Original Paper

Protective Effects of *Humulus lupulus* L. Extract on CCl_4-Induced Nephrotoxicity in Rats

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Keywords. Acute kidney injury; Carbon tetrachloride; *Humulus lupulus*; Nephrotoxicity; Oxidative stress

Introduction. Oxidative stress is a key contributor to acute kidney injury (AKI), a condition exacerbated by nephrotoxic agents like carbon tetrachloride (CCl_4). *Humulus lupulus* L. (hops), which is rich in polyphenols, exhibits potent antioxidant properties. This study evaluated the nephroprotective effects of *Humulus lupulus* L. extract on CCl_4-induced AKI in rats.

Methods. Twenty-four male Wistar rats were divided into four groups (n = 6): normal control, CCl_4 control, and two treatment groups receiving *Humulus lupulus* extract (100 or 200 mg/kg/day, intraperitoneally) for 14 days. On day 14, all groups except the normal control received CCl_4 (1 mL/kg, 1:1 v/v in olive oil, intraperitoneally). Forty-eight hours post-CCl_4 administration, serum and renal tissue samples were collected to assess biochemical markers (urea, creatinine, uric acid), oxidative stress parameters (malondialdehyde [MDA], total antioxidant capacity [TAC], catalase [CAT], superoxide dismutase [SOD], glutathione peroxidase [GPx]), and histopathological changes.

Results. CCl_4 significantly increased serum urea, creatinine, uric acid, and renal MDA levels while decreasing TAC, CAT, SOD, and GPx activities. Pretreatment with *Humulus lupulus* extract significantly attenuated these alterations, with the 200 mg/kg dose demonstrating superior efficacy. Histopathological analysis revealed reduced tubular and glomerular damage in treated groups.

Conclusion. *Humulus lupulus* extract attenuated CCl_4-induced nephrotoxicity in rats by enhancing antioxidant defenses. These preclinical findings suggest that hops extract warrants further investigation as a potential candidate for mitigating oxidative stress in AKI.

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INTRODUCTION

Oxidative stress, which arises from an imbalance between the activity of free radicals and antioxidants, damages biomolecules such as proteins, lipids, and nucleic acids, leading to impaired cellular function.[1] In kidney health, oxidative stress is a pivotal driver of renal disease progression, with studies demonstrating a strong inverse correlation between glomerular filtration rate and elevated oxidative stress and inflammatory markers.[2,3] This is particularly critical in acute kidney injury (AKI), a condition marked by a sudden impairment of kidney function or structure and characterized by elevated serum urea, creatinine, and uric acid levels.[4] The global incidence of AKI continues to rise, posing a significant burden on healthcare

systems, particularly in resource-constrained regions.[5]

While innovative therapeutic approaches hold promise for future renal therapies, kidney replacement therapy remains the primary treatment for AKI.[6] However, its associated complications—such as infection, vascular access issues, and high costs—limit its accessibility, particularly in certain countries. Consequently, early detection and prevention of AKI offer a more feasible and cost-effective strategy. Given the urgent need for affordable interventions to mitigate AKI, research into accessible therapeutic options is essential.

Herbal medicine, rooted in centuries of traditional practice, is increasingly recognized in modern science for its potential to provide cost-effective and accessible treatments. In the context of AKI, where conventional therapies are often prohibitively expensive or complex, plant-based interventions offer a promising alternative for prevention and management. Despite this potential, the nephroprotective effects of many medicinal plants remain underexplored. *Humulus lupulus* L. (hops), a member of the Cannabaceae family, stands out as a compelling candidate due to its well-documented antioxidant and anti-inflammatory properties, which directly target the oxidative stress and inflammation central to AKI pathogenesis.[7-12]

The therapeutic potential of *Humulus lupulus* L. stems from its diverse bioactive compounds, categorized into resins, essential oils, and polyphenols (Figure 1).[10] Polyphenols, including quercetin, xanthohumol, and catechin, are particularly effective in counteracting reactive oxygen species (ROS) and oxidative injuries implicated in AKI. Quercetin mitigates oxidative stress by modulating inflammatory pathways,[13,14] while xanthohumol and catechin protect against ROS-induced cellular damage.[15] These properties position hops as a promising agent for alleviating the oxidative burden on renal tissue, potentially preserving kidney function in AKI.

A CCl_4-induced AKI model in Wistar rats was utilized to investigate the nephroprotective effects of hops. This well-established experimental system replicates human AKI through ROS-mediated kidney damage.[16] Despite the known benefits of hops' constituents, their specific role in protection against CCl_4-induced nephrotoxicity remains largely unstudied, representing a critical knowledge gap. This study addresses the question: Can *Humulus lupulus* L. attenuate CCl_4-induced kidney damage through its antioxidant and anti-inflammatory properties? By answering this, we aim to validate hops as a cost-effective, plant-based strategy for AKI prevention, bridging traditional herbal knowledge with evidence-based medicine to address the global AKI burden.

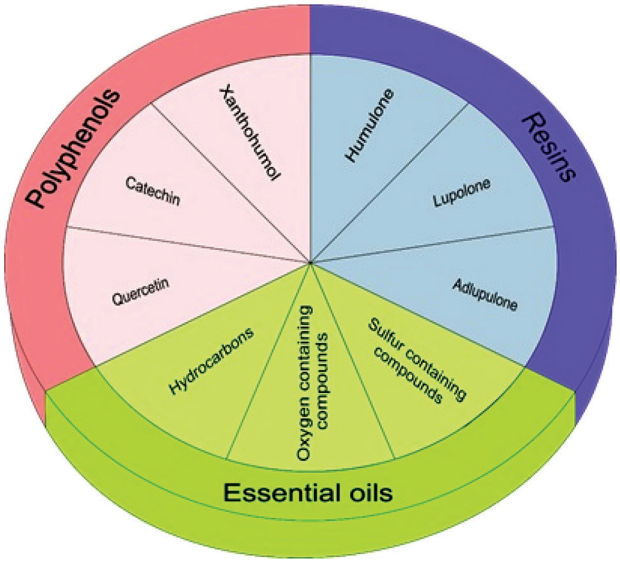

Figure 1. A handful of important constituents of Humulus lupulus are illustrated (36).

MATERIALS AND METHODS

Chemical substances

Bovine serum albumin, Coomassie blue, thiobarbituric acid, ferric chloride, ferric sulfate, hydrogen peroxide, butanol, methanol, and sodium acetate were purchased from Merck (Germany) were used as chemical substances. Ketamine was acquired from Alphasan (Netherlands) and TPTZ was obtained from Fulka (Netherlands). Commercial kits for urea, creatinine, uric acid, SOD, and GPx were obtained from Biomed Co., Iran.

Plant collection and extraction

The East Azerbaijan province Agricultural and Natural Resources Research Center (Iran) confirmed the genus and species of the collected hops plants (Herbarium No: 4193). The whole aerial parts of the hops were dried at room temperature in a dark place. The dried plants were ground into a fine powder (1:10 w/v, 100 g of powder per 1 L of water) and immersed in water for seven days. The

solution was then filtered and concentrated using a rotary evaporator to obtain the final aqueous extract (yield: 9.5% w/w). A single batch of hops extract was prepared from dried plants collected at the same time and processed under identical conditions.

Study design

In this study, 24 male Wistar rats aged eight weeks were purchased from the Pasteur Institute in Iran. They were kept without interference under standard conditions, including a temperature of 22 ºC, a 12-hour light/dark cycle, and a straw bed for two weeks for adaptation. All rats were matched for body weight (180–200 g). The rats were randomly divided into four different groups (n = 6) consisting of:

Normal Control group (C.N): In this group, rats received 0.5 ml of normal saline solution via intraperitoneal (i.p.) injection daily for 14 days, followed by a single i.p. injection of olive oil (1 ml/kg) on the 14th day.

CCl_4 control group: In this group, rats received 0.5 ml of normal saline solution via i.p. injection daily for 14 days and a single i.p. injection of olive oil and CCl_4 (1:1) at a dose of 1 ml/kg on the 14th day.

Treatment group 1 (T.H100): In this group, rats received a hops extract (100 mg/kg) via i.p. injection daily for 14 days, along with a single i.p. injection of olive oil and CCl_4 (1:1) solution (1 ml/kg) on the 14th day.

Treatment group 2 (T.H200): In this group, rats received hops extract (200 mg/kg) via i.p. injection daily for 14 days, followed by a single i.p. injection of a solution of olive oil and CCl_4 (1:1) (1 ml/kg) on the 14th day.

Doses of 100 mg/kg and 200 mg/kg of *Humulus lupulus* L. extract were selected based on prior in vivo studies demonstrating significant antioxidant activity and safety within this range.[17] The 48-hour post-CCl_4 injection time point for sample collection was chosen based on time-course studies of CCl_4-induced renal injury in Wistar rats, which identified this interval as optimal for detecting peak levels of oxidative stress markers (e.g., malondialdehyde [MDA]) and maximal histopathological damage (Figure 2).[18,19] This

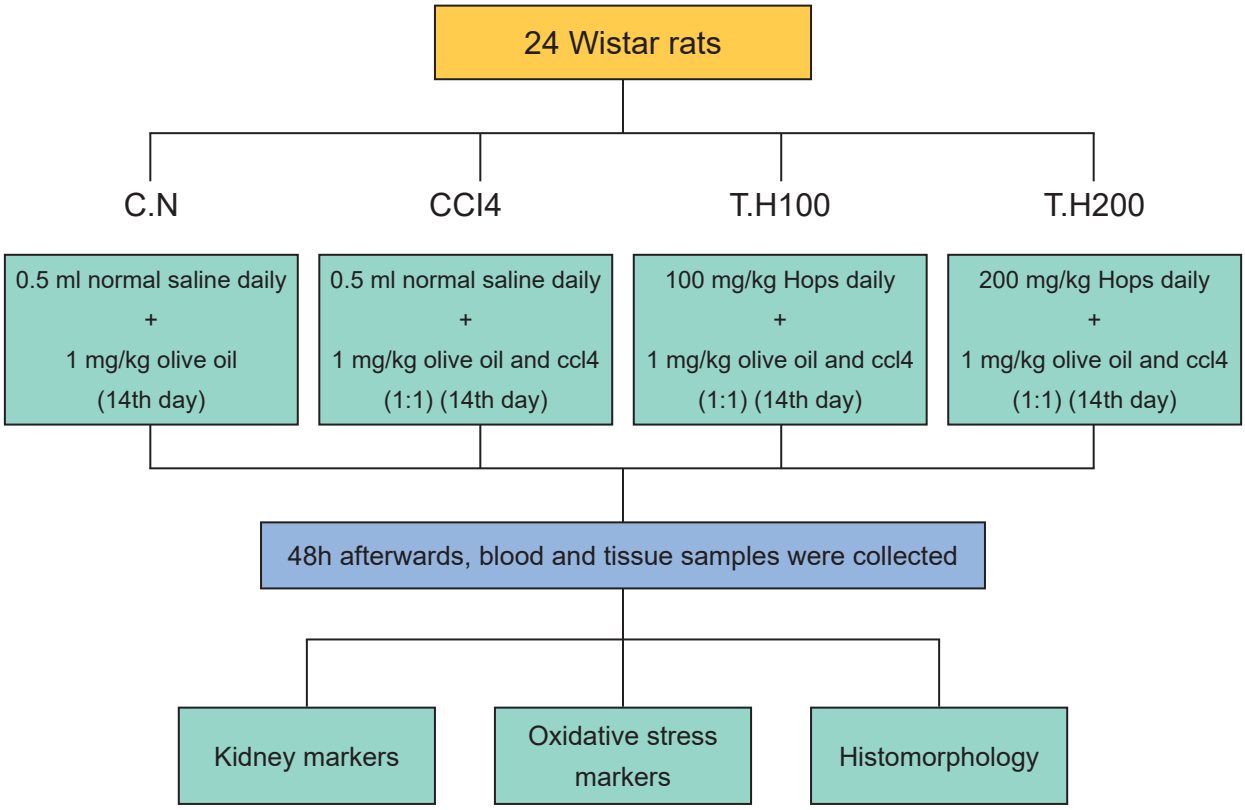

Figure 2. Schematic overview of experimental design.

timing ensured accurate assessment of both renal injury and the nephroprotective effects of *Humulus lupulus* L. extract.

Sample extraction and chemistry analyses

Forty-eight hours after the CCl_4 injection, rats were anesthetized with 200 µl composed of 160 µl of 10% ketamine and 40 µl of xylazine solution. Blood was collected directly from the heart and transferred to a blood clotting tube. The samples were centrifuged, and the serum was aliquoted into 0.5 ml portions and stored at -80 °C to measure serum parameters, including urea, creatinine, and uric acid, using commercially available kits.

Measurement of kidney antioxidant activity

200 mg of kidney tissue were removed, homogenized in 2 ml of 50 mM phosphate buffer (pH = 7.4), and immediately centrifuged at 12000 rpm at 4 °C. The total protein concentration in the supernatant was determined using the Bradford assay,[20] with bovine serum albumin as the standard. This allowed for the normalization of antioxidant enzyme activities and other parameters per gram of protein. The obtained supernatant was used for the measurement of total antioxidant capacity (TAC) via FRAP method (This assay measures the ability of antioxidants in the sample to reduce the Fe^{3+}–TPTZ complex to Fe^{2+}, producing a blue-green chromophore with an absorption maximum at 593 nm),[21] MDA concentrations using Mihara & Uchiyama's method with slight modifications (In this method, MDA reacts with thiobarbituric acid (TBA) under acidic conditions and high temperature to form an MDA-TBA adduct, which was measured spectrophotometrically at a wavelength of 532 nm.),[22] catalase (CAT) via Aebi method (Tissue homogenates are incubated with 10 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0). The decrease in absorbance at 240 nm is recorded every 15 s for 2),[23] as well as superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity according to the instructions provided via commercial kits (ZellBio GmbH, Germany) using StatFax 300 ELISA reader.

Morphological and histopathological studies

Following extraction, kidney tissue samples were immediately fixed in 10% neutral buffered formalin. The specimens were then dehydrated through a graded alcohol series (50% to 100%), cleared in xylene, and embedded in paraffin blocks. Sections of 4–5 µm thickness were prepared using a rotary microtome and stained with Hematoxylin and Eosin (H&E) for light microscopic examination. To ensure objectivity, all renal tissue slides were coded, and a pathologist evaluated them blindly, without prior knowledge of the treatment groups.

To quantitatively assess the extent of renal structural injury, a semi-quantitative histological damage index was calculated based on previously established criteria.[24] Key histopathological features of acute kidney injury—including tubular epithelial necrosis, loss of brush border, tubular dilatation, and intraluminal cast formation—were evaluated. For each tissue section, at least ten randomly selected, non-overlapping cortical fields were examined at 400× magnification. The severity of tubular damage was graded on a scale of 0 to 4 based on the percentage of affected tubules per field: 0 = normal histology (no damage); 1 = mild damage (< 25% of tubules affected); 2 = moderate damage (25–50% affected); 3 = severe damage (50–75% affected); and 4 = extensive damage (> 75% affected). The overall histological damage index for each animal was determined by averaging the scores from all examined fields.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. The results are reported as "mean ± standard deviation." A significance level of ($P < .05$) was established. The analyses were conducted using SPSS v. 21 software.

RESULTS

Serum biochemical analyses

CCl_4 intoxication significantly impaired renal function, evidenced by 30%%increase in serum urea, 44%%increase in creatinine, and 140% increase in uric acid levels compared to the C.N ($P < .0001$; Table 1). *Humulus lupulus* L.extract dose-dependently attenuated these markers. T.H 200 maintained near-normal levels of urea and creatinine to near-normal levels (decrease by15% and 25%, respectively compared to the CCl_4 group; $P < .0001$), while reducing uric acid by 43% ($P < .0001$). The BUN/Cr ratio, indicative of intrinsic renal injury, declined from 161.8 (C.N) to 142.4 in CCl_4 ($P < .01$), with T.H200 normalizing

Table 1. Effect of *Humulus lupulus* L. Pretreatment on Serum Renal Function in CCl_4-Treated Rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Urea/Creatinine ratio	Uric acid (mg/dl)
C.N	61.83 ± 1.72	0.39 ± 0.02	161.76 ± 7.22	1.68 ± 0.17
CCl4	80.33 ± 2.42†	0.56 ± 0.02†	142.35 ± 5.72†	4.03 ± 0.35†
T.H100	71.00 ± 2.28****	0.48 ± 0.01***	146.09 ± 7.29	3.05 ± 0.21****
T.H200	68.33 ± 1.86****	0.42 ± 0.03****	158.66 ± 7.11**	2.3 ± 0.26****

The data are represented as mean ± standard error. † represent a significant differences at ($P < .0001$). **, *** and **** represent a significant differences at ($P < .01$), ($P < .001$) and ($P < .0001$) respectively. C.N: normal control group, CCl_4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg.

this ratio to 158.7, confirming structural protection.

Malondialdehyde levels

CCl_4-induced lipid peroxidation was evidenced by a 3.8-fold increase in renal MDA (Figure 3). *Humulus lupulus* L.extract dose-dependently attenuated this damage, with T.H200 reducing MDA to near-normal levels (78% decrease compared to the CCl_4 group; $P < .001$), indicating potent inhibition of oxidative membrane damage ($P < .05$).

Total antioxidant capacity

CCl_4 exposure depleted systemic antioxidant reserves, reducing TAC by 39% versus C.N ($P < .0001$; Figure 4). *Humulus lupulus* L. extract dose-dependently reversed this deficit, with T.H200 elevating TAC by 42% relative to the CCl_4 group ($P < .0001$), indicating a significant but partial restoration of antioxidant capacity ($P < .05$ vs. C.N).

Catalase activity

CCl_4 severely compromised enzymatic antioxidant defenses, suppressing CAT activity by 48% compared to the C.N ($P < .0001$; Figure 5). *Humulus lupulus* L. extract elicited dose-dependent recovery, with T.H200 boosting CAT by 50% relative to the CCl_4 group ($P < .0001$).

Superoxide dismutase activity

CCl_4 critically impaired superoxide scavenging, depressing SOD activity by 64% compared to the C.N ($P < .0001$; Figure 6). *Humulus lupulus* L. extract stimulated dose-dependent recovery, with T.H200 enhancing SOD by 105% relative to the CCl_4 group ($P < .0001$).

Glutathione peroxidase activity

CCl_4 severely disrupted glutathione-dependent defenses, reducing GPx activity by 64% compared

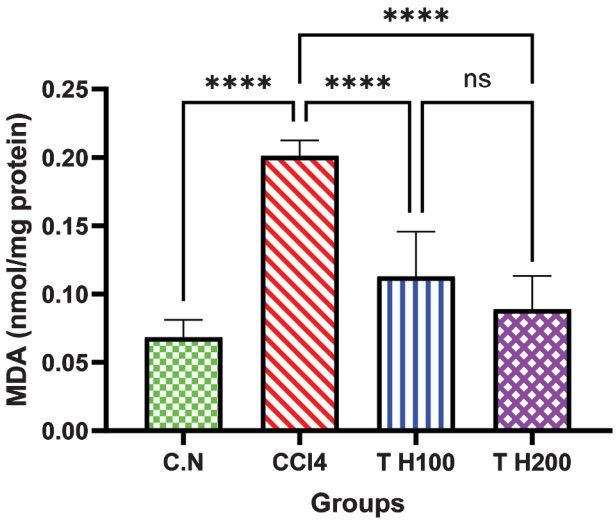

Figure 3. Effect of *Humulus lupulus* L. Pretreatment on malondialdehyde (MDA) level in CCl_4-treated rats. The data are represented as mean ± standard error. **** represents a significant difference ($P < .0001$). C.N: normal control group, CCl_4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg. MDA: malondialdehyde.

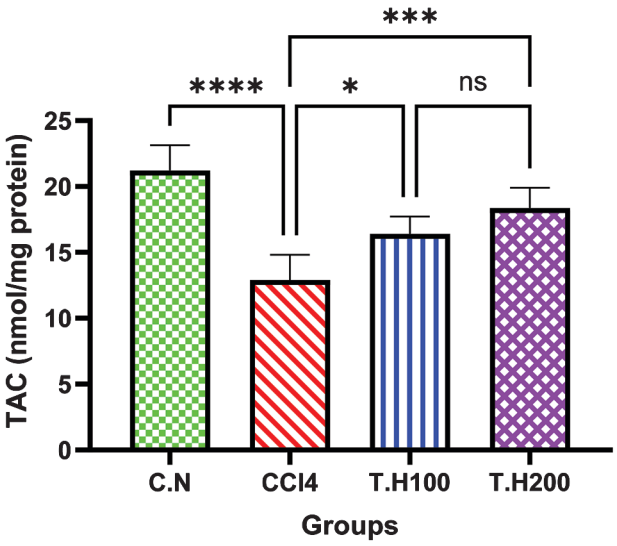

Figure 4. Effect of *Humulus lupulus* L. Pretreatment on total antioxidant capacity (TAC) in CCl_4-treated. The data are represented as mean ± standard error. *, ***, and **** represent a significant differences at ($P < .05$), ($P < .001$), and ($P < .0001$) respectively. C.N: normal control group, CCl_4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg. TAC: total antioxidant capacity.

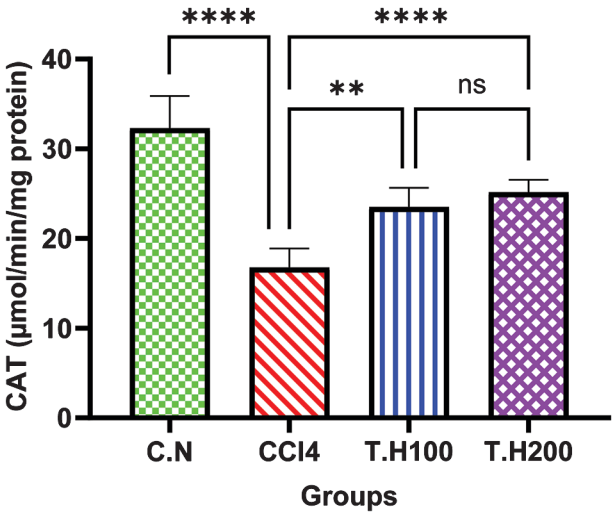

Figure 5. Effect of *Humulus lupulus* L. Pretreatment on catalase (CAT) activity in CCl_4-treated. The data are represented as mean ± standard error. ** and **** represent a significant differences at ($P < .01$) and ($P < .0001$) respectively. C.N: normal control group, CCl_4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg. CAT: Catalase.

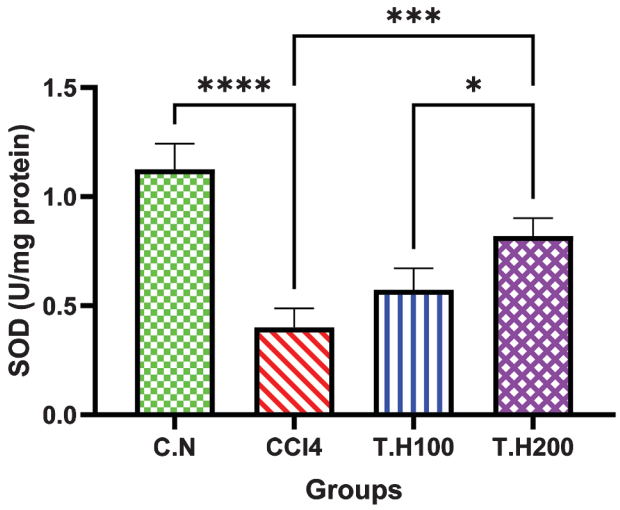

Figure 6. Effect of *Humulus lupulus* L. pretreatment on superoxide dismutase (SOD) activity in CCl4-treated rats. The data are expressed as mean ± standard error. *** and **** indicate significant differences at ($P < .001$) and ($P < .0001$), respectively. C.N: normal control group, CCl4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg. SOD: superoxide dismutase.

to the C.N ($P < .0001$; Figure 7). *Humulus lupulus* L. extract mediated dose-dependent restoration, with T.H200 elevating GPx by 103% relative to the CCl_4 group ($P < .0001$).

Histopathological findings

Histological studies indicated that the structures of the proximal and distal tubules, Bowman's capsule, glomeruli, and interstitial tissue were normal in the control group (Figure 8-A). CCl_4 induced histological and structural damage to the proximal and distal tubules, as well as the glomeruli (Figure 8-B), while pretreatment with T.H100 (Figure 8-C) and T.H200 (Figure 8-D) preserved the renal tissue architecture. Furthermore, a comparison of the histopathology index between groups showed that CCl_4 significantly increased the histological damage index, and treatment in the T.H200 group significantly prevented this damage ($P < .05$) (Figure 9).

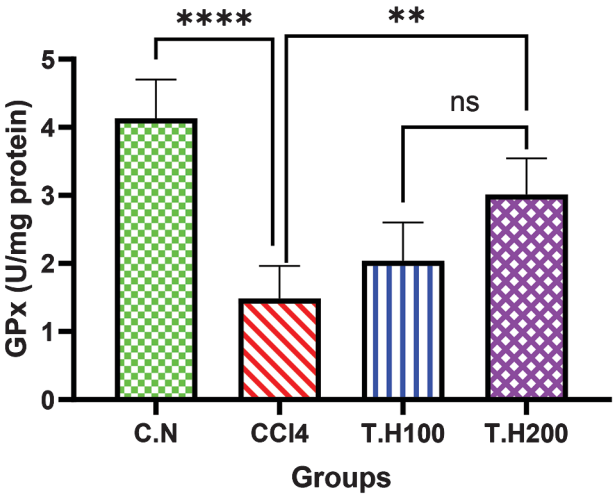

Figure 7. Effect of *Humulus lupulus* L. pretreatment on glutathione peroxidase (GPx) activity in CCl_4-treated rats is shown. The data are represented as mean ± standard error. ** and **** represent a significant differences at ($P < .01$) and ($P < .0001$) respectively. C.N: normal control group, CCl_4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg. GPx: glutathione peroxidase.

DISCUSSION

The prevalence of AKI is rising globally due to lifestyle changes. Oxidative stress is considered a major factor in the development of AKI.[25] While hops (*Humulus lupulus* L.) have been studied for hepatoprotection, this is the first report showing their prophylactic renoprotective effects in a CCl_4–induced AKI rat model.

In our study, we measured serum levels of urea, creatinine, and uric acid, all indicating impaired function and kidney injury. Urea and creatinine levels increase as a consequence of nephron damage.[26] Additionally, uric acid is used for renal damage assessment.[27] We found that CCl_4

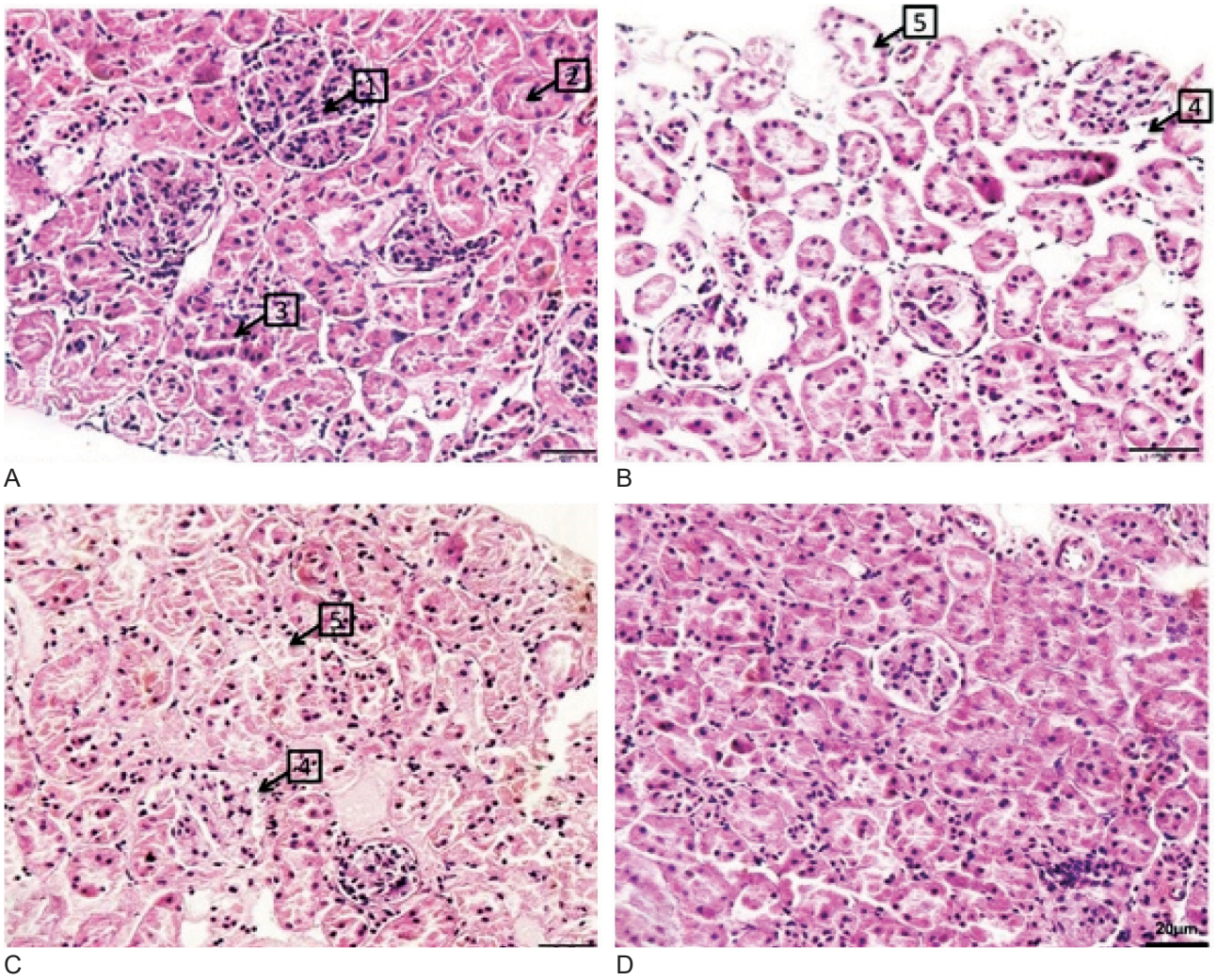

Figure 8. Histopathological structure of the kidney in the studied groups (× 20). The microscopic slides were stained with H&E. In the control group, the normal structure of the renal corpuscle (1), proximal tubules (2), and distal tubules (3) is shown (A). CCl_4 disrupted Bowman's capsule and caused tissue damage in the renal corpuscle (4) and proximal tubules (5) (B). In the treatment groups, T.H100 (C) and especially T.H200 (D) extracts prevented tissue damage caused by CCl_4.

intoxication significantly increased the serum levels of all three markers. Crucially, our results demonstrated that pre-treatment with Humulus lupulus significantly attenuated these elevations, restoring the markers toward normal levels. These findings are consistent with the results reported by several other groups,[28,29] which indicated that pre-treatment with hops led to significantly decreased urea, creatinine, and uric acid levels. These results suggest that *Humulus lupulus* mitigates CCl_4-induced kidney injury by enhancing antioxidant defenses..

MDA is the end product of lipid peroxidation, and higher concentrations of this substance indicate a shift towards excess free radicals production and failure of the antioxidant defense, ultimately leading to tissue injury.[30] In our study, CCl_4 administration significantly increased MDA levels. This finding is confirmed by other studies.[30,31] The results of our study showed that pre-treatment with hops significantly reduced MDA levels. These findings indicate that *Humulus lupulus* extract reduces lipid peroxidation and kidney injury, likely by decreasing MDA levels and boosting antioxidant enzyme activity.

The redox homeostasis in the cell is maintained through a balance between the oxidative and antioxidative axes. The cell's natural antioxidant defense system comprises two main biochemical branches: radical scavengers such as glutathione and vitamin C, and enzymes including SOD, GPx, and CAT.[32] Here, we report that CCl_4 administration significantly reduces the activity of SOD, GPx, and

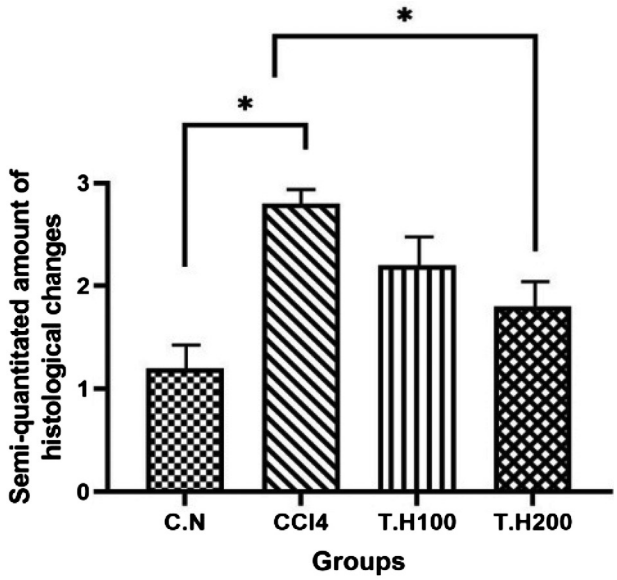

Figure 9. Effect of *Humulus lupulus* L. Pretreatment on Histopathological outcome in CCl_4-treated rats is shown. The data are represented as mean ± standard error. * represents a significant difference ($P < .05$). C.N: normal control group, CCl_4: carbon tetrachloride injury group. T.H100: hops extract 100 mg/kg. T.H200: hops extract 200 mg/kg.

CAT, as well as the overall TAC. These results align with those reported by other groups.[33,34] While baseline antioxidant levels were not measured, the normalization of CAT, SOD, and GPx activities to levels comparable with the normal control group, following treatment with hops, suggests that hops extract prevented the CCl_4-induced decline in antioxidant defenses. Hops pretreatment may decrease kidney injury by reducing the amount of free radicals, boosting antioxidant enzymes, and increasing the overall TAC.

CCl_4 administration induced severe histopathological damage to the Bowman's capsule and proximal tubules in Wistar rats, characteristic of AKI. These observations are consistent with prior studies documenting CCl_4-mediated renal injury.[35] Pretreatment with Humulus lupulus L. extract markedly reduced these histopathological changes, as evidenced by the preservation of tubular and glomerular integrity in the T.H100 and T.H200 groups (Figure 9). This nephroprotective effect is likely attributable to the extract's antioxidant properties, which significantly lowered MDA levels and enhanced the activities of CAT, SOD, and GPx (Figures 3–7). These findings suggest that Humulus lupulus L. mitigates CCl_4-induced oxidative stress, thereby preserving renal tissue architecture and supporting its protective potential against AKI.

Throughout our study, all biochemical and histopathological parameters improved in a dose-dependent manner, with 200 mg/kg (T.H200) consistently outperforming 100 mg/kg (T.H100). This suggests that higher dose deliver greater amounts of hops' bioactive constituents (e.g., flavonoids and polyphenols), which leads to more effective scavenging of CCl_4-generated free radicals and stronger up-regulation of endogenous antioxidant enzymes. Indeed, the T.H200 group demonstrated significantly higher antioxidant enzyme activities than the T.H100 group and a more pronounced preservation of the histopathological index ($P < 0.05$).

Our findings demonstrate that pretreatment with *Humulus lupulus* L. extract at 100–200 mg/kg protects against acute CCl_4-induced renal oxidative injury in rats. Translating these results to a clinical context suggests potential for hop-derived polyphenolic formulations as adjunctive nephroprotective agents in settings of acute toxin exposure or oxidative stress–mediated kidney injury (e.g., drug-induced nephrotoxicity, ischemia-reperfusion). Given the favorable safety profile of hops extracts in humans, future work could evaluate oral dosing regimens designed to achieve plasma levels of key prenylflavonoids comparable to those seen in our effective rat doses. Moreover, these data support the rationale for early prophylactic administration in high-risk patient populations (e.g., chemotherapy, contrast media use), to bolster endogenous antioxidant defenses before insult.

Limitation of the study

However, several caveats temper the direct extrapolation of our results. First, our model addresses only acute, single-dose CCl_4 toxicity; chronic or repeated exposures—and the efficacy of hops extract therein—remain unexplored. Second, we evaluated only male rats, limiting the assessment of sex differences. Third, pharmacokinetic parameters of key hops constituents were not measured, so optimal dosing windows and bioavailability in vivo require further characterization. Addressing these gaps will be essential before considering clinical trials.

CONCLUSION

In conclusion, this study provides preclinical

evidence that *Humulus lupulus* L. extract exerts significant protective effects against CCl_4-induced AKI in rats. Pretreatment with the extract, particularly at 200 mg/kg, mitigated renal dysfunction, reduced oxidative stress markers, enhanced antioxidant defenses, and preserved renal tissue architecture. These results demonstrate the efficacy of hops extract in this experimental rat model of AKI and support its potential for further exploration as a natural antioxidant intervention. However, translation to human AKI requires rigorous clinical evaluation. A limitation of this study is the absence of pre-exposure antioxidant measurements; thus, claims of 'restoration' are inferred from comparison to concurrent normal controls rather than baseline data.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the local Ethical Committee of Ardabil University of Medical Sciences IR.ARUMS.AEC.1401.037.

CONFLICT OF INTEREST

The authors declare no competing interests.

ABBREVIATIONS

AKI: Acute kidney injury
CCl_4: Carbon tetrachloride
TAC: Total antioxidant capacity
CAT: Catalase
MDA: Malondialdehyde
GPx: Glutathione peroxidase
SOD: Superoxide dismutase

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