotoxicity, aligning with research highlighting the crucial role of inflammatory pathways in mediating renal injury induced by nephrotoxic agents[20–22]. Our study offers novel insights by demonstrating GA's ability to attenuate renal injury and suppress key inflammatory pathways in a rat model. Pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 are central to CDDP-induced nephrotoxicity, contributing to inflammation and oxidative stress, which are key mechanisms of kidney damage[23,24]. The innate immune system mediates this inflammatory response, with persistent inflammation markers observed in affected tissues[25]. Anti-inflammatory agents have shown promise for mitigating renal damage by reducing inflammation and oxidative stress[23]. Consistent with this, our study demonstrated that GA treatment ameliorated tissue levels of TNF-α and IL-1β. Similarly, Topcu *et al*. found that apelin-13 administration reduced IL-1β levels in a rat model of CDDP-induced nephrotoxicity[26], further supporting the importance of targeting this pro-inflammatory cytokine in mitigating renal injury.

Therapeutic potential of GA is further supported by its beneficial effects in various injury models[27,28]. For example, in a mouse model of Con A-induced hepatitis, GA reduced kidney inflammation through the IL-25/M2 pathway. This process encourages the development of type 2 macrophages, which help to suppress inflammatory responses[27]. Additionally, GA and its analogs inhibited inflammatory markers in HK-2 cells, indicating anti-inflammatory potential in septic acute kidney injury[28]. This aligns with traditional uses of Glycyrrhiza glabra for kidney disorders[29]. The observed nephroprotective activity may stem from its combined antioxidant, anti-inflammatory, and anti-apoptotic effects[29]. These findings are consistent with previous reports demonstrating renoprotective effects of GA against CDDP-induced nephrotoxicity in mice, primarily attributed to its antioxidant and anti-inflammatory properties[13–15]. Pretreatment with GA has been shown to reduce DNA fragmentation, restore antioxidant enzyme levels, and mitigate oxidative stress and DNA damage[13]. In cisplatin-treated mice, administration of licorice extract containing GA has been associated with improvements in both renal and hepatic function, pointing to its potential in mitigating organ damage[14]. Additionally, studies in mice have shown that GA administration enhances renal function, alleviates histopathological damage, and modulates oxidative stress and inflammatory signaling pathways[15]. In our rat model, GA administration led to reductions in serum creatinine and BUN levels, alongside improvements in renal histopathology, thereby confirming its efficacy in attenuating cisplatin-induced kidney damage. These findings reinforce the therapeutic potential of GA as a renoprotective agent in the context of cisplatin nephrotoxicity.

In our study, the ethanol vehicle group exhibited moderate tubular epithelial alterations and focal necrotic changes, as also reflected by the semi-quantitative histopathological scores. Although these findings indicate a modest vehicle-associated tissue effect, the severity of injury in the ethanol group

remained substantially lower than that observed in the cisplatin (CDDP) group, and did not indicate overt nephrotoxicity. Importantly, GA administration did not exacerbate the histopathological changes observed in the ethanol group, supporting that the renoprotective effects in the GA + CDDP group are attributable to GA-mediated protection against cisplatin injury rather than a vehicle-related confounding effect.

Pro-inflammatory cytokines such as IL-1β and TNF-α are recognized as key mediators of cisplatin-induced nephrotoxicity. These cytokines promote activation of the NF-κB signaling pathway, a well-established regulator of inflammatory responses that contributes to renal injury in cisplatin-induced nephrotoxicity[7,30]. Activation of NF-κB has been implicated as a key step in initiating the inflammatory cascade that leads to renal cell injury and death in cisplatin-induced nephrotoxicity[30]. Consistently, Pei *et al*. demonstrated that isoliquiritin, a Glycyrrhiza-derived compound, attenuated cisplatin-induced proximal tubular injury by counteracting oxidative stress and inflammatory responses[31]. Consistent with previous findings highlighting the involvement of NF-κB in mediating the nephrotoxic effects of CDDP, our study demonstrated that GA treatment decreased the tissue levels of NF-κB. This suggests that renoprotective effects of GA may be involved, at least in part, in the modulation of NF-κB signaling and the subsequent attenuation of the inflammatory cascade triggered by CDDP.

Although IL-18 has been implicated in CDDP-induced acute kidney injury[32,33], its precise role remains complex and has not been fully elucidated. Some studies have suggested a potential protective role for IL-18 signaling[33], whereas others have demonstrated its contribution to renal damage[34]. Specifically, Okui *et al*. showed that IL-18 deficiency protects mice from CDDP-induced acute kidney injury[34]. In contrast, our study did not observe any significant changes in IL-18 levels between treatment groups. This discrepancy could be attributed to several factors, including species differences (mice vs. rats), variations in CDDP dosage and administration protocols, and the specific timing of sample collection. Furthermore, the genetic background of the animals used can also influence the inflammatory response. These variations in experimental design can significantly impact observed cytokine levels and may account for the differences between our findings and previous reports. Therefore, while IL-18 may play a role in CDDP nephrotoxicity in some contexts, our data suggests that the renoprotective effects of GA in our rat model were not mediated by alterations in IL-18 levels.

TLRs, which are integral components of innate immunity, are expressed in healthy renal tissues and contribute to immune surveillance[35]. However, chronic TLR activation can contribute to various kidney diseases, including nephrotoxicity, lupus nephritis, and diabetic nephropathy. TLR4, in particular, plays a significant role in CDDP-induced nephrotoxicity[36,37] by mediating the inflammatory response and exacerbating renal damage[38]. CDDP directly binds to TLR4, triggering downstream pro-inflammatory pathways like NF-κB and MAPK, leading to the production of cytokines such as TNF-α and IL-1β[39]. This direct interaction distinguishes the effects of CDDP from those of other metal allergens. Our study demonstrated that GA significantly reduced TLR4 expression in the kidneys of CDDP-treated rats, further supporting its anti-inflammatory and renoprotective potential. Supporting this mechanism, Emara *et al.* reported that glycyrrhizic acid ameliorated nephrotoxicity through suppression of the TLR4/NF-κB signalling pathway[40], consistent with the anti-inflammatory effects observed in the present study. This aligns with studies showing the protective effects of other compounds, such as linalool and vincamine, which also modulate the TLR4-mediated pathways[7,41]. Although TLR9 has been implicated in other renal pathologies, such as immune complex-mediated nephritis[42] and ischemia-reperfusion injury[43], its role in CDDP-induced nephrotoxicity remains unclear. TLR9 can enhance pro-inflammatory responses[43], but also appears to play a regulatory role by promoting regulatory T cell recruitment during AKI[44]. We did not observe a significant change in TLR9 expression following GA treatment. Further research is required to fully elucidate TLR9's role in CDDP-induced nephrotoxicity.

KIM-1 is a sensitive biomarker for proximal tubular injury, a hallmark of CDDP-induced nephrotoxicity[45]. KIM-1 has demonstrated its value in preclinical and clinical studies as a more sensitive and specific marker for early CDDP-induced acute kidney injury compared to traditional markers such as serum creatinine[45-48]. Our study demonstrated significantly elevated urinary KIM-1 levels on day 5 in the CDDP group, confirming proximal tubular damage. Importantly, GA treatment significantly attenuated

this KIM-1 elevation, suggesting a protective effect against CDDP-induced tubular injury. This aligns with KIM-1's established role as a crucial biomarker for evaluating renal injury in various nephrotoxicity models[45,48]. Beyond its role as a biomarker, KIM-1 actively contributes to the pathophysiology of renal injury. It has been shown to enhance hypoxia-triggered inflammation in the tubulointerstitial region by facilitating the uptake of extracellular vesicles by tubular epithelial cells, thereby amplifying the inflammatory response and contributing to kidney injury progression[49]. The observed presence of KIM-1-positive tubules surrounded by macrophages further underscores its role in mediating inflammatory responses and highlights the potential of GA to modulate these responses. In our study, the reduction in urinary KIM-1 levels after GA treatment further strengthens the evidence for its renoprotective potential, specifically by attenuating CDDP-induced proximal tubular damage. This finding, consistent with KIM-1's established role as an injury biomarker, suggests GA protects the kidneys by preserving tubular integrity.

The observed attenuation of biochemical markers of renal injury, BUN and creatinine in the GA + CDDP group suggests that GA can effectively mitigate CDDP-induced renal dysfunction. Furthermore, the reduction in KIM-1, a marker of kidney tubular injury, in the GA + CDDP group suggests a protective effect against tubular damage. The reduced expression of key pro-inflammatory mediators such as IL-1β, TNF-α, NF-κB and TLR4 in the kidneys of GA-treated rats suggests that its anti-inflammatory properties play an important role in mediating its renoprotective effects against cisplatin-induced kidney injury. These findings are consistent with earlier reports emphasizing the role of pro-inflammatory cytokines in cisplatin-induced nephrotoxicity[1] and further support the hypothesis that the anti-inflammatory effect of GA plays a key role in renal protection. Histological evaluation provided additional support for these findings by confirming the preservation of renal architecture in GA-treated rats and indicating that GA may help restore cellular integrity following cisplatin exposure.

In addition to its anti-inflammatory properties, GA may exert renoprotective effects through alternative mechanisms. Evidence from prior studies indicates that GA can influence oxidative stress pathways, which are critically involved in cisplatin-induced nephrotoxicity[13,15]. Elucidating these supplementary mechanisms may contribute to a more complete understanding of how GA confers renal protection. In line with our findings, Wang *et al*. reported that magnesium isoglycyrrhizinate, a glycyrrhizic acid derivative, mitigated cisplatin-induced mitochondrial injury through SIRT3-mediated pathways[50], suggesting an additional mechanism contributing to renoprotection. Similarly, Zhou *et al*. reported that glycyrrhizic acid glycosides attenuated nephrotoxicity by preserving tubular integrity and modulating the RhoA/ROCK1 signalling pathway[51], supporting a broader renoprotective potential of glycyrrhizic acid-related compounds.

While the present study contributes to the growing body of knowledge on the renoprotective effects of GA, several limitations should be acknowledged. First, the experiments were conducted using a rat model, and additional studies are needed to determine whether these findings are applicable to humans. Second, the current work focused on a single GA dose and administration schedule; investigating alternative dosages and timing in relation to cisplatin exposure may help optimize therapeutic outcomes. Third, although improvements in biochemical and histological parameters were evident, the precise molecular pathways through which GA exerts its protective effects remain to be clarified. In particular, further exploration of GA's interaction with specific signaling cascades related to inflammation and oxidative stress could enhance mechanistic understanding. Lastly, long-term investigations are necessary to evaluate both the durability of GA's protective effects and its potential for delayed adverse outcomes.

Despite these limitations, the results of this study indicate that GA has the potential to mitigate cisplatin-induced nephrotoxicity. However, further research, including well-designed clinical trials, will be required to confirm these findings and assess their relevance to human health.

Conclusion

This study demonstrated that glycyrrhizic acid has renoprotective effects in a rat model of cisplatin-induced nephrotoxicity. GA treatment led to reductions in serum creatinine, BUN, and pro-inflammatory mediators, along with preservation of renal structure, indicating protection of both function and tissue integrity. Although this work focused on cisplatin toxicity, the observed effects of GA may also be relevant in other forms of drug-induced kidney

injury. Further research should examine long-term outcomes and clarify how GA modulates pathways related to inflammation, oxidative stress, and cell death. Investigating its combined use with other nephroprotective agents may support the development of improved treatment approaches. Further clinical research is needed to determine whether GA can be safely translated into supportive care during chemotherapy.

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Conflict of interest

The authors declare no conflict of interest.

Authors' Contributions

All authors contributed to the conception and design of the study. Laboratory experiments and data collection were conducted by E.T., B.G., and O.C. Data analysis was carried out by E.T. and C.S. The manuscript was drafted by E.T. and critically reviewed by all authors. All authors approved the final version of the manuscript.

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