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Research Article
Exploring the Protective Effects of Astragaloside IV against Cisplatin-Induced Kidney Injury in Rats

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Abstract

Background and Objective: Cisplatin, a widely used chemotherapeutic agent, is associated with significant nephrotoxicity, leading to Acute Kidney Injury (AKI). Astragaloside IV (AS-IV), a compound derived from Radix Astragali, has shown promise in reducing renal fibrosis and oxidative stress. The objective of this study was to evaluate the protective effect of Astragaloside in cisplatin-induced kidney injury in rats. **Materials and Methods:** Thirty female Wistar rats were divided into three groups: A Normal control group (n = 10), a Cisplatin+Saline group (n = 10) and a Cisplatin+AS-IV group (n = 10). Cisplatin was administered intraperitoneally at 2.5 mg/kg twice weekly for four weeks to induce nephrotoxicity. The AS-IV was given at 80 mg/kg/day. Biochemical markers of kidney injury and oxidative stress, including MDA, TNF-α, KIM-1, NGAL, Nephrin, creatinine and urea, were measured. Histopathological analysis of kidney tissues was also performed. **Results:** Cisplatin administration significantly elevated plasma levels of MDA, TNF-α, KIM-1, NGAL, Nephrin, creatinine and urea, indicating oxidative stress and kidney injury. The AS-IV treatment significantly reduced these levels, suggesting its protective effects. Histopathological examination revealed severe tubular injury in the Cisplatin+Saline group, which was markedly attenuated in the AS-IV treated group. **Conclusion:** Astragaloside IV demonstrated significant protective effects against cisplatin-induced nephrotoxicity in rats. The compound reduced oxidative stress, inflammation and histopathological damage, highlighting its potential as an adjunct therapy to mitigate cisplatin-induced kidney injury in clinical settings.

Key words: Cisplatin, acute kidney injury, astragaloside, neutrophil gelatinase-associated lipocalin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cisplatin is a potent and efficacious chemotherapeutic agent that disrupts the DNA repair processes and triggers programmed cell death in cancer cells and which used in the clinical setting to treat a range of solid malignancies, including cancer of the lungs, stomach and ovary[1,2]. However, it is associated with unwanted adverse effects[1]. From a clinical perspective, the incidence of nephrotoxicity in patients receiving cisplatin is estimated to be between 20 and 35%. This can result in fatal Acute Kidney Injury (AKI) in affected patients[3,4]. Furthermore, it should be noted that pediatric patients are also prone to experiencing nephrotoxicity when cisplatin is administered[5]. Patients with Acute Kidney Injury (AKI) have clinical symptoms including impaired function of the renal tubules, acute renal failure, reduced red blood cell count, anemia, muscular tremors, weight loss, intestinal dysfunction, lethargy and tightness of the muscles around the eyes. These symptoms impede the efficacy of cisplatin as an anti-tumor therapy[6]. The compound cisplatin induces DNA damage and interferes with the normal operation of cytoplasmic organelles, particularly the endoplasmic reticulum and mitochondria. It activates apoptotic pathways and induces cellular damage by elevating oxidative stress and inducing inflammation[7,8].

The use of a combination of cisplatin and natural products in cancer chemotherapy has demonstrated promise in increasing the effectiveness of treatment and decreasing harmful side effects[9]. The Radix Astragali, a frequently employed Chinese medicine for enhancing vitality, has a history spanning over 2,000 years. Astragaloside IV (AS-IV), the primary constituent of Radix Astragal, has been utilized for the management of renal fibrosis. The AS-IV has a notable effect in decreasing the expression and accumulation of extracellular matrix (ECM) components in the kidney, thus slowing down the progression of tubulointerstitial fibrosis (TIF)[10]. There are studies in the literature on AS-IV in the case of neuronal damage[11,12], colorectal cancer[13,14], endometrial and ovarian cancer[14,15], liver damage[16] and regulating immunity[17]. Pharmacological research has demonstrated that AS-IV can reduce oxidative stress and inflammatory responses and hinder the development and advancement of renal fibrosis. The compound boosts the immune system and can enhance the responsiveness to other medications when used with other treatment modalities[17,18]. An experimental study on rats demonstrated that the intravenous administration of AS-IV primarily deposited in the kidney and liver[16,19].

Still, some studies are done for renal protectivity of AS-IV[20,21] but currently, there is no pharmacologically efficient medication available to prevent or treat nephrotoxicity caused by cisplatin. Several potent and safe medications derived from natural sources have been created to prevent cisplatin-induced Acute Kidney Injury (AKI). This study aimed to evaluate the protective and reparative effect of Astrogloside by using biochemical and histologic methods in cisplatin-induced kidney injury in rats.

MATERIALS AND METHODS

Study area: The investigation was carried out in the experimental animal laboratory of Demiroglu Bilim University, Gebze, Istanbul from February, 2024 to July, 2024.

Animals: Thirty adult female Wistar rats were obtained from Demiroglu Bilim University Laboratory. The research employed rats weighing between 200 and 210 g. The animals were confined in enclosures and kept in controlled environments with 12 hrs lighting and dark cycles at a temperature of 22±2°C. All animals were provided with a regular pellet meal and unrestricted access to tap water throughout the research.

Ethical consideration: The research has its protocol accepted by the Experimental Care of Animals and Ethics Committee of the University of Science University (Ethical Number: 19-02-2024/2824062216). All chemicals were obtained from Sigma-Aldrich Inc., unless otherwise noted.

Methodology: Thirty rats were studied. For the study, ten rats were chosen as controls. No medicine was given to this group. Twenty rats received 2.5 mg/kg/day cisplatin intraperitoneally (i.p.) twice a week for four weeks. A 20 mg/kg dosage was used to represent cisplatin-induced kidney injury. Two groups of cisplatin-treated rats were formed. Group 1 rats (n = 10) got 1 mL/kg/day 0.9% NaCl saline intraperitoneally for 4 weeks, whereas Group 2 rats received 80 mg/kg/day astragaloside IV. Four Cisplatin and Saline-treated rats died throughout the study. In rats given cisplatin and astragaloside IV, one died. At the end of the study, rats were sacrificed via cervical dislocation with a high dose of anesthesia. Blood was drawn by heart puncture for biochemical examination and organs were histologically examined.

Measurement of plasma lipid peroxidation (MDA): Thiobarbituric Acid Reactive Substances (TBARS), namely malondialdehyde (MDA), were quantified in plasma samples to evaluate lipid peroxidation. The plasma samples were treated with trichloroacetic acid and TBARS reagent, then combined and maintained at 100°C for 60 min. Following ice

chilling, the samples were subjected to centrifugation at 3000 revolutions per minute for 20 min. The liquid above the silt was then tested at 535 nm for absorbance. The MDA was measured in nanomolar units using tetraethoxypropane for calibration.

Measurement of plasma TNF-α, NGAL, KIM-1 and Nephrin: Quantification of plasma Tumour Necrosis Factor-Alpha (TNF-α), Neutrophil Gelatinase-associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1) and Nephrin levels were conducted using readily accessible ELISA kits (Sigma Aldrich in St. Louis, Missouri, USA) for enzyme-linked immunosorbent assay.

Blood biochemistry: Heart puncture was used to draw blood with a 1 mL syringe. The obtained blood was placed in heparin tubes. Keeping the samples at room temperature, they were centrifuged at 3000 rpm for 10 min. Plasma samples were stored at -20°C until testing. All groups' plasma BUN and creatinine levels were analyzed using the Beckman-Coulter AU 640 auto-analyzer and commercial kits from Beckman-Coulter Inc., California, United States.

Kidney biochemical analysis: The kidneys were promptly removed on decapitation and stored at a temperature of 20°C until biochemical analysis. Analyzing the kidney required blending it with a glass homogenizer in a solution of phosphate-buffered saline (PBS) that was five times its volume. The pH value of the solution was 7.4. The mixture was subjected to centrifugation at 5,000 g for 15 min. The Bradford method quantified nerve tissue protein by use of bovine serum albumin[21]. The quantification of TGF-beta1 in kidney tissue supernatants was performed using conventional rat ELISA kits.

Histopathological examination of the kidney: Immediately after the sacrifice, the kidneys were removed and stored at -20°C until they could undergo biochemical analysis. Liver and kidney pieces (4 µm) maintained in formalin were stained with Hematoxylin and Eosin (H&E). The slices were acquired via an Olympus C-5050 multi-purpose camera connected to an Olympus BX51 (Olympus Inc., Tokyo, Japan) optical microscope. Morphological evaluation was performed utilizing computerized software for image analysis developed by Media Cybernetics, Inc., USA. Analysis was conducted on ten microfields per slice at a magnification of ×20. The assessor was oblivious to the research group's existence. A semi-quantitative approach was used to evaluate the incidence of tubular epithelial necrosis, luminal necrotic debris, tubular dilatation and interstitial inflammation in the kidney sections of rats from all groups. The evaluation was based on the following rating scale:

- 0-5% = Score 0
- 6-20% = Score 1
- 21- 40% = Score 2
- 41-60% = Score 3
- 61-80% = Score 4
- 81-100% = Score 5

Statistical analysis: The SPSS 15.0 for Windows (IBM Corp., Armonk, New York, USA) was used for statistical analysis. Student's t-test and analysis of variance compared parametric variable groups. Mann-Whitney U test was used to compare nonparametric variable groups. Also utilized for parametric-non-parametric distinction was the Shapiro-Wilk test. Results are Mean+SEM. Statistical significance was determined at $p<0.05$.

RESULTS

Biochemical analysis: Astragaloside IV treatment showed a significant protective effect against cisplatin-induced damage. Plasma MDA levels were notably higher in the Cisplatin+Saline group compared to the Normal control ($p<0.001$), but Astragaloside IV significantly reduced these levels ($p<0.01$). The TNF-α, KIM-1, NGAL and Nephrin levels were all substantially elevated in the Cisplatin+Saline group ($p<0.01$ to $p<0.001$), but Astragaloside IV treatment significantly decreased these markers ($p<0.01$ to $p<0.001$). Additionally, Astragaloside IV effectively reduced the elevated plasma creatinine, urea and kidney TGF-beta1 levels induced by cisplatin ($p<0.01$ to $p<0.001$) (Table 1).

Histopathology analysis: Comparatively, kidney sections taken from the Cisplatin+Saline group exhibited tubular epithelial necrosis, luminal necrotic debris, tubular dilatation and interstitial inflammation, in contrast to the Normal control group. Assessments for tubular epithelial necrosis, luminal necrotic debris, tubular dilatation and interstitial inflammation were found to be significantly higher in the Cisplatin+Saline group; 2.5±0.2, 2.8±0.1, 3.2±0.2 and 0.9±0.2, respectively, compared to 0.2±0.1, 0.2±0.1, 0.1±0.1 and 0.1±0.1 in the Normal control group (p Astragaloside IV treatment substantially lowered histopathological scores to 1.2±0.1, 1.5±0.2, 1.4±0.1 and 0.4±0.1 ($p<0.01$ for all Cisplatin+Saline group comparisons).

Figure analysis: Figure 1 shows the kidney histopathology H&E staining at x10 and x40 magnification.

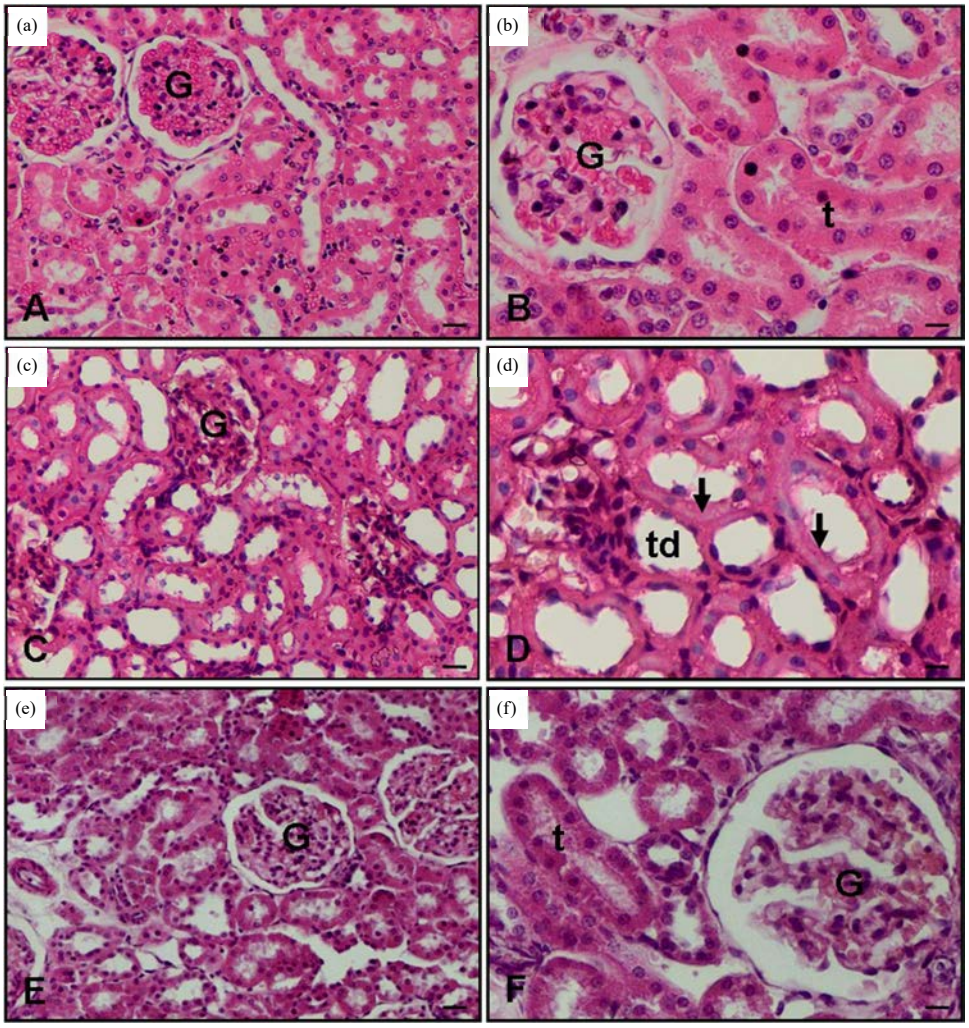

Fig. 1(a-f): Kidney histopathology H&E (x10 and x40 Magnification), (a-b) Normal kidney (control group), glomerul (G), tubules (t), (c-d) Cisplatin and Saline group kidney have acute tubular injury findings that tubular cell necrosis (arrow) and tubular dilatation (td) and (e-f) Cisplatin and Astragaloside IV group have diminished acute tubular injury findings

Table 1: Biochemical analysis of the experimental groups

	Normal control group	Cisplatin+Saline group	Cisplatin+Astragaloside IV group
Plasma MDA (nM)	43.5±1.8	116.4±11.3**	73.4±7.9#
Plasma TNF-α (pg/mL)	14.2±1.1	69.5±2.6*	48.3±5.1#
Plasma KIM-1 (pg/mL)	35.8±1.7	64.3±1.5**	55.8±3.2#
Plasma NGAL (pg/mL)	49.6±0.9	108.4±4.7**	81.3±2.4##
Nephrin (ng/mL)	169.5±8.8	266.2±7.5*	203.4±5.3#
Plasma creatinine (mg/dL)	0.5±0.1	2.1±0.2*	1.4±0.1#
Plasma urea (mg/dL)	23.2±0.5	85.3±2.9*	47.6±1.8##
Kidney TGF-beta1 (pg/mg protein)	11.8±0.6	67.4±1.7**	48.7±3.3##

Results were presented as Mean±SEM, *p<0.01, **p<0.001: Different from control group and #p<0.01, ##p<0.001: Different from Cisplatin and Saline group

Table 2: Histopathological examination scores of the kidneys of experimental groups

	Normal control group	Cisplatin+Saline group	Cisplatin+Astragaloside IV group
Tubular epithelial necrosis	0.2±0.1	2.5±0.2*	1.2±0.1#
Luminal necrotic debris	0.2±0.1	2.8±0.1*	1.5±0.2#
Tubular dilatation	0.1±0.1	3.2±0.2*	1.4±0.1#
Interstitial inflammation	0.1±0.1	0.9±0.2*	0.4±0.1#

Results were presented as Mean±SEM, *p<0.01: Different from control group and #p<0.01: Different from Cisplatin and Saline group

Figure 1a-b shows a normal kidney (Control group) showing normal glomeruli (G) and tubules (t), Fig. 1c-d shows kidney sections from the Cisplatin+Saline group exhibit acute tubular injury, characterized by tubular cell necrosis (indicated by arrows) and tubular dilatation (td). In Fig. 1e-f, the kidney sections from the Cisplatin+Astragaloside IV group show diminished acute tubular injury, with reduced tubular cell necrosis and tubular dilatation compared to the Cisplatin+Saline group.

The biochemical and histopathological results demonstrate the protective effects of Astragaloside IV against cisplatin-induced nephrotoxicity. The significant reduction in MDA, TNF-α, KIM-1, NGAL, Nephrin, creatinine, urea and TGF-beta1 levels in the Astragaloside IV treated group indicates its potent antioxidant, anti-inflammatory and renoprotective properties (Table 2). The histopathological findings further support these results, showing reduced tubular epithelial necrosis, luminal necrotic debris, tubular dilatation and interstitial inflammation in the Astragaloside IV treated group. These findings suggested that Astragaloside IV can mitigate the adverse renal effects of cisplatin, potentially improving the therapeutic index of cisplatin in clinical settings.

DISCUSSION

The current study findings demonstrate that AS-IV significantly ameliorates cisplatin-induced kidney injury, as evidenced by both biochemical and histopathological analyses. The MDA is a well-known marker of lipid peroxidation and oxidative stress. In the present study, cisplatin administration significantly increased plasma MDA levels, indicating enhanced lipid peroxidation and oxidative damage. This was consistent with previous studies by Pabla and Dong[7], that have shown cisplatin-induced nephrotoxicity is mediated through oxidative stress. The AS-IV treatment significantly reduced MDA levels, suggesting its potent antioxidant properties. This reduction in oxidative stress is likely due to AS-IV's ability to enhance mitophagy and maintain mitochondrial function, as supported by the upregulation of the Mfn2/Pink1/Parkin axis[22]. Mitophagy, the selective autophagy of damaged mitochondria, is crucial for maintaining cellular homeostasis and preventing oxidative damage[23].

The TNF-α is a pro-inflammatory cytokine that plays a crucial role in the inflammatory response associated with kidney injury. The results showed a significant increase in plasma TNF-α levels in the Cisplatin-treated group, indicating a strong inflammatory response. This finding aligned with previous research by Dasari *et al.*[8], indicating that, cisplatin induces inflammation through the activation of various signaling pathways. The AS-IV treatment significantly decreased TNF-α levels, demonstrating its anti-inflammatory effects. This reduction in inflammation may be attributed to AS-IV's ability to modulate inflammatory pathways, including the inhibition of NF-κB signaling, which is known to regulate the expression of pro-inflammatory cytokines[5,9]. The KIM-1 is a sensitive biomarker for detecting kidney injury. In the present study, cisplatin administration significantly increased plasma KIM-1 levels, indicating renal tubular damage. This was consistent with previous studies by Zhang *et al.*[24], that have shown elevated KIM-1 levels in cisplatin-induced nephrotoxicity. The AS-IV treatment significantly reduced KIM-1 levels, suggesting its protective effect on renal tubular cells. This protective effect may be due to AS-IV's ability to enhance mitophagy and reduce oxidative stress, thereby preventing tubular cell apoptosis[22]. Additionally, AS-IV has been shown to inhibit the activation of the MAPK/ERK pathway, which is involved in the regulation of cell survival and apoptosis[25]. The NGAL is another biomarker for acute kidney injury. The findings indicated a substantial rise in plasma NGAL levels in the Cisplatin-treated group, indicating acute kidney damage. This finding was in line with previous research indicating that NGAL levels are elevated in response to renal injury[4]. The AS-IV treatment significantly reduced NGAL levels, demonstrating its renoprotective effects. This reduction in NGAL levels may be attributed to AS-IV's ability to reduce inflammation and oxidative stress. The AS-IV has been shown to modulate the Nrf2/ARE pathway, enhancing the expression of antioxidant enzymes and reducing oxidative damage[26].

Nephrin is a key protein in the slit diaphragm of podocytes and its levels are indicative of podocyte injury. In our study, cisplatin administration significantly increased plasma Nephrin levels, indicating podocyte damage. This was consistent with previous studies by Liu *et al.*[27], that have shown Nephrin loss in cisplatin-induced nephrotoxicity. The AS-IV treatment significantly decreased Nephrin levels, suggesting its protective effect on podocytes. This protective effect may be due to AS-IV's ability to enhance mitophagy and maintain mitochondrial function in podocytes[13]. Furthermore, AS-IV has been shown to inhibit the TGF-β/Smad signaling pathway, which is involved in the regulation of fibrosis and podocyte injury[28]. Plasma urea and creatinine levels serve as reliable markers for assessing renal function. The findings revealed significant elevations in plasma urea and creatinine concentrations in the group treated with cisplatin, suggesting compromised renal function. This finding aligned with previous research by Ji *et al.*[9], indicating that, cisplatin induces

renal dysfunction[9]. The AS-IV treatment significantly reduced plasma creatinine and urea levels, demonstrating its renoprotective effects. This improvement in renal function may be attributed to AS-IV's ability to reduce oxidative stress, inflammation and tubular cell apoptosis[29]. The TGF-beta1 is a key mediator of renal fibrosis. In this study, cisplatin administration significantly increased kidney levels of TGF-beta1, indicating the onset of renal fibrosis. This was consistent with previous studies that have shown elevated TGF-beta1 levels in cisplatin-induced nephrotoxicity[10]. The AS-IV treatment significantly reduced TGF-beta1 levels, suggesting its anti-fibrotic effects. This reduction in TGF-beta1 levels may be due to AS-IV's ability to inhibit the TGF-β/Smad signaling pathway, thereby reducing fibrosis and promoting renal repair[28]. Histopathology of kidney sections showed severe tubular epithelial necrosis, luminal necrotic debris, tubular dilatation and interstitial inflammation in the cisplatin-treated group. These pathological changes were significantly attenuated by AS-IV treatment, indicating its protective effect on renal tissue. The reduction in tubular injury and inflammation is likely due to AS-IV's ability to enhance mitophagy and maintain mitochondrial function, as suggested by the upregulation of the Mfn2/Pink1/Parkin axis[22].

The protective effects of AS-IV can be attributed to its ability to modulate key signaling pathways involved in kidney injury. The AS-IV has been shown to enhance mitophagy via the Mfn2/Pink1/Parkin pathway, which is crucial for the removal of damaged mitochondria and maintenance of mitochondrial quality control[28]. This mechanism is supported by our findings of reduced oxidative stress and improved mitochondrial function in AS-IV treated rats.

Sepsis-Associated Acute Kidney Injury (S-AKI) is a significant clinical problem characterized by inflammation and oxidative stress[29]. The study by Xu *et al.*[30] demonstrated that Hyperhomocysteine (HHcy) exacerbates S-AKI by promoting inflammation and cell death through the Gpr97-TPL2 pathway. Although the current study did not focus on this pathway, the anti-inflammatory and antioxidant properties of AS-IV suggest that it could be beneficial in treating S-AKI. The AS-IV's ability to inhibit the Gpr97-TPL2 pathway, as shown in the study by Xu *et al.*[30], indicates its potential to reduce renal tubular damage and inflammation in sepsis-induced kidney injury.

Furthermore, the study by Li *et al.*[26] demonstrated that AS-IV attenuates acetaminophen-induced liver injuries by activating the Nrf2 signaling pathway, which enhances the expression of antioxidant enzymes and reduces oxidative damage. This finding supports the potential use of AS-IV in other conditions characterized by oxidative stress and inflammation, such as S-AKI. The findings of this study have significant clinical implications. Cisplatin is a widely used chemotherapeutic agent, but its nephrotoxicity limits its clinical utility. The ability of AS-IV to mitigate cisplatin-induced kidney injury suggests that it could be used as an adjuvant therapy to enhance the therapeutic index of cisplatin. This is particularly important for patients who are at high risk of nephrotoxicity, such as those with pre-existing kidney conditions or pediatric patients[31]. Additionally, the potential use of AS-IV in treating S-AKI could provide a new therapeutic approach for managing sepsis-related complications.

This research supports AS-IV's protective benefits, however, it has drawbacks. The study used rats, thus human trials are required to corroborate these results. The AS-IV's long-term effects and combinations with other chemotherapeutics should also be examined. The AS-IV's protective molecular processes should be studied further. Knowing the pathways might help develop tailored treatments for cisplatin-induced nephrotoxicity and sepsis-induced kidney damage.

CONCLUSION

The AS-IV significantly ameliorates cisplatin-induced nephrotoxicity by reducing oxidative stress, inflammation and renal tissue damage. These protective effects are mediated through the enhancement of mitophagy and the reduction of inflammatory responses. The AS-IV holds promise as a potential adjuvant therapy to improve the safety and efficacy of cisplatin chemotherapy. Additionally, its potential use in treating sepsis-induced kidney injury warrants further investigation.

SIGNIFICANCE STATEMENT

This study was necessary to address the critical challenge of cisplatin-induced nephrotoxicity, a major limitation in cancer chemotherapy, by exploring potential protective strategies. The research uniquely contributes to the academic understanding of nephroprotection by demonstrating that Astragaloside IV (AS-IV) significantly mitigates oxidative stress, inflammation and histopathological damage in cisplatin-induced kidney injury in rats. These findings suggest that AS-IV could be a promising adjunct therapy to reduce renal complications in patients undergoing cisplatin treatment, expanding therapeutic options in clinical oncology.

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