

Research Article:

Nephroprotective Effects of *Citrus spp.* Fruit-Cuticle Extracts, Melatonin, and Hydrochlorothiazide on HgCl₂-Induced Acute Renal Failure in Albino Rats

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Abstract

Background: The management of renal failure is a global challenge and roles of medicinal plants in treating acute renal failure (ARF) are still under consideration. Most research focused on their benefits in chronic kidney disease (CKD), leaving a gap in how these natural remedies could help in ARF management.

Aim: To find out how *Citrus sinensis*, *Citrus limon* fruit cuticles and melatonin protect the kidneys from HgCl₂-induced nephrotoxicity at rat model and comparing them with routine-diuretic thiazide drug.

Patients and Methods: Thirty-six healthy male Wistar rats were evenly allocated into six groups (n=6): Control, Model (ARF), *Citrus limon* Extract (CLE), *Citrus sinensis* Extract (CSE), Melatonin (MEL), and Hydrochlorothiazide (HCTZ). The rats were provided with regular food and water ad libitum. except for the control group, both the model and treatment groups were injected with HgCl₂ every 72 hours for a duration of three weeks to induce ARF. Treatment protocols were assigned to the treatment groups throughout the trial duration. At the end of the study, serum renal biomarkers including Urea, Creatinine, Uric acid, Electrolytes (Na⁺, K⁺, and Cl⁻), Packed Cell Volume, and Malondialdehyde (MDA) were quantified from blood samples.

Result: HgCl₂-treated animals exhibited a substantial elevation (p<0.05) in serum urea, creatinine, and MDA levels, alongside a non-significant reduction in serum Na⁺ and PCV levels, indicating HgCl₂-induced renal impairment in comparison to control rats. Moreover, treatments of CLE and CSE, MEL and HCTZ in conjunction with HgCl₂ significantly (P<0.05) ameliorated HgCl₂-induced nephrotoxicity, in comparison to model rats, indicating a reduction in HgCl₂'s toxic effects and safeguarding of renal tubules from oxidative stress.

Conclusion: All treatments from the current study showed a mild renal protective effect across biomarkers of urea, creatinine and MDA and CSE showed most significant potential effect.

Keywords: Mercury Chloride, Nephrotoxicity, Renal failure, *Citrus sinensis*, *Citrus limon*, Melatonin and Hydrochlorothiazide.

1. Introduction

Acute renal failure (ARF) is a critical clinical condition marked by a rapid decline in glomerular filtration rate, leading to the accumulation of nitrogenous waste products and disturbances in electrolyte and fluid balance [1]. Despite progress in supportive care, the burden of ARF worldwide remains significant with high rates of morbidity

and mortality [2, 3]. It is estimated to be the cause of 1.7 million deaths annually and over 13.3 million new cases of ARF are reported every year, making renal disease one of the top ten leading causes of death in the globe [4]. A significant and frequently undetected cause of ARF is exposure to heavy metals, notably mercury, particularly in the occupational and environmental contexts [5].

Mercury chloride (HgCl₂) has been extensively investigated for its ability to induce renal injury through a cas-

cade of oxidative stress mechanisms that include the generation of reactive oxygen species (ROS), depletion of intracellular glutathione and inhibition of antioxidant defense mechanisms, resulting in DNA damage, lipid peroxidation, and cellular apoptosis, tubular necrosis and mitochondrial dysfunction [6, 7]. Therefore, usage of antioxidant-rich medicines has become promising approach to attenuate mercury-induced kidney impairment. Due to significant levels of flavonoids, essential oils and phenolic compounds, phytochemicals have been demonstrated to have strong antioxidant, anti-inflammatory and metal-chelating activities [8]. Sweet orange (*Citrus sinensis*) and lemon (*Citrus limon*) are rich in flavanones such as hesperidin, naringin and naringenin, which have demonstrated powerful antioxidant activities [9].

Another bioactive molecule, the pineal indoleamine melatonin, has well-characterized antioxidant and cytoprotective properties [10]. Its distinctive amphiphilic nature enables it to cross cell membranes and directly scavenge free radicals, making it very effective in reducing ROS-mediated renal failure [11]. Thiazide diuretics such as hydrochlorothiazide (HCTZ) are extensively utilized in clinical settings for the management of hypertension and edema. Although not primarily known for antioxidant effects [12]. Its use has been proposed for improving nephroprotection through reduction of functional and structural renal impairment. HCTZ is cost-effective, however deleterious effects may occur with long-term use.

Considering the complex pathophysiology of HgCl_2 -induced kidney injury, treatment with phytochemicals from citrus fruit cuticle, natural antioxidants and traditional diuretics may be protective. Hence, the present investigation was designed to evaluate the nephroprotective efficacy of *Citrus sinensis*, *Citrus limon* fruit cuticles, melatonin and HCTZ in albino rats with HgCl_2 produced ARF. The present work is aimed to investigate the therapeutic potential of phytomedicine and adjunct pharmacologic drugs in the management of renal damage generated by toxins.

2. Materials and Methods

2.1. Plant Materials

Fruits of *Citrus spp.* were collected from Sulaymaniyah local market in January 2025. The peel parts were separated and desiccated in an oven at 60 °C for 48 hours, away from sunlight, then pulverized and provided as powdered plant samples.

2.2. Preparation of plant extracts

Powders from the peels of *Citrus sinensis* and *Citrus limon* were extracted as described by [13] with 95% ethanol using a Soxhlet extractor (100 ml. Version, China). After comprehensive extractions, the extract was filtered and completely concentrated utilizing a rotary evaporator distillation method [14]. The yields (28 gm) were preserved in an airtight dark container in the refrigerator until required.

2.3. Animals and Housing

The present investigation was performed on mature male albino rats (*Rattus norvegicus*) weighing 195-210 grams, 8 weeks old. To avoid physiological changes associated with the estrous cycle, which repeats every 4-6 days, male rats were used [15]. The experiment was conducted from January to February 2025 at Komar University of Science and Technology.

The animals were housed in plastic enclosures lined with wood shavings. Throughout the trial duration, six animals were housed per cage. The animals were maintained under conventional laboratory settings with a 12-hour light and 12-hour dark photoperiod, at a temperature of 22 ± 4 °C, and were provided with food and water ad libitum in accordance with the study design. All experimental protocols were evaluated and sanctioned by the Animal Research Ethics Committee of the College of Science, Raparin University, Sulaymaniyah-Ranya, Iraq.

2.4. Toxicity investigations

Toxicity tests for each fruit cuticle extract were conducted on six randomly selected animals. Rats were subjected to overnight fasting, then exposed to 1 mL plant extract (25 mg/Kg BW), the dose was delivered orally via gavage. The animals were then monitored constantly for three hours, thereafter, any nighttime mortality was confirmed.

2.5. Induction of renal injury

ARF was induced by administering HgCl_2 solution in experimental rats via intraperitoneal route (5 mg per kg of body weight) every 72 hours for duration of 21 days. The current study aimed to investigate the effects of *Citrus sinensis* and *Citrus limon* fruit cuticles, melatonin, and HCTZ on HgCl_2 -induced nephrotoxicity in albino rats. The 36 experimental rats were randomly divided into six groups (n = 6) and subjected to the following treatments:

Group 1: Control (CT): received standard rat diet and drinking water for three weeks.

Group 2: Model (Nephrotoxicity): received HgCl_2 (5 mg/kg BW, i.p.) [16] every 72 hours for 3 weeks.

Group 3: CLE: received CLE (25 mg/kg body weight, 6 days/ week) via oral gavage for 3 weeks, concurrently received HgCl_2 (5 mg/kg body weight i.p.) every 72 hours.

Group 4: CSE: received CSE (25 mg/kg body weight, 6 days/ week) via oral gavage for 3 weeks, concurrently received HgCl_2 (5 mg/kg body weight i.p.) every 72 hours.

Group 5: MEL: received MEL (10 mg/kg body weight, 6 days/ week) via oral gavage for 3 weeks, concurrently received HgCl_2 (5 mg/kg body weight i.p.) every 72 hours.

Group 6: HCTZ: received HCTZ (10 mg/kg body weight, 6 days/ week) via oral gavage for 3 weeks, concurrently were received HgCl_2 (5 mg/kg body weight i.p.) every 72 hours.

Rats have unrestricted access to food and drinking water. The control group rats were received an equivalent volume of distilled water via oral gavage for three weeks. Body

weights were measured weekly to ascertain the administration doses throughout the study period.

2.6. Blood sample collections

At the study's completion, all rats were exposed to an overnight fasting followed by anesthesia with a ketamine-xylazine combination (90 mg/kg, i.p. and 10 mg/kg, i.p., respectively) [17]. Blood samples were then collected using cardiac puncture and transferred to sterile EDTA tubes for PCV testing after 10 minutes of gentle inversion and to gel tubes for 15 minutes at room temperature for complete clot formation, followed by a centrifugation at 3000 rpm for 15 minutes. The serum acquired was stored at deep freeze and were analyzed for biochemical parameters using a fully automated analyzer (cobas-6000).

2.7. Statistical analysis

The corresponding data of this investigation was analyzed using a computer program (GraphPad Prism software, Version 9.0). Results were expressed as mean $\pm$ standard error (mean $\pm$ SE). One-way ANOVA was followed by Tukey's multiple comparison test to determine

group differences with a value of $P < 0.05$ considered to be statistically significant.

3. Results and Discussion

The present study showed that repeated intraperitoneal administration of HgCl_2 successfully induced acute renal injury in rats as evidenced by marked elevations in serum urea, creatinine and malondialdehyde (MDA) levels together with mild disturbances in electrolyte balance and PCV in comparisons with control rats. Most of these biochemical abnormalities were greatly mitigated by co-administration of CLE, CSE, MEL and HCTZ showing various levels of nephroprotection against mercury-induced damage. Among all treatments, CSE exhibited the most prominent restorative effect on oxidative stress and renal biomarkers. (Table 1; Figures 1 and 2). HgCl_2 -induced acute renal failure is multifactorial, involving oxidative stress via ROS generation and GSH depletion, mitochondrial and transport dysfunction, and inflammation-mediated cytotoxicity [18]. These mechanisms synergistically impair renal filtration and tubular function, resulting in acute tubular necrosis and renal failure [19, 20].

Table 1: Effects of CLE, CSE, MEL, and HCTZ on serum biochemical parameters in HgCl_2 -induced nephrotoxicity in rats.

Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. A value of $P < 0.05$ was considered statistically significant. Significance levels are indicated as follows:

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. Superscript (a) indicates significantly different from the control group ($P < 0.05$)

Serum Parameters	HgCl ₂ -Induced Nephrotoxicity Groups					
	Control	Model	CLE	CSE	MEL	HCTZ
Urea (mg/dL)	40.27 $\pm$ 1.53	139.80 $\pm$ 25.83 ****	44.95 $\pm$ 2.58 ****	48.65 $\pm$ 3.52 ****	24.28 $\pm$ 1.62 ****	24.60 $\pm$ 1.90 ****
Creatinine (mg/dL)	0.32 $\pm$ 0.03	1.79 $\pm$ 0.19 ****	0.33 $\pm$ 0.02 ****	0.54 $\pm$ 0.16 ****	0.42 $\pm$ 0.02 ****	0.37 $\pm$ 0.01 ****
Uric acid (mg/dL)	1.13 $\pm$ 0.09	1.65 $\pm$ 0.23	1.90 $\pm$ 0.18	1.40 $\pm$ 0.12	1.93 $\pm$ 0.21	2.20 $\pm$ 0.26
Na ⁺ (mmol/L)	139.6 $\pm$ 0.59	137.60 $\pm$ 0.96	139.80 $\pm$ 0.83	140.0 $\pm$ 1.55	140.30 $\pm$ 0.02	140.90 $\pm$ 0.23 *
K ⁺ (mmol/L)	4.97 $\pm$ 0.16	5.53 $\pm$ 0.40	4.92 $\pm$ 0.32	4.18 $\pm$ 0.91 *	5.20 $\pm$ 0.39	5.03 $\pm$ 0.30
Cl ⁻ (mmol/L)	99.43 $\pm$ 0.40	100.63 $\pm$ 0.84	102.60 $\pm$ 0.55	102.20 $\pm$ 2.43	102.50 $\pm$ 0.84	103.10 $\pm$ 1.14
PCV (%)	39.67 $\pm$ 1.66	38.00 $\pm$ 1.12	32.17 $\pm$ 1.70	41.17 $\pm$ 1.81	39.33 $\pm$ 2.09	38.00 $\pm$ 2.74
MDA (μ mol/l)	1.84 $\pm$ 1.90 ^a	3.68 $\pm$ 0.08 ****	2.36 $\pm$ 0.24 ***	2.17 $\pm$ 0.25 ****	2.44 $\pm$ 0.23 ***	2.69 $\pm$ 0.08 **

The significant increase in serum urea and creatinine observed in HgCl_2 -treated rats reflects impaired glomerular filtration and renal tubular dysfunction. In the current study, serum urea and creatinine increased in HgCl_2 model group in comparisons with control group (Table 1; Figure 1A and 1B). These results are consistent with earlier reports indicating that mercury preferentially accumulates in the proximal tubular epithelial cells by organic anion and amino acid transport systems, where Hg^{2+} exhibits strong

binding to sulfhydryl-containing proteins and glutathione, resulting in mitochondrial dysfunction, tubular necrosis and reduction in glomerular filtration rate (GFR). Mercury exposure also impairs membrane permeability and inhibits Na^+/K^+ -ATPase activity, impairing renal excretory function and tubular transport. Studies by [21, 22] confirmed that mercuric chloride induces acute renal failure primarily through oxidative injury and proximal tubular degeneration.

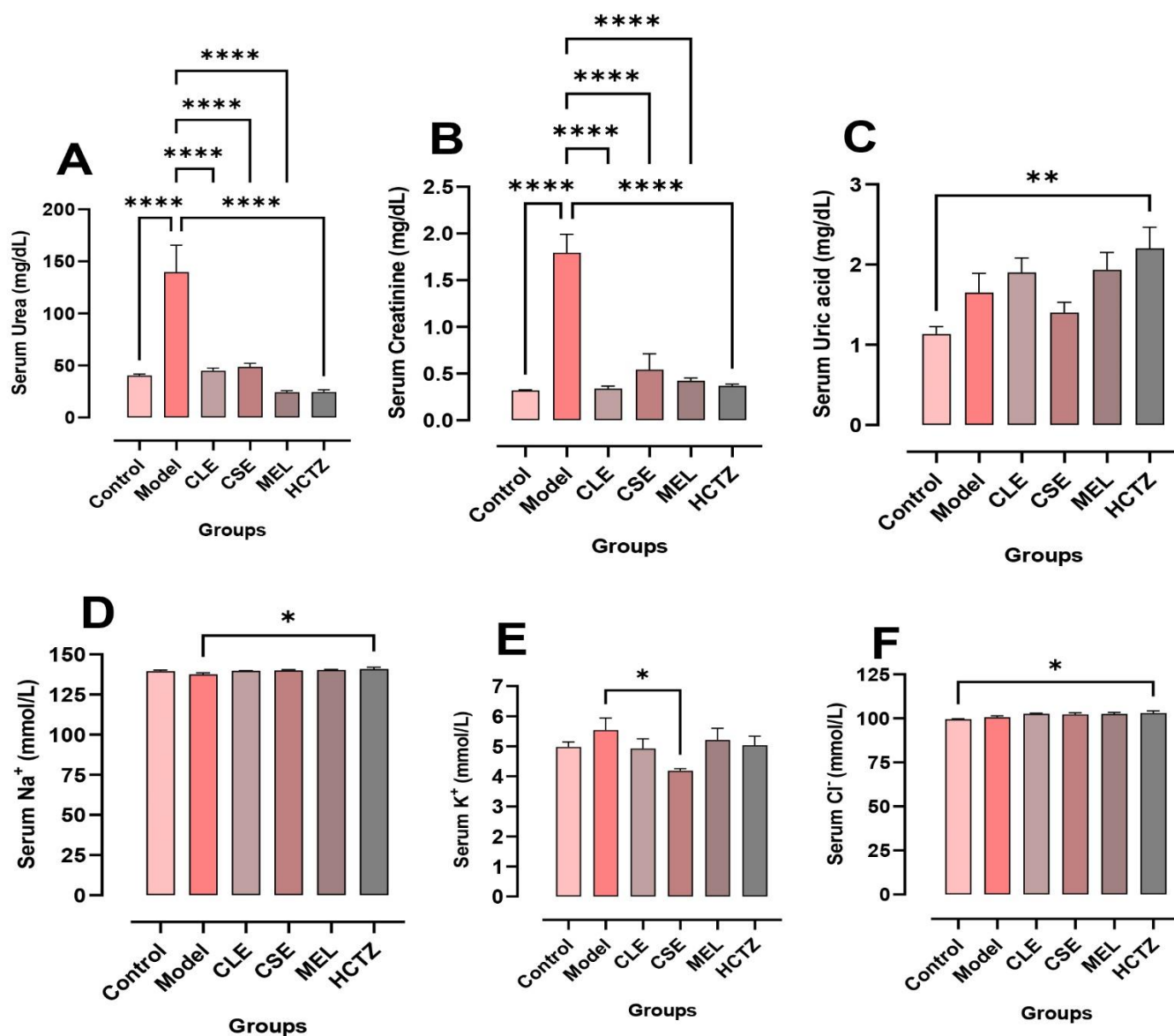


Figure 1: Influences of CLE, CSE, MEL, and HCTZ on serum biochemical parameters in HgCl₂-induced nephrotoxicity in rats. (A) serum urea, (B) serum creatinine, (C) serum uric acid, (D) serum Na⁺, (E) serum K⁺, and (F) serum Cl⁻ levels. Data are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. A value of $P < 0.05$ was considered statistically significant. Significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; "ns" indicates non-significant differences.

Treatment with CLE, CSE, MEL and HCTZ significantly reduced serum urea and creatinine levels in comparison to the HgCl₂-only group (Figures 1A and 1B). This shows a nephroprotective effect probably mediated by antioxidant and hemodynamic processes [23]. The substantial decrease in MDA values also offers more evidence about the ability of these medicines to inhibit lipid peroxidation and maintain the integrity of cell membranes against oxidative stress. This finding supports the well-known reno-protective actions of citrus flavanones like as hesperidin and naringenin. These bioactive compounds

have been proved to protect against mercury-induced renal damage via various mechanisms, including scavenging of ROS, boost of endogenous antioxidant defenses like superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), inhibition of inflammatory signaling pathways including NF- κ B and TNF- α , stabilization of mitochondrial function, and chelation of mercury ions. Collectively, these actions contribute to the restoration of renal redox homeostasis and the preservation of kidney structure and function [24, 25].

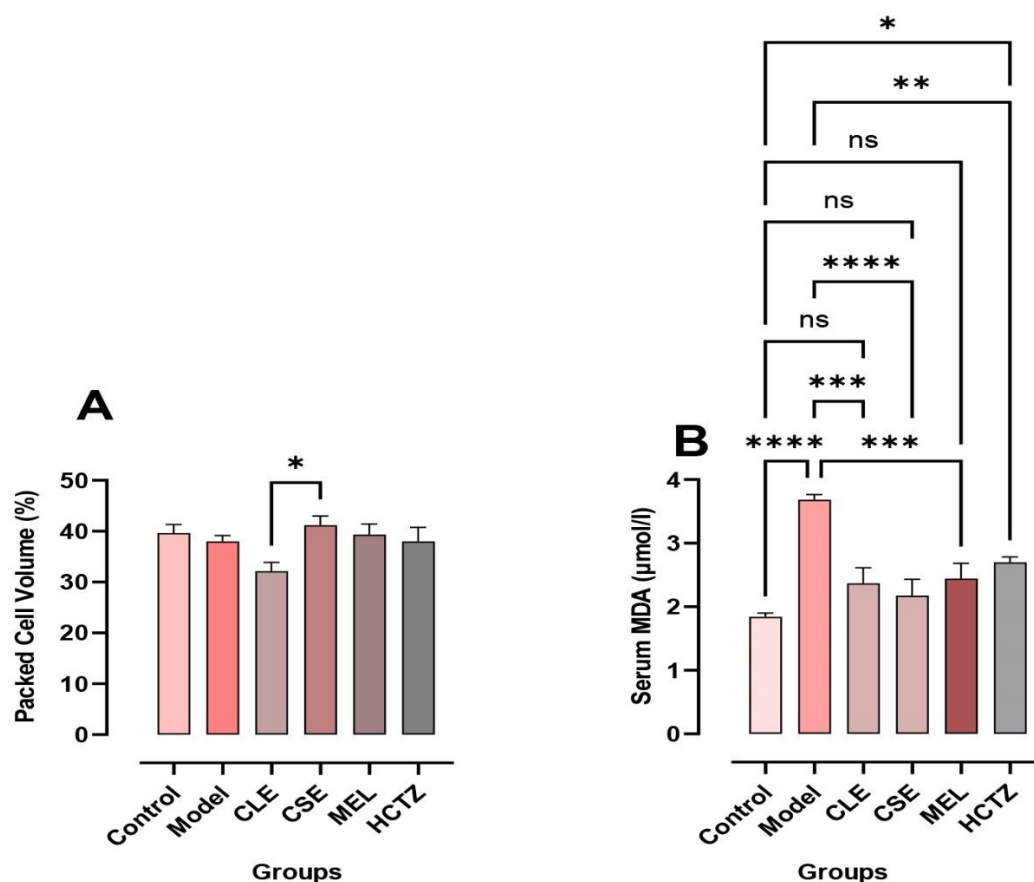


Figure 2: Influences of CLE, CSE, MEL, and HCTZ on packed cell volume (A) and oxidative stress marker (MDA) (B) in HgCl_2 -induced renal injury rats. Results are expressed as mean $\pm$ SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. A value of $P < 0.05$ was considered statistically significant. Significance levels are indicated as follows: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****); "ns" indicates non-significant differences.

The greater efficacy of CSE over CLE may depend on the different phytochemical content and antioxidant power of *Citrus sinensis* and *Citrus limon*. Sweet orange peel contains hesperidin and polymethoxyflavones, which are known to have higher free radical scavenging and anti-inflammatory properties than numerous other citrus bioactives. Moreover, citrus essential oils can maintain the mitochondrial membranes and prevent the oxidative damage of mercury exposure. This mechanism may be contributing to the more significant decrease in serum MDA in the CSE-treated group (Figure 2B).

In rats treated with HgCl_2 , the serum uric acid levels were moderately increased, but these changes were not statistically significant. Interestingly, CSE treatment slightly decreased uric acid levels (Table 1; Figure 1C) according to study [26]. These results may reflect a moderate uricosuric or xanthine oxidase-inhibitory action, but further studies should be performed to clarify this mechanism. Sodium (Na^+) in the model group showed a mild non-significant decrease and all treatments showed an upward trend towards normalization. HCTZ caused the greatest sodium restoration which is consistent with its well-known natriuretic effects and established mechanism of inhibition of the sodium-chloride cotransporter in the distal convoluted tubule, thus promoting natriuresis [27]. HCTZ provided a remarkable level of nephroprotection,

even though it is not traditionally considered an antioxidant. HCTZ is a thiazide diuretic that primarily inhibits the sodium-chloride cotransporter (NCC) in the distal convoluted tubule, leading to decreased sodium and chloride reabsorption and increased natriuresis and diuresis. In addition, HCTZ causes hypocalciuria. It decreases intracellular sodium, indirectly leading to increased calcium reabsorption. Furthermore, the increased sodium load to the collecting duct stimulates aldosterone mediated processes, further affecting sodium retention and potassium excretion [28].

There were no significant differences in potassium (K^+) and chloride (Cl^-) levels among experimental groups (Table 1; Figures 1E and 1F) showing minor tubular damage. Interestingly, the PCV was dramatically lowered in the CLE treated group, which may be attributed to its diuretic or volume regulating effects. The reductions in PCV for the other treatment groups were not statistically significant, however, indicating a modest hematological impact at the doses administered (Table 1; Figure 2A). Hesperidin, naringenin and melatonin are rich in antioxidants and are known to improve endogenous antioxidant defenses, therefore decreasing oxidative insult to RBCs and maintaining their lifespan and function. This antioxidant buffering likely kept RBCs alive and prevented significant variations in PCV [29].

The present investigation showed that melatonin has an active role in renal protection by its capacity to decrease creatinine and MDA levels efficiently (Figures 1.B and 2B). Melatonin exerts powerful cytoprotective effects that counteract pathophysiological processes induced by mercury as stated by study [30] that melatonin upregulates glutathione peroxidase (GPx), SOD, and CAT, which are typically downregulated during HgCl₂ toxicity. It also maintains intracellular glutathione (GSH) levels and restores redox homeostasis in renal tissues. In addition, melatonin is amphiphilic, enabling it to pass through cellular and mitochondrial membranes freely and penetrate intracellular compartments rapidly. This allows for the direct scavenging of reactive species such as hydroxyl radicals ($\cdot\text{OH}$) and peroxynitrite (ONOO^-). Melatonin possesses a remarkable ability to trigger a 'scavenger cascade' via its metabolites AFMK and AMK, which themselves are capable of neutralizing reactive species even beyond the capability of the parent chemical [31].

In the present study, serum sodium levels showed a mild non-significant decline following HgCl₂ administration, whereas treatment groups demonstrated partial restoration toward normal values (Figure 1D) Hyponatremia in mercury toxicity may result from impaired tubular sodium reabsorption secondary to proximal tubular damage and inhibition of Na⁺/K⁺-ATPase activity. HCTZ administration was associated with the largest sodium restoration, likely due to its pharmacologic effects on renal electrolyte transport and renal hemodynamics. Hydrochlorothiazide inhibits the Na⁺/Cl⁻ cotransporter in the distal convoluted tubule, changing sodium handling and lowering intraglomerular pressure. Although HCTZ is not primarily considered an antioxidant agent, emerging evidence suggests that thiazide diuretics may indirectly reduce renal oxidative stress by improving renal perfusion and decreasing tubular workload under nephrotoxic conditions. Study [28] suggested that improved hemodynamic stability may contribute to preservation of renal function in toxic renal injury.

The potential mechanism of Citrus peel extracts in restoring renal indicators reveals that bioactive substances like flavonoids, phenolic acids and essential oils have considerable protective effects against HgCl₂-induced kidney injury, at least partly, via improving endogenous antioxidant defense mechanisms. Study [32] reported that Citrus peels are rich in flavonoids, phenolic acids, and essential oils, which collectively contribute to significant antioxidant, anti-inflammatory, and metal-chelating effects. These compounds protect renal tissues from oxidative and inflammatory damage, especially under conditions of heavy metal exposure.

4. Conclusions

The study investigated the potential nephroprotective effects of *Citrus sinensis* and *Citrus limon* fruit cuticle extracts, melatonin, and hydrochlorothiazide in a rat model of HgCl₂-induced acute renal failure. Our findings demonstrate that co-administration of citrus peel extracts and melatonin with HgCl₂ significantly

attenuated renal damage through reducing serum urea, creatinine, and malondialdehyde levels. CSE showed the most prominent protective effect, suggesting a high content of bioactive antioxidant compounds capable of mitigating HgCl₂-induced renal oxidative injury. The study suggests that natural antioxidants and phytochemicals can modulate renal responses during acute toxic exposure.

Limitations of this study

A few limitations should be acknowledged in this study. The relatively small sample size (n = 6) may limit statistical power and generalizability. The study relied primarily on biochemical assessments, without incorporating advanced molecular analyses: e.g., apoptotic, mitochondrial, or gene expression pathways) to fully elucidate the underlying mechanisms of protection. Additionally, the dose response relationships were not conducted. Finally, long-term safety and efficacy were not investigated.

Conflict of interest

The authors declare no conflict of interest in this study.

Declaration of Generative AI Use

The authors used generative artificial intelligence (AI) tools solely for English language editing, grammar improvement, and manuscript formatting assistance. All scientific information, interpretations analysis and findings were independently created and verified by the authors.

5. References

- [1] M. Ostermann, N. Lumlertgul, R. Jeong, E. See, M. Joannidis, and M. James, "Acute kidney injury," *The Lancet*, vol. 405, no. 10474, pp. 241–256, 2025: [https://doi.org/10.1016/S0140-6736\(24\)02385-7](https://doi.org/10.1016/S0140-6736(24)02385-7).
- [2] X. Xu *et al.*, "Acute kidney injury: pathogenesis and therapeutic interventions," *Molecular biomedicine*, vol. 6, no. 1, p. 61, 2025: <https://doi.org/10.1186/s43556-025-00293-4>.
- [3] D. Ponce, N. Kazan, A. Pereira, and A. Balbi, "Acute kidney injury: risk factors and management challenges in low-and middle-income countries," *NEPHROLOGY*, vol. 8, no. 1, pp. 60–67, 2020: <https://doi.org/10.2147/IJNRD.S104209>.
- [4] A. Zuk and J. V. Bonventre, "Acute kidney injury," *Annual review of medicine*, vol. 67, no. 1, pp. 293–307, 2016: <https://doi.org/10.1146/annurev-med-050214-013407>.
- [5] P. Lentini, L. Zanolli, A. Granata, S. S. Signorelli, P. Castellino, and R. Dellaquila, "Kidney and heavy metals-The role of environmental exposure," *Molecular medicine reports*, vol. 15, no. 5, pp. 3413–3419, 2017: <https://doi.org/10.3892/mmr.2017.6389>.
- [6] C. G. Y. Paul B Tchounwou, Anita K Patlolla, and Dwayne J Sutton, "Heavy Metals Toxicity and the Environment," *NIH*, vol. 1, pp. 1–26, 2012, doi: https://doi.org/10.1007/978-3-7643-8340-4_6.
- [7] C. Caglayan, F. M. Kandemir, S. Yildirim, S. Kucukler, and G. Eser, "Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level,

- oxidative stress, apoptosis and inflammation in rats," *Journal of Trace Elements in Medicine and Biology*, vol. 54, pp. 69-78, 2019/07/01/ 2019, doi: <https://doi.org/10.1016/j.jtemb.2019.04.007>.
- [8] V. Unsal, "Natural Phytotherapeutic Antioxidants in the Treatment of Mercury Intoxication-A Review," (in eng), *Adv Pharm Bull*, vol. 8, no. 3, pp. 365-376, Aug 2018, doi: <https://doi.org/10.15171/apb.2018.043>.
- [9] M. A. Alam, N. Subhan, M. M. Rahman, S. J. Uddin, H. M. Reza, and S. D. Sarker, "Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action," (in eng), *Adv Nutr*, vol. 5, no. 4, pp. 404-17, Jul 2014, doi: <https://doi.org/10.3945/an.113.005603>.
- [10] M. Markowska, S. Niemczyk, and K. Romejko, "Melatonin Treatment in Kidney Diseases," (in eng), *Cells*, vol. 12, no. 6, Mar 8 2023, doi: <https://doi.org/10.3390/cells12060838>.
- [11] A. Tarocco *et al.*, "Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care," *Cell Death & Disease*, vol. 10, no. 4, p. 317, 2019/04/08 2019, doi: <https://doi.org/10.1038/s41419-019-1556-7>.
- [12] W. Jo, E. S. Koh, and S. Chung, "Therapeutic roles of thiazides and loop diuretics in blood pressure control and renal protection against chronic kidney disease," *Clin Hypertens*, vol. 29, no. 1, 0/ 2023, doi: <https://doi.org/10.1186/s40885-023-00238-5>.
- [13] Z. H. Ibrahim, "Anti-obesity effects of Arum maculatum, Nasturtium officinale plant extracts and exercise in high fat diet-induced obese rats," *Kurdistan Journal of Applied Research*, vol. 6, no. 2, pp. 190-198, 2021: <https://doi.org/10.24017/science.2021.2.18>.
- [14] N. Bennour, H. Mighri, H. Eljani, T. Zammouri, and A. Akrou, "Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of Moringa oleifera cultivated in Southern Tunisia," *South African Journal of Botany*, vol. 129, pp. 181-190, 2020: <https://doi.org/10.1016/j.sajb.2019.05.005>.
- [15] K. Mahmood and S. Kadir, "Protective Effects of Exogenous Melatonin on Cisplatin-Induced Acute Nephrotoxicity in Rats," *Journal of Zankoy Sulaimani-Part A*, vol. 19, no. 1, pp. 17-26, 2017: <https://doi.org/10.17656/jzs.10581>.
- [16] P. R. Augusti *et al.*, "Effect of lycopene on nephrotoxicity induced by mercuric chloride in rats," *Basic & clinical pharmacology & toxicology*, vol. 100, no. 6, pp. 398-402, 2007: <https://doi.org/10.1111/j.1742-7843.2007.00067.x>.
- [17] Z. O. Khudhur and I. M. Maulood, "Lead acetate deteriorates the improvement effect of L-arginine and tetrahydrobiopterin on endothelin-1 receptors activity in rat aorta," *Baghdad Science Journal*, vol. 20, no. 5, p. 43, 2023: <https://doi.org/10.21123/bsj.2023.7594>.
- [18] E. C. de Almeida *et al.*, "Metalloproteomic Investigation of Hg-Binding Proteins in Renal Tissue of Rats Exposed to Mercury Chloride," *International Journal of Molecular Sciences*, vol. 25, no. 1, p. 164, 2023: <https://doi.org/10.3390/ijms25010164>.
- [19] S. E. Orr and C. C. Bridges, "Chronic kidney disease and exposure to nephrotoxic metals," *International journal of molecular sciences*, vol. 18, no. 5, p. 1039, 2017: <https://doi.org/10.3390/ijms18051039>.
- [20] E. Onan, S. Ulu, and Ö. Güngör, "Heavy Metals and Kidney," *Turk J Nephrol*, vol. 33, no. 3, pp. 244-251, 2024: <https://doi.org/10.5152/turkjnephrol.2024.22497>.
- [21] R. S. Almeer, G. Albasher, F. Alotibi, S. Alarifi, D. Ali, and S. Alkahtani, "Ziziphus spina-christi Leaf Extract Suppressed Mercury Chloride-Induced Nephrotoxicity via Nrf2-Antioxidant Pathway Activation and Inhibition of Inflammatory and Apoptotic Signaling," *Oxidative medicine and cellular longevity*, vol. 2019, no. 1, p. 5634685, 2019: <https://doi.org/10.1155/2019/5634685>.
- [22] C. C. Bridges and R. K. Zalups, "Mechanisms involved in the transport of mercuric ions in target tissues," *Archives of toxicology*, vol. 91, no. 1, pp. 63-81, 2017: <https://doi.org/10.1007/s00204-016-1803-y>.
- [23] M. Kahramanoğullari, M. Erişir, M. Yaman, and T. Parlak Ak, "Effects of naringenin on oxidative damage and apoptosis in liver and kidney in rats subjected to chronic mercury chloride," *Environmental Toxicology*, vol. 39, no. 5, pp. 2937-2947, 2024: <https://doi.org/10.1002/tox.24164>.
- [24] A. M. Mahmoud, R. J. Hernandez Bautista, M. A. Sandhu, and O. E. Hussein, "Beneficial effects of citrus flavonoids on cardiovascular and metabolic health," *Oxidative medicine and cellular longevity*, vol. 2019, no. 1, p. 5484138, 2019: <https://doi.org/10.1155/2019/5484138>.
- [25] A. Abd-Eltawab Tammam *et al.*, "Hesperidin protects rats' liver and kidney from oxidative damage and physiological disruption induced by nickel oxide nanoparticles," *Frontiers in Physiology*, vol. 13, p. 912625, 2022, doi: <https://doi.org/10.3389/fphys.2022.912625>.
- [26] A. Ota-Kontani, H. Hirata, M. Ogura, Y. Tsuchiya, and M. Harada-Shiba, "Comprehensive analysis of mechanism underlying hypouricemic effect of glucosyl hesperidin," *Biochemical and biophysical research communications*, vol. 521, no. 4, pp. 861-867, 2020: <https://doi.org/10.1016/j.bbrc.2019.10.199>.
- [27] N. J. Frenkel *et al.*, "Thiazide-induced hyponatraemia is associated with increased water intake and impaired urea-mediated water excretion at low plasma antidiuretic hormone and urine aquaporin-2," *Journal of Hypertension*, vol. 33, no. 3, pp. 627-633, 2015, doi: <https://doi.org/10.1097/hjh.0000000000000423>.
- [28] Y. Zhao *et al.*, "Structural bases for Na⁺-Cl⁻ cotransporter inhibition by thiazide diuretic drugs and activation by kinases," *Nature Communications*, vol. 15, no. 1, p. 7006, 2024, doi: <https://doi.org/10.1038/s41467-024-51381-y>.
- [29] R. Vinodhini and M. Narayanan, "The impact of toxic heavy metals on the hematological parameters in common carp (Cyprinus carpio L.)," *Journal of Environmental Health Science & Engineering*, vol. 6, no. 1, pp. 23-28, 2009, doi: <https://doi.org/10.5555/20093168474>.
- [30] G. Şener, A. Ö. Şehirli, and G. Ayanog˘lu-D˘ulger, "Melatonin protects against mercury (II)-induced oxidative tissue damage in rats," *Pharmacology & toxicology*, vol. 93, no. 6, pp. 290-296, 2003, doi: <https://doi.org/10.1111/j.1600-0773.2003.pto930607.x>.
- [31] R. Kołodziejska *et al.*, "Melatonin—A Powerful Oxidant in Neurodegenerative Diseases," *Antioxidants*, vol. 14, no. 7, p. 819, 2025, doi: <https://doi.org/10.3390/antiox14070819>.
- [32] X. Lu, C. Zhao, H. Shi, Y. Liao, F. Xu, H. Du, H. Xiao, and J. Zheng, "Nutrients and bioactives in citrus fruits: Different citrus varieties, fruit parts, and growth stages," *Critical Reviews in Food Science and Nutrition*, vol. 63, no. 14, pp. 2018–2041, 2023. doi: <https://doi.org/10.1080/10408398.2021.1969891>.