Check for updates

OPEN ACCESS

DOI: 10.46903/gjms/23.1.Special.1844

ORIGINAL ARTICLE

DOSE-DEPENDENT PROTECTIVE EFFECTS OF SESAMIN AGAINST RENAL INFLAMMATION AND OXIDATIVE STRESS IN CISPLATIN-TREATED RATS: AN EXPERIMENTAL STUDY

Kumayl Abbas Meghji[1], Samia Siddiqui[1], Safiya Javed[2], Ali Abbas Thalho[3], Raima Kalhoro[4], Ahsan Aslam Memon

Departments of [1]Physiology, [2]Pathology & [3]Pharmacology, Isra University Hyderabad, [4]Department of Pathology, Peoples University of Medical and Health Sciences for Women, Nawabshah, [5]Department of Pharmacology, Indus Medical College, Tando Muhammad Khan, Sindh, Pakistan

ABSTRACT

Background: Cisplatin, widely used for treating cancers, often induces nephrotoxicity, limiting clinical use. Sesamin, a lignan from sesame seeds, shows antioxidant and anti-inflammatory effects that may protect renal function. The objective of this study was to assess the dose-dependent anti-oxidative and anti-inflammatory effects of sesamin on cisplatin-induced nephrotoxicity.

Materials & Methods: This quasi-experimental study was performed at Isra University Hyderabad between April and October 2024. Forty male albino Wistar rats were grouped into: Group A (control), Group B (cisplatin 20 mg/kg intraperitoneally), Group C (cisplatin 20 mg/kg intraperitoneally + sesamin 10 mg/kg orally for 10 days), and Group D (cisplatin 20 mg/kg intraperitoneally + sesamin 20 mg/kg orally for 10 days). Following treatment, blood and kidney tissue samples were collected for biochemical and histological analysis. Data were analyzed in SPSS with significance at $P \leq 0.05$.

Results: Cisplatin treatment caused significant weight loss ($p<0.05$) and elevated serum urea, creatinine, inflammatory markers (IL-1, IL-6, TNF-α), and oxidative stress markers (MDA) in Group B ($p<0.05$). Groups C and D exhibited less pronounced elevations in these parameters. Group D showed the most favorable results, with near-normal histological architecture and improved antioxidant enzyme levels (SOD, GPx), compared to group B ($p<0.05$). Histopathological analysis revealed severe renal damage in Group B, while groups C and D displayed less pronounced renal injury, with Group D showing the best preservation of kidney structure.

Conclusion: Sesamin demonstrated dose-dependent protection against cisplatin-induced nephrotoxicity by reducing oxidative stress, inflammatory markers, and improving renal function.

KEY WORDS: Cisplatin; Inflammation; Nephrotoxicity; Oxidative Stress; Sesamin.

Cite as: Meghji KA, Siddiqui S, Javed S, Thalho AA, Kalhoro R, Memon AA. Dose-dependent protective effects of sesamin against renal inflammation and oxidative stress in cisplatin-treated rats: an experimental study. Gomal J Med Sci 2025 Jan-Mar;23(1)Special:140-45. https://doi.org/1046903/gjms/23.1.Special.1844

INTRODUCTION

Cisplatin, a platinum-based chemotherapeutic agent, is widely used in the treatment of various cancers, including those of the breast, testes, bladder, head and neck, lungs, and cervix.[1] It is also effective in treating high-grade malignancies such as osteosarcoma and squamous cell carcinoma when used in combination therapies.[2] Cisplatin's cytotoxic mechanism involves entering the cell and undergoing aquation, a chemical process that generates hydrolyzed products. These products bind to the N7 position of purine residues, leading to DNA replication impairment, cell cycle arrest, and ultimately apoptosis in cancer cells.[3]

Despite its potent anticancer properties, the clinical use of cisplatin is significantly restricted due to its severe side effects, including nephrotoxicity, cardiotoxicity, ototoxicity, peripheral neuropathy, leukemia, and

Corresponding Author:
Dr. Kumayl Abbas Meghji
Assistant Professor
Department of Physiology
Isra University
Hyderabad, Pakistan
E-mail: dr.kumaylabbas@gmail.com

Date Submitted: 10-11-2024
Date Revised: 02-04-2025
Date Accepted: 08-04-2025

neurotoxicity.[4] Among these, nephrotoxicity remains the most critical challenge, as cisplatin induces acute kidney injury (AKI) by damaging renal tubular cells and triggering cell death, leading to rapid declines in renal function in up to 30% of patients undergoing therapy.[5,6] Despite these toxicities, cisplatin remains a cornerstone in cancer treatment due to the lack of more effective alternatives, spurring ongoing research into compounds that may mitigate its harmful effects.

In recent years, naturally occurring plant-based compounds with potent anticancer properties have garnered attention. One such compound is sesamin, a fat-soluble lignan extracted from sesame oil and seeds, which is known for its antioxidant, antihypertensive, anti-inflammatory, and neuroprotective properties.[7] Sesamin exhibits anticancer effects by targeting and disrupting multiple cancer-related signaling pathways.[8] Recent studies have also shown that sesamin can alleviate renal injury in hyperlipidemic rats by enhancing antioxidant enzyme activity and reducing oxidative stress.[9,10] Additionally, sesamin has been found to inhibit neutrophil infiltration, suppress pro-inflammatory cytokine release, and promote adenosine CD39-A2AR signaling, further supporting its potential therapeutic role in mitigating cisplatin-induced nephrotoxicity.[11]

Therefore, the objective of this study was to assess the dose-dependent anti-oxidative and anti-inflammatory effects of sesamin on cisplatin induced nephrotoxicity.

MATERIALS AND METHODS

Between April and October 2024, this study was carried out at Isra University in Hyderabad in the Postgraduate Research Laboratory and the Department of Physiology. Using a purposive sampling technique, forty male Albino Wistar rats weighing between 250 and 300 grams and 8 to 10 weeks of age were purchased from the Agriculture University in Tando Jam, Sindh. The sample size was calculated through power analysis for animal studies.[12,13] All animals were treated in accordance with the International Research Council's guidelines for laboratory animals, and the Ethics Review Committee of Isra University provided ethical approval (IU/RR-10-IRC-24/N/2024/109).

After a one-week acclimatization period, the rats were randomly assigned to four groups of 10 each: Group A (control), Group B (cisplatin 20 mg/kg intraperitoneally), Group C (cisplatin 20 mg/kg intraperitoneally + sesamin 10 mg/kg orally for 10 days), and Group D (cisplatin 20 mg/kg intraperitoneally + sesamin 20 mg/kg orally for 10 days). Sesamin, sourced from Nanjing NutriHerb BioTech Co., Ltd., China, was dissolved in carboxymethylcellulose (CMC, 0.5%) as a vehicle, while cisplatin was obtained from Pfizer Pakistan Limited. The doses of sesamin and cisplatin were based on previous studies.[5,6,11]

On the 11th day, rats were anesthetized using chloroform in an inverted jar. Cardiac puncture was performed to collect a sample of blood which was then transferred to gel tubes where it was allowed to clot for 30 minutes, followed by centrifugation at 3000 rpm for 15 minutes at 4°C. The separated serum was stored for biochemical analysis. Serum urea and creatinine levels were assessed using a Cobas c311 automated analyzer from Hitachi Roche. Levels of IL-1 (Catalogue no: MBS264984), IL-6 (Catalogue no: MBS2020158), and TNF-α (Catalogue no: MBS175904), along with the biochemical markers in renal tissue homogenates such as Malondialdehyde (MDA) (Catalogue no: MBS268427), Reduced Glutathione (GSH) (Catalogue no: MBS724319), Glutathione Peroxidase (GPx) (Catalogue no: MBS732529), and Superoxide Dismutase (SOD) (Catalogue no: MBS036924), were all assessed using PicoKine® kits, following the manufacturer's instructions. The rats were sacrificed via cervical dislocation, and kidneys were extracted, rinsed with saline, and fixed in 10% neutral buffered formalin. After fixation, tissues underwent routine histological processing, including dehydration in ethanol (70-100%), clearing in xylene, and embedding in paraffin wax. Thin sections (5-7µm) were cut using a semi-automatic microtome (BK-2238, BIOBASE, China) and stained with Hematoxylin and Eosin (H&E) for histopathological examination.

Data were analyzed using SPSS version 25.0. Quantitative differences between groups were evaluated using one-way ANOVA followed by post hoc Tukey's test, with a significance threshold of $P \leq 0.05$.

RESULTS

The mean pre-experimental weights were noted to be 259.27±5.98, 260.16±3.18, 259.62±4.49, and 258.94±3.37 in group A, B, C, and D respectively ($p>0.05$). The mean post-experimental weights of group A, B, C, and D were noted to be 262.65±5.98, 213.43±4.75, 225.24±3.15, and 237.53±2.58, respectively. The difference among the post-experimental body weight among the experimental animals was statistically significant ($p<0.05$), with a marked drop in body weight in group B. Groups C and D also displayed weight loss, however, it was less pronounced as compared to group B, with Group D showing best results, Figure 1.

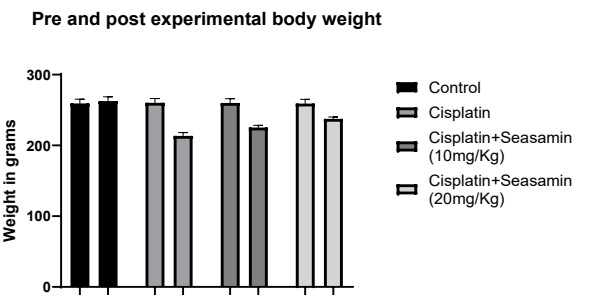

Figure 1. Pre and Post experimental body weights of experimental animals

Table 1. Distribution of markers of renal function and inflammation among experimental groups.

Group	Group A	Group B	Group C	Group D	p-value
Urea (mg/dL)	21.89±0.98 B,C,D	61.04±2.34 A,C,D	37.03±1.01 A,B,D	27.42±1.24 A,B,C	0.000*
Creatinine (mg/dL)	0.60±0.01 B,C,D	3.01±0.17 A,C,D	1.62±0.10 A,B,D	1.15±0.29 A,B,C	0.000*
IL1 (pg/ml)	133.43±2.59 B,C,D	571.57±5.75 A,C,D	310.68±4.59 A,B,D	163.11±3.99 A,B,C	0.000*
IL-6 (pg/ml)	95.46±2.19 B,C,D	600.54±3.60 A,C,D	310.26±5.38 A,B,D	164.34±3.39 A,B,C	0.000*
TNFα (pg/ml)	94.88±1.59 B,C,D	373.62±2.60 A,C,D	194.51±2.48 A,B,D	139.74±94.73A,B,C	0.000*

Table 2. Distribution of markers of oxidative stress among experimental groups.

Group	Group A	Group B	Group C	Group D	p-value
MDA (nmol/mg)	81.40±1.27 B,C,D	153.50±2.68 A,C,D	114.52±2.12 A,B,D	95.67±2.01 A,B,C	0.000*
SOD (U/mg)	63.35±2.25 B,C,D	32.78±1.17 A,C,D	50.32±1.20 A,B,D	57.81±0.55 A,B,C	0.000*
GPx (U/mg)	38.21±0.73 B,C,D	19.71±0.81 A,C,D	27.43±1.41 A,B,D	33.84±1.10 A,B,C	0.000*

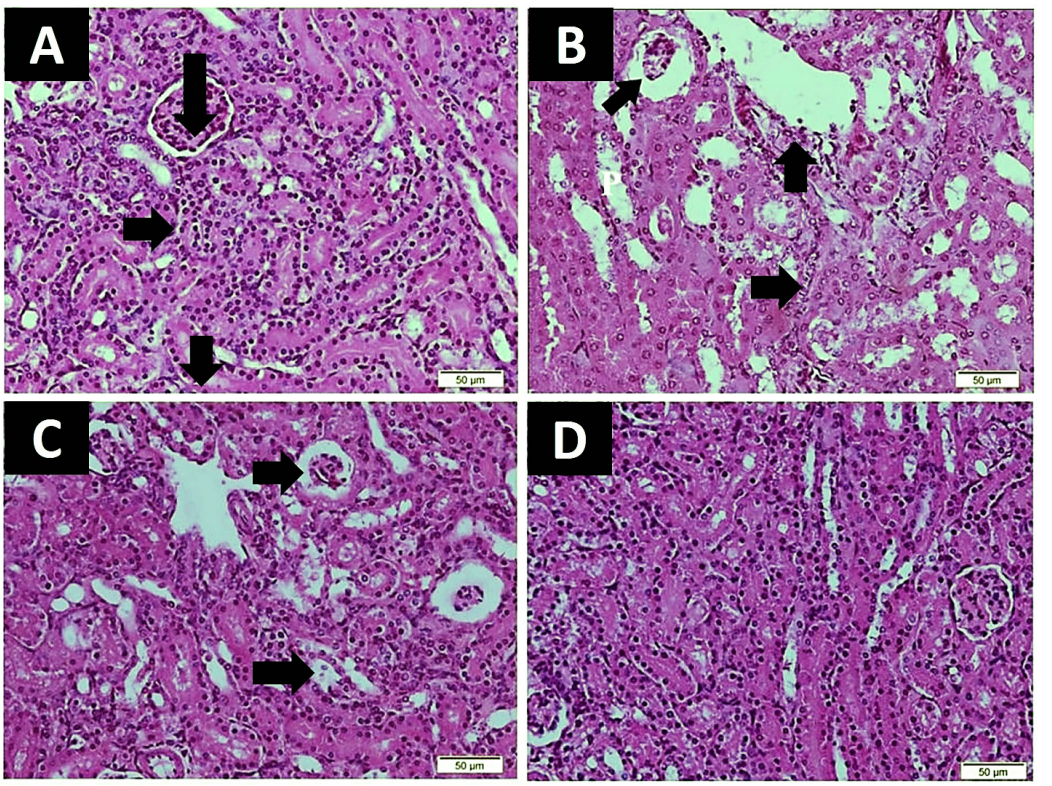

Figure 2. Photomicrographs of renal tissue sections of experimental groups (H&E stain)

Table 1 displays the renal function and inflammatory marker levels across the experimental groups. Group B showed a marked rise in mean serum urea and creatinine levels compared to the control group, A ($p < 0.05$). Similar elevations were observed in groups C and D, though less pronounced than in group B, with group D showing the most favorable results. Likewise, the mean levels of inflammatory markers (IL-1, IL-6, and TNF-α) rose significantly in group B compared to control group A ($p < 0.05$). Groups C and D also demonstrated elevated levels of these markers, though the increases were less marked than in group B, with group D again showing the best outcomes.

Table 2. displays the oxidative stress marker levels across the experimental groups. Group B showed a noticeable rise in MDA levels compared to the control group, A ($p < 0.05$). Similar elevations were observed in groups C and D, though less pronounced than in group B, with group D showing the most favorable

results. Similarly, the levels of SOD and GPx were significantly decreased in group B as compared to control group A ($p<0.05$). A similar decline was observed in groups C and D, though less pronounced than in group B, with group D showing the most favorable results.

Figure 2 shows the renal cut sections of experimental animals. Figure 2A shows the renal sections of untreated control rats with normal renal glomeruli, proximal and distal tubules, as marked by arrows. Figure 2B shows the renal cut sections of cisplatin treated nephrotoxic rats with patchy infiltration of inflammatory cells along with tubular injury and cytoplasmic vacuolization, as marked by arrow heads. Figure 2C shows the renal cut sections of group C rats, with less pronounced degenerative changes in the renal tubules and glomeruli. Figure 2D shows the renal cut sections of group D rats which displayed near normal histological architecture with no marked alterations.

DISCUSSION

This study aimed to examine sesamin's protective impact on cisplatin-induced kidney damage in rats, assessing both chemical and tissue-level indicators of renal health in untreated and sesamin-treated groups. Cisplatin-treated rats showed significant reduction in body weight as well as impairments in renal function and histology, which were mitigated by sesamin administration.

As mentioned earlier, cisplatin serves as a "double-edged sword" in cancer therapy: while it effectively targets cancer cells, it also damages normal host cells, with kidneys being especially vulnerable. This vulnerability is attributed to kidneys being tasked with the perilous chore of eliminating toxic waste from the body, exposing them to free radicals.[6] Within the renal tubular cells, cisplatin undergoes metabolism, generating highly toxic components through a series of chemical reactions.[14] This disrupts the balance between free radical production and elimination, leading to oxidative stress, and in turn, nephrotoxicity.[15] In this study, cisplatin administration led to a significant reduction in body weight among the experimental animals, aligning with findings from prior research.[5,6,16] However, sesamin treated rats showed significantly lesser weight loss, consistent with observations by Badreldin H. et al., who reported that sesamin therapy alleviates cisplatin-induced weight reduction.[16]

Cisplatin therapy was also associated with a marked elevation in the serum levels of urea and creatinine in the present study, indicating a decline in renal function. This aligns with findings by Badreldin et al. and Hakimizadeh et al., who also reported significant renal impairment, evidenced by elevated serum urea and creatinine levels following cisplatin therapy..[17,18] Serum urea and creatinine levels were notably lower in experimental rats treated with sesamin adjunct therapy than those in rats treated with cisplatin alone, aligning with findings by Badreldin H. et al.[16] Altyar et al. reported that the nephroprotective effect of sesamin is dose-related, with higher doses associated with notably reduced serum urea and creatinine levels, consistent with the results of the current study.[11]

Cisplatin treated rats also showed a significant increase in the levels of pro-inflammatory cytokines, such as IL1, IL6, and TNF-α as compared to controls, in the present study. This aligns with findings from Tripathi et al. and Anwer et al., who both noted a significant increase in serum inflammatory markers following cisplatin therapy.[19,20] This is attributed to the fact that one of the key mechanisms through which cisplatin exerts its nephrotoxic effects is by mediating programmed necrosis and inflammation.[21] On the other hand, serum levels of IL-1, IL-6, and TNF-α in experimental rats receiving adjunct sesamin therapy were markedly lower than those observed in rats treated solely with cisplatin. These findings align with those reported by Badreldin H. et al.[16] Moreover, high dose of sesamin was associated with a lower level of inflammatory markers as compared to low dose in the current study, which is in accordance with the findings of Altyar et al.[11]

As mentioned, a key mechanism driving the pathogenesis of cisplatin-induced renal damage is the generation of ROS and oxidative stress.[22] This was evident in the current study, as cisplatin-treated rats exhibited a significant increase in lipid peroxidation coupled with a reduction in renal antioxidant enzyme levels, indicating oxidative stress. This is similar to the findings reported by Siqing et al. and Ijaz et al. both of whom reported marked oxidative stress and significant depletion of oxidative markers in experimental rats treated with cisplatin.[23,24] Concomitant sesamin therapy, however, was associated with significantly lower levels of oxidative markers in the current study, consistent with the findings reported by Badreldin H. et al.[16] Additionally, the antioxidant effects of sesamin were found to be dose related in the current study with higher dose showing significantly better results, similar to the findings of Altyar et al.[11]

Additionally, Siqing et al.[23] and Ijaz et al.[24] reported that cisplatin induces significant alterations in renal histology, such as infiltration of inflammatory cells and tubular necrosis, findings that align with the results of the present study. These changes were however not as pronounced in sesamin treated rats, corroborating the findings of Badreldin H. et al. and Altyar et al.[11,16]

There were certain limitations in the current study, particularly related to time and financial resources, which restricted further investigations, such as immunohistochemical assays. Additionally, the protective effects of sesamin on other cisplatin-in-

duced toxicities, including hepatotoxicity, testicular toxicity, and ototoxicity, were not examined. Therefore, further studies are recommended to explore the dose-dependent protective effects of sesamin on these additional toxicities, which could provide a more comprehensive understanding of its therapeutic potential.

CONCLUSION

Sesamin exhibits dose-dependent protective effects against cisplatin-induced nephrotoxicity. Sesamin therapy significantly mitigated oxidative stress, reduced inflammatory marker levels, and improved renal function and histopathological outcomes. The results suggest that higher doses of sesamin offer enhanced protection, making it a promising adjunct therapy for preventing cisplatin-induced renal damage. Further studies are warranted to explore the underlying mechanisms and optimize dosing strategies for potential clinical application in cancer patients undergoing cisplatin chemotherapy.

REFERENCES

1. Kopacz-Bednarska A, Król T. Cisplatin-Properties and clinical application. Oncol Clin Pract. 2022;18(3):166–76. https://doi.org/10.5603/OCP.2022.0020
2. Solernó LM, Sobol NT, Gottardo MF, Capobianco CS, Ferrero MR, Vásquez L, et al. Propranolol blocks osteosarcoma cell cycle progression, inhibits angiogenesis and slows xenograft growth in combination with cisplatin-based chemotherapy. Sci Rep. 2022;12(1):15058. https://doi.org/10.1038/s41598-022-18324-3
3. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Exp Pharmacol. 2021:303–28. https://doi.org/10.2147/JEP.S267383
4. Meghji KA, Memon TF, Aslam A, Noor N, Abbas A, Memon RA. The novel role of resveratrol in attenuating cisplatin induced testicular toxicity in rats. Prof Med J. 2020;27(09):2023–9. https://doi.org/10.29309/TPMJ/2020.27.09.4469
5. Meghji KA, Memon TF, Ahmed I, Memon SG, Noor N, Abbas A. Nephroprotective effects of L-Arginine against chemotherapy induced acute kidney injury in wistar rats. J Islamabad Med Dent Coll. 2020;9(4):249–55. https://doi.org/10.35787/jimdc.v9i4.535
6. Meghji KA, Talpur RA, Uqaili AA, Nizammani YM, Kazi N, Nizammani GS. Resveratrol attenuates oxidative stress in chemotherapy induced acute kidney injury: An experimental rat model. KMUJ. 2019;11(2):85–9. https://doi.org/10.35845/kmuj.2019.19114
7. Oboulbiga EB, Douamba Z, Compaoré-Sérémé D, Semporé JN, Dabo R, Semde Z, et al. Physicochemical, potential nutritional, antioxidant and health properties of sesame seed oil: a review. Front Nutr. 2023;10:1127926. https://doi.org/10.3389/fnut.2023.1127926
8. Khuanphram N, Taya S, Kongtawelert P, Wongpoomchai R. Sesame extract promotes chemopreventive effect of hesperidin on early phase of diethylnitrosamine-initiated hepatocarcinogenesis in rats. Pharmaceutics. 2021;13(10):1687. https://doi.org/10.3390/pharmaceutics13101687
9. Farouk SM, Gad FA, Emam MA. Comparative immuno-modulatory effects of basil and sesame seed oils against diazinon-induced toxicity in rats; a focus on TNF-α immunolocalization. Environ Sci Pollut Res. 2021;28:5332–46. https://doi.org/10.1007/s11356-020-10840-x
10. Mahzari S, Hosseinian S, Mohebbati R, Noshahr ZS, Rad AK. Kidney dysfunction and oxidative stress in doxorubicin-induced nephrotic rat: protective role of sesame oil. Saudi J Kidney Dis Transpl. 2021;32(5):1243–52. https://doi.org/10.4103/1319-2442.344743
11. Altyar AE, Albadrani GM, Farouk SM, Alamoudi MK, Sayed AA, Mohammedsaleh ZM, et al. The antioxidant, anti-inflammatory, and anti-apoptotic effects of sesamin against cisplatin-induced renal and testicular toxicity in rats. Ren Fail. 2024;46(2):2378212. https://doi.org/10.1080/0886022X.2024.2378212
12. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med. 2013;35(2):121–6. https://doi.org/10.4103/0253-7176.116232
13. Hanif MS, Baloch MS, Meghji KA, Abbas A, Kashif S, Qureshi R. Histopathological changes in the gastric mucosa induced by carbaryl toxicity: an experimental rat model. KMUJ. 2020;12(2). https://doi.org/10.35845/kmuj.2020.19896
14. Zhang X, Peng X, Wang C, Olatunji OJ, Famurewa A. Tiliacora triandra attenuates cisplatin triggered hepatorenal and testicular toxicity in rats by modulating oxidative inflammation, apoptosis and endocrine deficit. Front Biosci (Landmark Ed). 2022;27(2):44. https://doi.org/10.31083/j.fbl2702044
15. Neamatallah T, El-Shitany NA, Abbas AT, Ali SS, Eid BG. Honey protects against cisplatin-induced hepatic and renal toxicity through inhibition of NF-κB-mediated COX-2 expression and the oxidative stress dependent BAX/Bcl-2/caspase-3 apoptotic pathway. Food Funct. 2018;9(7):3743–54. https://doi.org/10.1039/C8FO00653A
16. Badreldin HA, Al Salam S, Al Suleimani Y, Ashique M, Manoj P, SUDHADEVI M, et al. Ameliorative effect of sesamin in cisplatin-induced nephrotoxicity in rats by suppressing inflammation, oxidative/nitrosative stress, and cellular damage. Physiol Res. 2019;69(1):61. https://doi.org/10.33549/physiolres.934142
17. Ali BH, Abdelrahman A, Al Suleimani Y, Manoj P, Ali H, Nemmar A, et al. Effect of concomitant treatment of curcumin and melatonin on cisplatin-induced nephrotoxicity in rats. Biomed Pharmacother. 2020;131:110761. https://doi.org/10.1016/j.biopha.2020.110761

18. Hakimizadeh E, Kaeidi A, Hassanshahi J, Mehrbani M, Rahmani M. Ameliorating effect of pistachio hydroalcoholic extract on cisplatin-induced nephrotoxicity in mice. Res J Pharmacogn. 2021;8(1):73–9. https://doi.org/10.22127/rjp.2020.119426
19. Tripathi P, Alshahrani S. Mitigation of IL-1β, IL-6, TNF-α, and markers of apoptosis by ursolic acid against cisplatin-induced oxidative stress and nephrotoxicity in rats. Hum Exp Toxicol. 2021;40(12 Suppl):S397–405. https://doi.org/10.1177/09603271211045953
20. Anwer T, Alshahrani S, Somaili AMH, Khubrani AH, Ahmed RA, Jali AM, et al. Nephroprotective Effect of Diosmin against Cisplatin-Induced Kidney Damage by Modulating IL-1β, IL-6, TNFα and Renal Oxidative Damage. Molecules. 2023;28(3):1302. https://doi.org/10.3390/molecules28031302
21. Tang C, Livingston MJ, Safirstein R, Dong Z. Cisplatin nephrotoxicity: new insights and therapeutic implications. Nat Rev Nephrol. 2023;19(1):53–72. https://doi.org/10.1038/s41581-022-00631-7
22. Alshahrani S, Ali Thubab HM, Ali Zaeri AM, Anwer T, Ahmed RA, Jali AM, et al. The Protective Effects of Sesamin against Cyclophosphamide-Induced Nephrotoxicity through Modulation of Oxidative Stress, Inflammatory-Cytokines and Apoptosis in Rats. Int J Mol Sci. 2022;23(19):11615. https://doi.org/10.3390/ijms231911615
23. Ma S, Xu H, Huang W, Gao Y, Zhou H, Li X, et al. Chrysophanol relieves cisplatin-induced nephrotoxicity via concomitant inhibition of oxidative stress, apoptosis, and inflammation. Front Physiol. 2021;12:706359. https://doi.org/10.3389/fphys.2021.706359
24. Ijaz MU, Aziz S, Faheem M, Abbas K, Nasir S, Naz H, et al. Orientin Attenuates Cisplatin-Induced Renal Toxicity by Reducing Oxidative Stress and Inflammation. Pak Vet J. 2021;41(4). https://doi.org/10.29261/pakvetj/2021.076

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	KAM, SS
Acquisition, Analysis or Interpretation of Data:	KAM, SS, SJ, AAT, RK
Manuscript Writing & Approval:	KAM, SS, SJ, AAT, AAM

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.