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Multifaceted Nephroprotection: Exploring Nettle*(Urtica dioica)* extract Role in Combating Cisplatin-Induced Renal Injury

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- Oxidative stress
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- Apoptosis

Abstract

Abstract: Cisplatin is a widely used chemotherapeutic agent, but its clinical application is often limited by nephrotoxicity, characterized by oxidative stress, inflammation, and apoptosis in renal tissues. This study investigates the potential protective effects of *Urtica dioica* (nettle) ethanolic extract against cisplatin-induced kidney damage in a rat model. Thirty-two rats were divided into four groups: control, nettle-treated, cisplatin-treated, and cisplatin plus nettle extract-treated. Renal function markers, oxidative stress parameters, inflammatory mediators, and apoptotic markers were analyzed. The results show that cisplatin administration led to significant renal dysfunction, evident from elevated serum creatinine and BUN levels, increased oxidative stress markers (MDA), and decreased antioxidant enzyme activities (SOD, CAT, and GSH). Additionally, cisplatin upregulated NF-κB and proinflammatory cytokines (IL-1β, TNF-α, IL-6), as well as intrinsic and extrinsic apoptotic markers (caspase-3, caspase-8, caspase-9, cytochrome c, and Bax), while downregulating the anti-apoptotic protein Bcl-2. Conversely, nettle extract significantly ameliorated cisplatin-induced renal impairment by improving kidney function, enhancing antioxidant enzyme activity, reducing NF-κB and proinflammatory cytokine levels, and restoring apoptotic balance. These findings suggest that *Urtica dioica* possesses strong nephroprotective, anti-inflammatory, antioxidant, and anti-apoptotic properties, potentially offering a therapeutic approach to mitigate cisplatin-induced nephrotoxicity. Further research is necessary to elucidate its precise mechanisms and clinical applicability.

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Introduction

Cisplatin (Cis) is one of the most well-known anticancer drugs, widely prescribed for the treatment of various solid tumors. However, its clinical use is significantly limited by its nephrotoxic side effects [1,2]. While cisplatin is highly effective in treating tumors, its application is often associated with several adverse effects, including neurotoxicity, ototoxicity, and nephrotoxicity [3,4]. Approximately 20-30% of patients undergoing cisplatin treatment develop post-treatment acute kidney injury (AKI) [5,6]. Moreover, patients with AKI frequently progress to chronic renal disease, leading to an increased risk of tumor-related mortality [7,8].

Cisplatin-induced nephrotoxicity predominantly affects the epithelial cells of the proximal tubules, manifesting as oxidative damage, renal inflammation, and tubular necrosis [4]. The primary mechanisms underlying this renal pathology include oxidative stress, inflammation, and apoptosis [9]. Cisplatin is metabolized in the renal tubules into highly reactive components that deplete natural antioxidants and nitrosative defenses. These components interact with various cellular elements, causing damage to structural and functional proteins, as well as inducing apoptosis, autophagy, and necrosis [3]. Furthermore, cisplatin administration triggers the release of several cytokines, exacerbating inflammation and contributing to renal degeneration [3].

Natural compounds have been previously recognized for their promising role in the treatment of various diseases, including cardiovascular diseases, tumors, diabetes, neurodegenerative disorders, and aging. This is largely attributed to their minimal side effects and variations in efficacy, mechanisms of action, and accuracy [10,11].

Nettle (Urtica dioica), a member of the Urticaceae family, has traditionally been used to treat a wide range of conditions such as rheumatoid arthritis, urinary tract infections, Alzheimer's disease, gingivitis, allergies, bursitis, kidney stones, tendinitis, hair growth, cough, and gout [12]. Nettle contains several phenolic compounds, including rutin, chlorogenic acid, quercetin, caffeic acid, and kaempferol 3-Orutinoside [13]. Additionally, it is rich in fatty acids such as linolenic acid, palmitic acid, and cis-9,12-linoleic acid, as well as various amino acids [14].

Previous studies have highlighted the antioxidant potential of Urtica dioica (UD) leaves, alongside their diverse biological activities, including anticarcinogenic, antigenotoxic, anti-inflammatory, hepatoprotective, and cardioprotective effects [15,16]. Furthermore, UD has demonstrated nephroprotective, neuroprotective, and hepatoprotective properties against potassium bromate-induced oxidative damage in rodent models [17,18].

The aim of our study is to investigate the nephroprotective effects of UD against cisplatin-induced nephrotoxicity, focusing on its antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

2. Material and methods

2.1. Preparation of nettle ethanolic extract

For preparation of the ethanolic extract from nettle (Urtica dioica), *Nettle* L was purchased from a local market, Elnekityelattar, Mansoura City, Egypt. The leaves were thoroughly washed with cool water to remove any dirt or debris and air-dried in a well-ventilated area, away from direct

sunlight, until they became crisp. Alternatively, they can be dried using an oven set to a low temperature (40–50°C) or a food dehydrator. Once dried, the leaves were ground into a fine powder using a blender or grinder. The powdered nettle leaves were mixed with ethanol (70–95%) in a 1:10 ratio (one part plant material to ten parts ethanol). The mixture was placed in a sealed container and left to macerate for 24–48 hours at room temperature, with occasional shaking. After the maceration process, the mixture was filtered using fine mesh, cheesecloth, or vacuum filtration to separate the liquid extract from the plant residue. For concentration of the extract, the ethanol was evaporated under reduced pressure using a rotary evaporator or air-dried in a fume hood, yielding a concentrated extract. The extract was then stored in an amber glass bottle in a cool, dark place to effectively preserve its bioactive compounds. The describedprocedure was carried out following references [19,20], with certain modifications.

2.2. Animals

Our study included thirty-two rats weighing between 180 and 200 grams, housed in plastic cages under controlled environmental conditions with a temperature of 22 ± 2°C, a continuous dark/light cycle, and 45% relative humidity. Diet pellets were provided ad libitum. All experimental procedures were conducted in the laboratory of Al-Qunfudah College of Medicine, Umm Al-Qura University, Al-Qunfudah, Saudi Arabia. The study's animal protocols were reviewed and approved by the Scientific Research Ethics Committee of Umm Al-Qura University, Makkah, Saudi Arabia, under approval number (HAPO-02-K-012-2025-05-2706).

2.3. Experimental design

The rats were divided into four groups of eight (n=8): Group I (Control), receiving normal saline via oral gavage for three weeks; Group II (Nettle), administered nettle extract at an oral dose of 300 mg/kg by gavage, based on a previous study by Yousuf et al. [21]; Group III (Cis), receiving a single intraperitoneal injection of cisplatin at 5 mg/kg on the first day, a dosage known to induce nephrotoxicity [22]; and Group IV (Cis+Nettle), which received both cisplatin and nettle extract according to the previously established dosages and routes of administration.

2.4 Sample collection

One day post-experiment, the rats were anesthetized using a ketamine-xylazine mixture, after which blood samples were collected via cardiac puncture, coagulated, and centrifuged to obtain serum. The rats were sacrificed via cervical dislocation, and both kidneys were removed and rinsed with cold phosphate-buffered saline (PBS). Kidney samples were either immersed in 10% buffered formalin for histological examination or homogenized (10% w/v) in cold Tris-HCl buffer, centrifuged, and the obtained supernatant stored at -80°C for further biochemical analysis.

2.5. Assay of BUN, Creatinine, and Pro-Inflammatory Cytokines

Serum creatinine and blood urea nitrogen (BUN) levels were measured using Spinreact kits (Spinreact, Girona, Spain). Meanwhile, renal supernatant levels of pro-inflammatory cytokinesinterleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6)were assessed using ELISA kits from ABclonal Technology (Wuhan, China): Cat# RK00009 for IL-1β (62.5–4000 pg/mL), RK00029 for TNF-α (31.2–2000 pg/mL), and RK00020 for IL-6 (125–8000 pg/mL). The inflammation-related transcription factor NF-κB was quantified using a

MyBioSource ELISA kit (San Diego, CA, USA; Cat# MBS453975; 0.312–20 ng/mL). Apoptosis markers were measured using the following ELISA kits: caspase-3 (ABclonal, Cat# RK09074; 0.312–20 ng/mL), Bcl-2 (ABclonal, Cat# RK03525; 1.56–100 ng/mL), Bax (Cusabio, Wuhan, China; Cat# CSB-EL002573RA; 62.5–4000 pg/mL), caspase-8 (Novus Biologicals, Centennial, CO, USA; Cat# NBP2-75041; 0.16–10 ng/mL), cytochrome c (Abbexa Ltd., Cambridge, UK; Cat# abx256861; 0.39–25 ng/mL), and caspase-9 (Novus Biologicals, Cat# NBP2-75044; 1.56–100 ng/mL). All assays were conducted according to the manufacturers' protocols, and absorbance was measured at 450 nm using a microplate reader.

2.6. Assay of oxidative stress markers

Malondialdehyde (MDA) levels were assessed according to the procedure described by Ohkawa et al. [23]. Moreover, the activities of renal supernatant superoxide dismutase (SOD) and catalase (CAT), as well as the content of glutathione (GSH), were measured based on the methodologies outlined in references [24, 25, 26], respectively.

2.7. Histopathological examination

After the sacrifice of the animals, the isolated kidney was washed with a saline solution and then fixed in 10% paraformaldehyde. Subsequently, the specimens were dehydrated in absolute ethanol and embedded in paraffin blocks. The samples were then sectioned into 5-micrometer-thick slices and stained with hematoxylin and eosin (H&E) for microscopic examination [27].

2.8. Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 8 software. Data are expressed as mean ± standard deviation (SD). Group differences were assessed through analysis of variance (ANOVA), followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Protective Effect of Nettle Extract on Cisplatin-Induced Renal Function Deterioration

A single-dose intraperitoneal injection of cisplatin (Cis) significantly ($p< 0.05$) increased renal function markers, including creatinine and blood urea nitrogen (BUN), compared to control animals (Fig. 1A, B). However, co-administration of nettle extract with Cis in Group IV markedly improved renal function by reducing serum creatinine and BUN levels compared to the Cis group. These findings suggest that nettle extract exhibits a nephroprotective effect against cisplatin-induced renal degeneration.

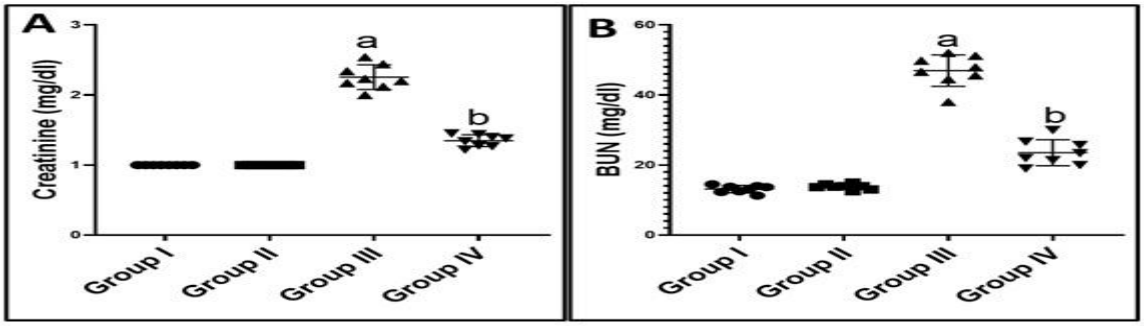

Fig.1. A graph illustrates the effect of cisplatin (Cis) and nettle extract on renal function across different groups, specifically measuring (A) creatinine and (B) blood urea nitrogen (BUN). *ap*< 0.05 vs. Groups I and II rats; *bp*< 0.05 vs. Group III (Cis group).

3.2. preventive effect of nettle on Cis-induced kidney injury

As illustrated in Figure 2, H&E-stained sections of the control and nettle extract groups (Fig. 2A, B) demonstrate normal renal medulla histology. In contrast, the cisplatin group (Fig. 2C) exhibits significant renal injury, characterized by marked tubular dilation, infiltration of numerous inflammatory cells, epithelial cell degeneration within the tubule lumens, and cast formation. However, the Cis+nettle group (Fig. 2D) shows a clear reduction in tubular inflammation, suggesting a protective effect. This observed improvement in renal function may explain the corresponding enhancement in renal morphology.

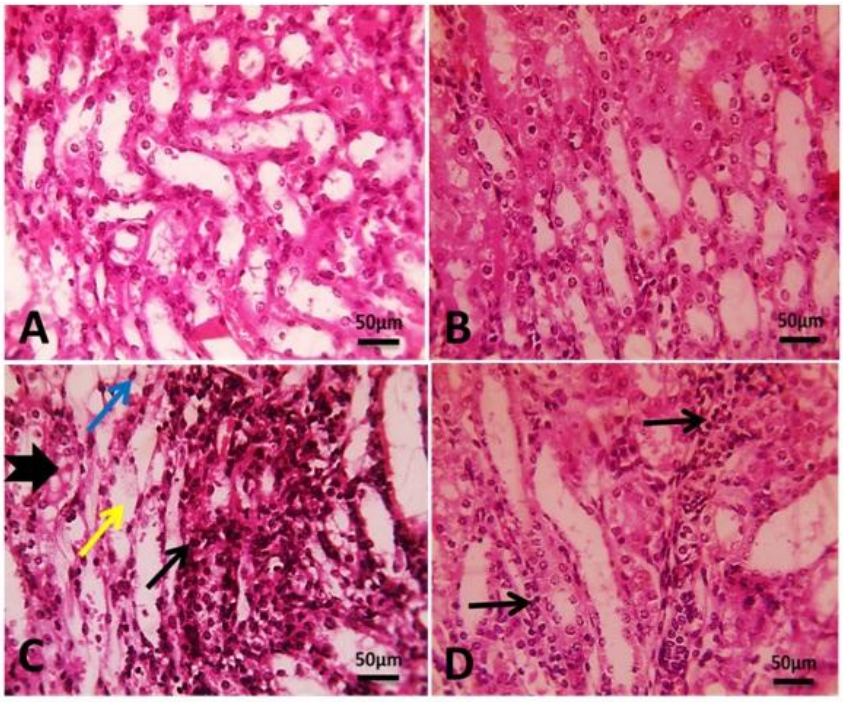

Fig. 2. Microscopic examination of HE-stained renal sections revealed distinct histopathological differences among the study groups. Control and nettle extract groups (A, B) displayed a normal medulla with no signs of inflammation. In contrast, renal sections from the Cis group (C) exhibited marked medullary tubular dilation (yellow arrow), interstitial inflammation (thin black arrows), cast formation (thick black arrow), and detached epithelial cells (blue arrow), indicating significant renal damage. However, renal sections from the treated Cis+nettle group (D) showed only mild interstitial inflammation (thin black arrows) in the medulla, suggesting a potential protective effect of the nettle extract. Images were taken at high magnification (X400) with a scale bar of 50 µm.

3.3. Mitigative effect of nettle extract against Cis-induced renal oxidation

As shown in **Figure 3**, administration of cisplatin induces severe oxidative stress in renal tissue, significantly elevating malondialdehyde (MDA), a well-known lipid peroxidation marker. This is accompanied by a marked reduction in the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), as well as a decrease in glutathione (GSH) levels, compared to control rats. Conversely, co-administration of nettle extract with cisplatin significantly reversed these oxidative stress markers. Based on these findings, nettle extract exhibited a potent antioxidant effect in the renal tissues of cisplatin-treated rats.

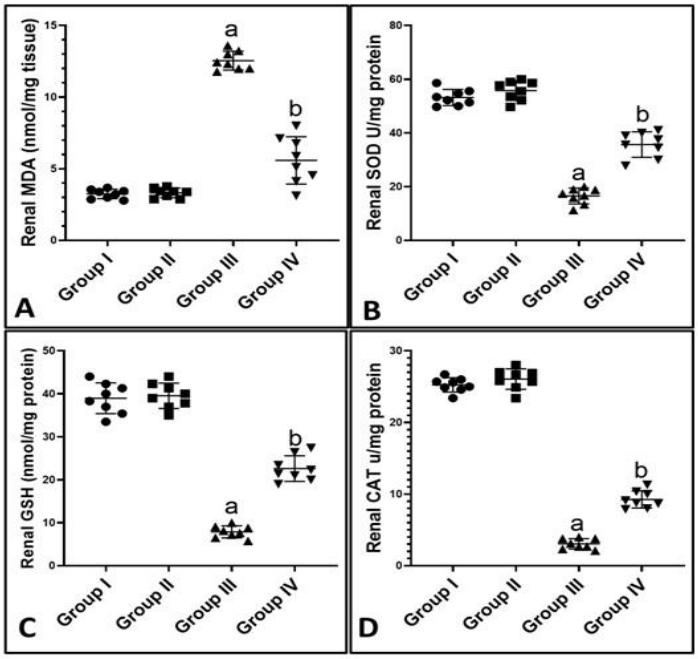

Fig.3. A graph illustrates the effects of cisplatin (Cis) and nettle extract on oxidative stress markers, including (A) MDA, (B) SOD, (C) GSH, and (D) CAT. *ap*< 0.05 compared to control groups; *bp*< 0.05 compared to the Cis group.

3.4. Cisplatin-induced Renal Inflammation and the Anti-inflammatory Effect of Nettle Extract

Intraperitoneal administration of cisplatin (Cis) significantly elevated the protein levels of the nuclear inflammatory transcription factor NF-κB, leading to a corresponding increase in proinflammatory cytokines IL-1β, TNF-α, and IL-6, compared to control animals (Fig. 4A-D). However, co-administration of nettle extract with Cis markedly reduced NF-κB levels and proinflammatory cytokines in comparison to the Cis group. These findings suggest that nettle extract exerts a potent anti-inflammatory effect against cisplatin-induced renal inflammation.

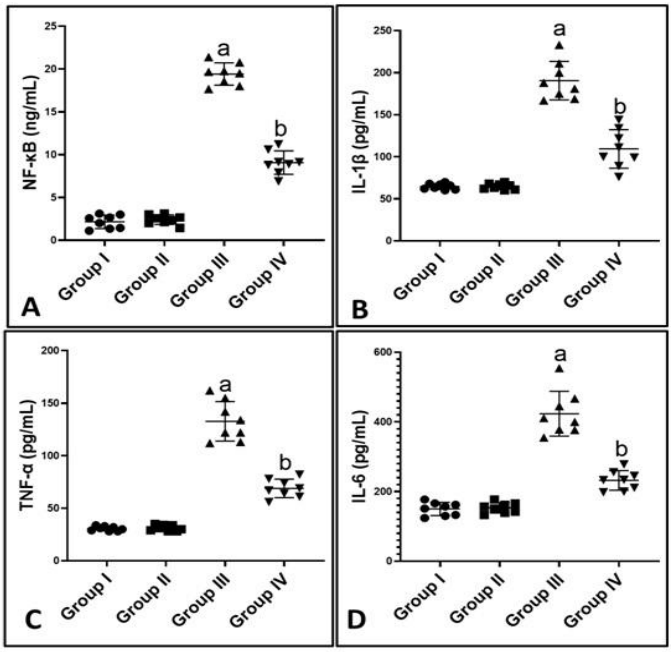

Fig.4. An illustrative graph presents the effects of cisplatin (Cis) and nettle extract on inflammatory markers: (A) NF-κB, (B) IL-1β, (C) TNF-α, and (D) IL-6. *ap*< 0.05, significant compared to control animals; *bp*< 0.05, significant compared to Cis-treated animals.

3.5. Anti-apoptotic Effect of Nettle on Renal Tubular Epithelial Cells

Compared to the control group, cisplatin (Cis) administration resulted in a significant elevation in the protein levels of both intrinsic and extrinsic apoptotic pathway markers, including caspase-9, cytochrome c, Bax, caspase-8, and caspase-3, while showing a notable decline in the antiapoptotic protein Bcl-2 (Figure 5). However, co-administration of nettle extract with cisplatin in Group IV effectively reversed these apoptotic alterations. Based on these findings, nettle extract demonstrated a strong anti-apoptotic effect, protecting renal tubular epithelial cells from cisplatin-induced apoptosis.

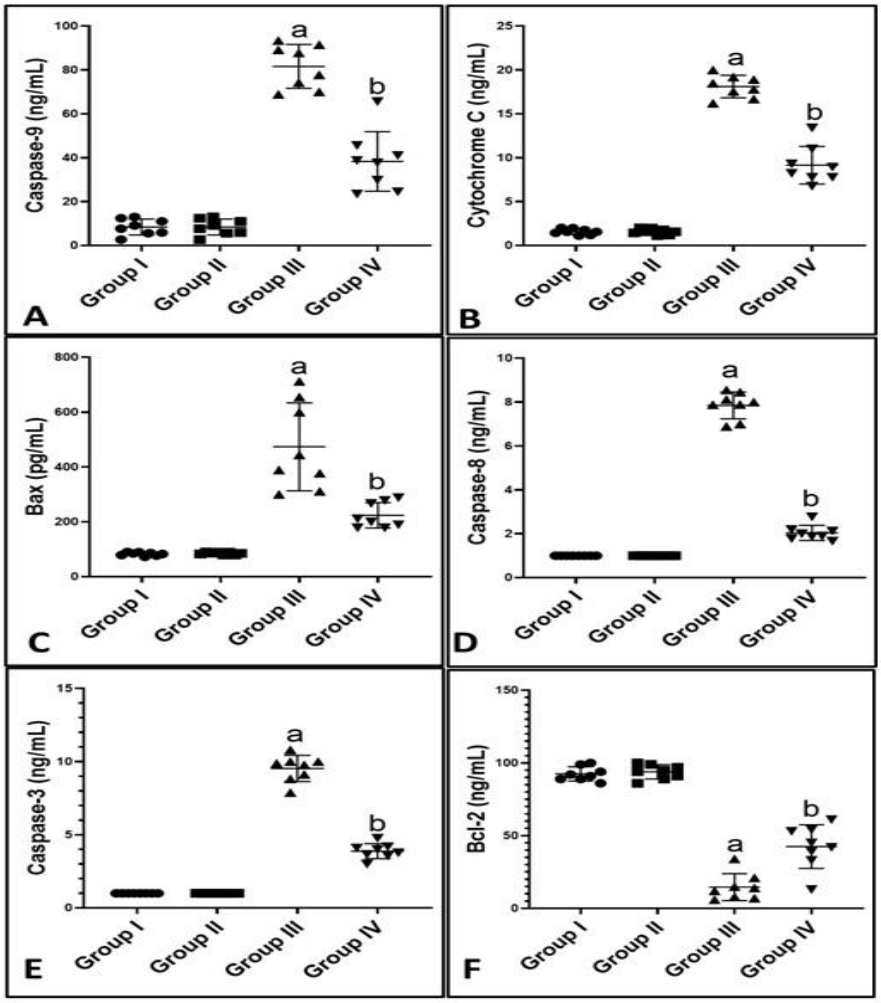

Fig. 5. A graph represents the effects of cisplatin (Cis) and nettle extract on apoptotic and antiapoptotic markers: (A) caspase-9, (B) cytochrome c, (C) Bax, (D) caspase-8, (E) caspase-3, and (F) Bcl-2. *ap*< 0.05, significant compared to Groups I and II rats; *bp*< 0.05, significant compared to Group III rats.

4. Discussion

In this study, we investigated the potential nephroprotective effects of nettle ethanolic extract against cisplatin-induced acute kidney injury. Our findings indicate that coadministration of nettle with cisplatin provides renoprotective benefits, modulating renal failure, as evidenced by histological improvements in the renal medulla's tubular structures. This protective effect is attributed to nettle extract's antioxidant properties,

which enhance the endogenous antioxidant system, its anti-inflammatory activity, which suppresses the inflammatory cascade within renal tubules, and its anti-apoptotic effects, which inhibit both intrinsic and extrinsic apoptotic pathways.

In the present study, intraperitoneal injection of cisplatin led to significant deterioration in renal function, as evidenced by elevated blood urea nitrogen (BUN) and creatinine levels, along with pronounced structural damage to the renal morphology—indicative of nephrotoxicity. These findings are consistent with previous research documenting the toxic effects of cisplatin on renal tissues [3,4,6,28,29]. Conversely, the combined intake of nettle extract with cisplatin demonstrated a restorative effect on renal function and morphology. This aligns with the findings of Salih [30], who reported the beneficial impact of nettle extract on renal function and histology in a model of gentamicin-induced nephrotoxicity in rabbits.

Several mechanisms contribute to the nephrotoxic effects of cisplatin on kidney tissues. One such mechanism is the depletion of the endogenous antioxidant system [3, 31], which results from cisplatin's metabolic conversion into reactive molecules [3]. This depletion leads to a buildup of reactive oxygen species (ROS), causing oxidative damage to epithelial cells [6].

Our research findings revealed that a single-dose injection of cisplatin significantly elevated malondialdehyde (MDA) levels while reducing the activity of key antioxidant enzymes, including superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). In contrast, nettle extract demonstrated notable antioxidant properties, enhancing the activity of these enzymes while suppressing lipid peroxidation. These findings are in line with previous studies by Salih [30], who reported a decline in renal tissue MDA levels and an upregulatory effect on GSH content in a model of gentamicin-induced renal oxidative injury. Additionally, the work by Dhouibi et al. [17] documented the antioxidant effects of *Urtica dioica* against potassium bromate-induced renal oxidative stress, showing restoration of SOD, CAT, and glutathione peroxidase activity, along with a reduction in MDA levels. NF-κB plays a crucial role in regulating the transcription of several genes involved in inflammation, particularly through the production of inflammatory cytokines [32]. It has been established that NF-κB is associated with ROS overproduction, suggesting that targeting NF-κB stimulation could mitigate severe inflammatory conditions, including acute kidney injury (AKI). These findings align with our study, which demonstrates that cisplatin (Cis) injection modulates the protein levels of key renal inflammatory mediators, including NF-κB, TNF-α, IL-6, and IL-1β. Our results are consistent with previous studies reporting that cisplatin-induced kidney inflammation leads to increased levels of NF-κB and proinflammatory cytokines in a rat model [33-35]. Moreover, cisplatin administration has been associated with elevated renal levels of IL-1β, IL-6, IL-18, and neutrophil infiltration [6]. On the other hand, oral administration of nettle extract alongside cisplatin for three weeks effectively mitigated renal inflammation by suppressing the rise in inflammatory markers. These findings align with those of Albadawi et al. [36], who reported that *Urtica dioica* counteracts rotenone-induced neuroinflammation in a rat model of Parkinson's disease by downregulating

the gene expression of IL-6 and TNF-α. Similarly, Genc et al. [37] documented the anti-inflammatory effects of *Urtica dioica* in a rat model of colitis, demonstrating its ability to suppress the levels of proinflammatory mediators.

Renal tubular apoptosis is considered one of the key mechanisms in the pathology of cisplatin-induced nephrotoxicity [31]. In this study, the protein levels of caspase-3, caspase-8, caspase-9, cytochrome c, and Bax in renal supernatant were markedly increased, while Bcl-2 levels decreased. These results align with the findings of Mohamed et al. [38], who reported that cisplatin injection elevates apoptotic markers such as caspase-9, caspase-3, and Bax while reducing Bcl-2 expression. Additionally, research by Alanezi et al. [39] documented the upregulatory effect of cisplatin on renal caspase-3 and Bax immunoexpression, along with its suppressive impact on Bcl-2 immunoexpression.

In contrast, our study demonstrated that the administration of nettle ethanolic extract in Group IV significantly suppressed the protein levels of renal supernatant extrinsic and intrinsic apoptotic pathways while enhancing the expression of the anti-apoptotic marker Bcl-2. These findings are in line with the work of Albadawi et al. [36], who reported that *Urtica dioica* inhibits neuronal apoptosis in a rat model of parkinsonism through its regulation of apoptotic markers such as Bcl-2, Bax, and caspase-3. Similarly, Keleş et al. [40] documented that *Urtica dioica* counteracts diethyl nitrosamine-induced renal apoptosis by inhibiting the immunoexpression of caspase-3.

5. Conclusion

This study highlights the protective effects of nettle (*Urtica dioica*) extract against cisplatin-induced kidney damage. Cisplatin treatment led to renal dysfunction, increased oxidative stress, heightened inflammation, and enhanced apoptosis, as evidenced by elevated levels of creatinine, BUN, MDA, NF-κB, and apoptotic markers. Additionally, the activity of antioxidant enzymes and anti-apoptotic proteins was significantly reduced. Co-administration of nettle extract effectively countered these adverse effects by improving renal function, reducing oxidative stress markers, suppressing inflammatory cytokines, and restoring apoptotic balance. These findings suggest that nettle extract possesses nephroprotective, anti-inflammatory, antioxidant, and anti-apoptotic properties, making it a promising candidate for mitigating drug-induced kidney injury. Further research is needed to explore its therapeutic potential and underlying mechanisms.

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