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# Histopathological and immunohistochemical evaluation of renal tissue in rats following chronic low-dose cadmium exposure and the nephroprotective potential of N-acetylcysteine

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Cadmium is a nephrotoxic heavy metal that progressively accumulates within renal tissue, inducing structural injury. This study evaluated the histopathological, morphometric, and immunohistochemical effects of chronic low-dose cadmium exposure on rat kidneys and investigated the nephroprotective potential of N-acetylcysteine. Thirty adult male Sprague-Dawley rats were divided into three groups: control; cadmium chloride (0.5 mg/kg orally twice weekly for eight weeks); and cadmium chloride concurrently with N-acetylcysteine (150 mg/kg/day intraperitoneally). Renal sections were processed for hematoxylin-eosin, Periodic Acid–Schiff, and Masson's trichrome staining, and for immunohistochemical localization of Caspase-3 and Ki-67. Quantitative morphometric analysis demonstrated that chronic cadmium exposure increased the kidney-to-body weight ratio by 29.6 %, reduced mean glomerular diameter by 22 % (82.4±3.1 versus 105.6±4.2 micrometers; p=0.004), caused a 38 % decrease in tubular lumen area (p<0.001), and triggered a 4.1-fold expansion of interstitial fibrosis (15.8 ± 1.2% versus 3.9±0.5 %; p=0.001). Immunohistochemical data revealed that cadmium provoked a 3.2-fold elevation in Caspase-3 expression (18.2±2.1 % versus 5.6±1.0 %; p=0.002) and a 45% suppression of the Ki-67 proliferative labeling index (9.3±1.4 % versus 17.0±1.8 %; p=0.008), indicating accelerated apoptosis and compromised regeneration. A strong positive correlation was identified between interstitial fibrosis and Caspase-3 expression (Pearson r = 0.89, p<0.001). Co-administration of N-acetylcysteine significantly attenuated these alterations: interstitial fibrosis was reduced by 62% (6.7±0.9 %; p=0.003), mean glomerular diameter was partially restored to 96.1±3.8 micrometers, tubular lumen area recovered to 61.2±2.9 % (p=0.02), Caspase-3 expression decreased to 8.3±1.2 % (p=0.01), and the Ki-67 labeling index rose to 13.5±1.3 % (p=0.04). Collectively, these findings prove that chronic cadmium exposure induces apoptosis-driven fibrosis, whereas N-acetylcysteine provides substantial nephroprotection by suppressing cell death, preventing fibrosis, and partial restoring proliferative capacity.

**Keywords:** cadmium nephrotoxicity, N-acetylcysteine, renal histopathology, oxidative stress, rat model, immunohistochemistry, tubular injury, interstitial fibrosis, apoptosis, cellular proliferation.

# Гістопатологічна та імуногістохімічна оцінка тканини нирок щурів за хронічного впливу низьких доз кадмію та нефропротекторний потенціал N-ацетилцистеїну

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Кадмій є нефротоксичним важким металом, який поступово накопичується в тканинах нирок, спричиняючи їхнє структурне ушкодження. У цьому дослідженні оцінено гістопатологічні, морфометричні та імуногістохімічні ефекти хронічного впливу низьких доз кадмію на нирки щурів, а також вивчено нефропротекторний потенціал N-ацетилцистеїну. Тридцять дорослих самців щурів лінії Спрег-Доулі були розділені на три групи: контрольну; групу кадмію, яка отримувала хлорид кадмію (0,5 мг/кг перорально двічі на тиждень протягом восьми тижнів); та групу лікування, яка отримувала хлорид кадмію одночасно з N-ацетилцистеїном (150 мг/кг/добу внутрішньочеревно). Зрізи нирок обробляли для фарбування гематоксиліном-еозином, за методом Шиффа та трихромом за Массоном, а також для імуногістохімічного виявлення Каспази-3 та Ki-67. Кількісний морфометричний аналіз показав, що хронічний вплив кадмію збільшив відношення маси нирок до маси тіла на 29,6 %, зменшив середній діаметр клубочків на 22 % (82,4±3,1 порівняно з 105,6±4,2 мікрометра; p=0,004), спричинив звуження площі просвіту канальців на 38 % (p<0,001) та викликав 4,1-кратне розростання фіброзної тканини (15,8±1,2 % порівняно з 3,9±0,5 %; p=0,001). Імуногістохімічні дані свідчать, що кадмій викликав 3,2-кратне підвищення експресії Каспази-3 (18,2±2,1 % порівняно з 5,6±1,0 %; p=0,002) та 45 % пригнічення індексу проліферативного маркування Ki-67 (9,3±1,4 % порівняно з 17,0±1,8 %; p=0,008), що вказує на прискорений апоптоз та порушення регенерації. Було виявлено сильний позитивний кореляційний зв'язок між інтерстиціальним фіброзом та експресією Каспази-3 (коефіцієнт Пірсона r = 0,89, p<0,001). Супутнє введення N-ацетилцистеїну нівелювало ці зміни: інтерстиціальний фіброз зменшився на 62 % (6,7±0,9 %; p=0,003), середній діаметр клубочків частково відновився до 96,1±3,8 мікрометра, площа просвіту канальців відновилася до 61,2±2,9 % (p=0,02), експресія Каспази-3 знизилася до 8,3±1,2 % (p=0,01), а індекс маркування Ki-67 зріс до 13,5±1,3 % (p=0,04). У сукупності ці результати доводять, що хронічний вплив кадмію викликає фіброз, зумовлений апоптозом, тоді як N-ацетилцистеїн забезпечує суттєву нефропротекцію шляхом пригнічення загибелі клітин, запобігання фіброзу та часткового відновлення проліферативної здатності.

**Ключові слова:** нефротоксичність кадмію, N-ацетилцистеїн, гістопатологія нирок, оксидативний стрес, модель на щурах, імуногістохімія, ушкодження канальців, інтерстиціальний фіброз, апоптоз, клітинна проліферація.



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## Introduction

Cadmium (Cd) is a non-essential toxic heavy metal recognized as one of the most hazardous environmental pollutants due to its persistence, bioaccumulation, and long biological half-life [1, 2].

Human exposure occurs through contaminated food, drinking water, industrial emissions, cigarette smoke, batteries, pigments, electroplating processes, and phosphate fertilizers [3, 4]. Because cadmium is poorly eliminated from the body, chronic exposure results in progressive accumulation, particularly within the liver and kidneys, where it may remain for decades [2, 5].

The kidney is considered the principal target organ of chronic cadmium toxicity. After absorption, cadmium binds to metallothionein and is transported to the renal cortex, where it accumulates within proximal tubular epithelial cells [6].

Once intracellular storage capacity is exceeded, free cadmium ions induce cellular injury through oxidative stress, mitochondrial dysfunction, membrane damage, and disruption of intracellular signaling pathways [7, 8]. Consequently, prolonged exposure may lead to tubular degeneration, impaired reabsorption, proteinuria, glucosuria, aminoaciduria, and progressive deterioration of renal function [9].

Chronic cadmium nephropathy is characterized by structural and functional alterations involving both tubular and glomerular compartments. Histopathological studies have demonstrated tubular epithelial degeneration, loss of brush borders, tubular necrosis, inflammatory infiltration, glomerular atrophy, and interstitial fibrosis following sustained cadmium exposure [10, 11]. These pathological changes contribute to the progression of chronic kidney disease and may eventually culminate in renal failure [12].

Oxidative stress is considered a central mechanism underlying cadmium-induced renal injury. Cadmium indirectly stimulates the generation of reactive oxygen species while simultaneously depleting endogenous antioxidant defenses, including glutathione and antioxidant enzymes [13]. The resulting imbalance promotes lipid peroxidation, protein oxidation, DNA damage, inflammation, apoptosis, and extracellular matrix accumulation [14].

Activation of apoptotic pathways, particularly those involving caspase-3 – a key executioner protease whose cytoplasmic expression serves as a definitive molecular signature of programmed cell death – has been consistently reported in experimental models of cadmium nephrotoxicity [15].

This study, by combining immunohistochemical detection of caspase-3 with quantification of the proliferative marker Ki-67, aimed to characterize both the apoptotic burden and the regenerative capacity of renal tissue under cadmium exposure. In parallel, suppression of cellular proliferation may impair tissue regeneration and delay recovery from injury [16].

N-acetylcysteine is a well-established antioxidant and precursor of glutathione synthesis. In addition to directly scavenging reactive oxygen species, it restores intracellular redox balance and modulates signaling pathways associated with inflammation, fibrosis, and apoptosis [17, 18].

Several experimental studies have reported beneficial effects of N-acetylcysteine against toxicant-induced renal injury; however, comprehensive evaluations combining histopathological, morphometric, and immunohistochemical assessments in chronic low-dose cadmium exposure models remain limited [19, 20–24].

## The aim of the study

Therefore, the present study aimed to evaluate the histopathological, morphometric, and immunohistochemical effects of chronic low-dose cadmium chloride exposure on rat kidneys, and to assess the nephroprotective efficacy of N-acetylcysteine by quantifying renal structural integrity, interstitial fibrosis, caspase-3-mediated apoptosis, and Ki-67-indexed cellular proliferation.

## Materials and methods

### *Experimental animals and study design*

Thirty healthy adult male Sprague-Dawley rats weighing 200–220 g were obtained from the Animal House Unit of the College of Veterinary Medicine, University of Wasit. Following a one-week acclimatization period, animals were randomly allocated into three equal groups (n = 10 per group) and maintained under standard laboratory conditions (22±2°C; 12 h light/12 h dark cycle) with unrestricted access to food and water throughout the experimental period.

The study groups were designated as follows:

*Group I (Control)*: Animals received normal saline orally and intraperitoneally for eight weeks.

*Group II (Cadmium)*: Animals received cadmium chloride ($CdCl_2$) at a dose of 0.5 mg/kg body weight orally, twice weekly for eight weeks.

*Group III (Cadmium + N-acetylcysteine)*: Animals received cadmium chloride as described for Group II together with N-acetylcysteine at a dose of 150 mg/kg/day administered intraperitoneally throughout the experimental period.

The selected cadmium dosage was based on previously validated protocols capable of inducing chronic renal injury without causing significant mortality.

### *Tissue collection and processing*

At the end of the eight-week exposure period, animals were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg). Both kidneys were surgically excised and carefully examined.

The right kidney was weighed for determination of the kidney-to-body weight ratio. Tissue samples were fixed in 10 % neutral buffered formalin for 48 hours, dehydrated through graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. Serial sections of 4 µm thickness were prepared using a rotary microtome.

### *Histological and immunohistochemical examination*

Renal tissue sections were subjected to routine histological and immunohistochemical staining as follows:

- Hematoxylin and Eosin (H&E) staining: evaluation of general renal architecture and pathological alterations.
- Periodic Acid-Schiff (PAS) staining: assessment of basement membrane integrity and proximal tubular brush borders.
- Masson's Trichrome staining: visualization and quantification of collagen deposition and interstitial fibrosis.

For immunohistochemistry, the following primary antibodies were employed:

- Rabbit anti-rat caspase-3 antibody (Abcam, ab13847; dilution 1 : 200): assessment of apoptotic activity.
- Mouse monoclonal anti-Ki-67 antibody (Dako, M7240; dilution 1 : 150): evaluation of cellular proliferative activity.

Antigen retrieval was performed using citrate buffer (pH 6.0), followed by visualization using a diaminobenzidine chromogen system and hematoxylin counterstaining.

***Histopathological scoring***

Histopathological lesions were evaluated independently by two blinded veterinary histopathologists using a semi-quantitative scoring system adapted from previously published renal injury assessment protocols. Tubular damage, glomerular alterations, and interstitial inflammatory changes were graded according to lesion severity.

Tubular injury was graded from 0 to 4, where 0 indicated normal morphology and 4 represented extensive tissue involvement. Glomerular alterations and interstitial inflammation were similarly assessed using standardized severity scales.

***Morphometric analysis***

Morphometric measurements were performed using *ImageJ* software (Version 1.53, National Institutes of Health, USA). Digital images were captured using an *Olympus BX53* microscope equipped with a *DP27* digital camera.

The following parameters were quantitatively assessed:

- Mean glomerular diameter (μm).
- Tubular lumen area expressed as a percentage of total tubular cross-sectional area.
- Percentage of interstitial fibrotic area in Masson's trichrome-stained sections.

For each animal, measurements were obtained from multiple randomly selected non-overlapping microscopic fields, and the mean value was used for statistical analysis.

***Statistical analysis***

Statistical analyses were performed using *SPSS* software version 28. Data were expressed as mean ± standard error of the mean (SEM). Data distribution was evaluated using the Shapiro-Wilk test.

Comparisons among experimental groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. Correlations between quantitative variables were assessed using Pearson's correlation coefficient. Statistical significance was considered at $p<0.05$.

## Results and discussion

***Gross and biometric findings***

Macroscopic examination of kidneys obtained from the control group revealed normal renal morphology characterized by a smooth surface, firm consistency, and a uniform reddish-brown appearance.

In contrast, kidneys from cadmium-exposed rats exhibited a pale discoloration, mild enlargement, and cortical mottling, indicating early toxic injury to the renal parenchyma.

These observations were supported by biometric analysis (***Table 1***). The kidney-to-body weight ratio increased significantly in the cadmium-treated group (0.92 %) compared with the control group (0.71 %; $p$=0.01), representing an increase of approximately 29.6 %. This elevation is commonly associated with renal edema, inflammatory infiltration, and cellular hypertrophy induced by toxic injury [1, 4].

**Table 2**

Kidney-to-body weight ratio (%) in control, cadmium-exposed, and N-acetylcysteine-treated rats after 8 weeks of intervention (n = 10)

| Experimental Group | Kidney / Body Weight Ratio (%) | Mean ± SEM | *p*-value (vs. Control) |
|---|---|---|---|
| Control | 0.68–0.74 | 0.71±0.03 | – |
| Cd | 0.86–0.98 | 0.92±0.04 | 0.01** |
| Cd + NAC | 0.73–0.83 | 0.78±0.03 | 0.09 |

*Note:* Significant increase in kidney weight ratio in the Cd group indicates edema, inflammatory infiltration, or cellular hypertrophy. NAC co-administration partially reversed this effect. **p < 0.01.

Administration of N-acetylcysteine partially reversed this alteration, reducing the kidney-to-body weight ratio to 0.78 %. Although this value remained slightly higher than that of the control group, it was significantly lower than that observed in cadmium-exposed animals, suggesting attenuation of renal inflammation and tissue swelling.

The observed increase in renal mass following cadmium exposure agrees with previous reports demonstrating that chronic cadmium accumulation induces inflammatory responses and structural remodeling within renal tissue [3, 6]. The improvement observed in the N-acetylcysteine-treated group may be attributed to restoration of intracellular glutathione levels and suppression of oxidative injury pathways [8, 12]. Subsequent microstructural changes and tissue alterations were graded using specific semi-quantitative evaluation criteria (***Table 2***).

To systematically evaluate the structural severity of cadmium-induced nephrotoxicity and the potential protective effects of N-acetylcysteine, a comprehensive semi-quantitative scoring system was utilized (***Table 2***). This assessment specifically focused on three vulnerable renal compartments: tubular damage (including vacuolation, necrosis, and brush border loss), glomerular alterations (such as shrinkage and capillary tuft

condensation), and the extent of interstitial inflammation. The high inter-observer agreement (Cohen's κ = 0.89) confirms the reliability and reproducibility of the scoring criteria employed across all experimental groups.

**Table 2**
Histopathological scoring criteria used for semi-quantitative assessment of renal tissue injury in experimental rats

| Parameter | Score | Description |
|---|---|---|
| Tubular damage | 0 | No detectable change |
| | 1 | Mild injury (<10 % of tubules affected: slight vacuolation, minimal casts) |
| | 2 | Moderate injury (10–25 %: focal necrosis, brush border loss) |
| | 3 | Severe injury (25–50 %: widespread necrosis, proteinaceous casts) |
| | 4 | Extensive injury (>50 %: tubular collapse, marked dilation) |
| Glomerular changes | 0 | Normal glomerular architecture |
| | 1 | Mild shrinkage or mild mesangial expansion |
| | 2 | Moderate collapse or capillary tuft condensation |
| | 3 | Severe sclerosis or global collapse |
| Interstitial inflammation | 0 | Absent |
| | 1 | Focal mononuclear infiltration |
| | 2 | Multifocal infiltration |
| | 3 | Diffuse inflammatory cell infiltrate |

*Note:* Scoring was performed by two blinded veterinary histopathologists; inter-observer agreement (Cohen's κ) = 0.89.

***Histopathological alterations***

Histopathological examination of hematoxylin and eosin-stained renal sections revealed marked differences among experimental groups (***Figure 1***).

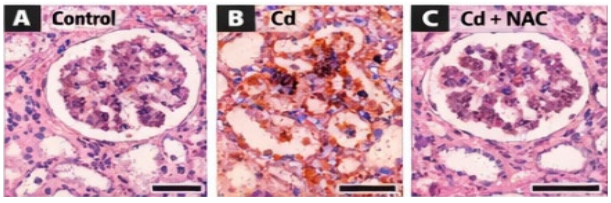


***Figure 1.*** Representative histopathological alterations in renal cortex sections (H&E, ×200):
(A) Control group showing normal renal architecture and intact glomeruli; (B) cadmium-exposed group showing glomerular shrinkage, widening of Bowman's space, and interstitial alterations; (C) cadmium + N-acetylcysteine group showing partial restoration of renal histological architecture. Scale bar = 100 µm

Kidneys from the control group displayed normal renal architecture characterized by intact glomeruli, preserved Bowman's spaces, normal proximal and distal tubules, and minimal interstitial cellularity. Proximal tubular epithelial cells exhibited normal morphology with intact brush borders and eosinophilic cytoplasm.

In contrast, cadmium-exposed animals demonstrated severe renal injury characterized by glomerular shrinkage, collapse of capillary tufts, widening of Bowman's space, associated interstitial changes, proteinaceous cast formation, and marked inflammatory cell infiltration. In addition, glomeruli exhibited varying degrees of shrinkage and collapse, accompanied by expansion of the interstitial compartment.

Representative glomerular alterations are shown in ***Figures 1***, ***2***, ***6***, and ***7***, while tubular lesions were assessed throughout the examined renal cortex sections.

Periodic Acid-Schiff staining further confirmed severe disruption of proximal tubular brush borders and basement membrane integrity in cadmium-treated animals (**Figure 2**). These findings are consistent with the known susceptibility of proximal tubular epithelial cells to cadmium accumulation and toxicity [7, 10].

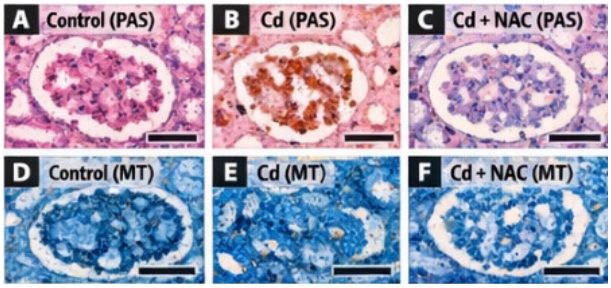


**Figure 2.** Histochemical evaluation of renal cortex sections using Periodic Acid-Schiff (PAS) and Masson's trichrome (MT) staining (×400):
(A–C) PAS-stained sections: (A) control group demonstrating intact basement membranes and preserved proximal tubular brush borders; (B) cadmium-exposed group displaying severe disruption of the brush border and basal laminas; (C) cadmium + N-acetylcysteine group showing restoration of tubular boundary integrity. (D–F) Masson's trichrome-stained sections: (D) control group showing minimal physiological collagen deposition; (E) cadmium-exposed group revealing marked interstitial fibrosis and expanded extracellular matrix accumulation (blue color); (F) cadmium + N-acetylcysteine group demonstrating significantly reduced collagen deposition.
Scale bar = 50 µm

Semi-quantitative histopathological scoring demonstrated a significant increase across all evaluated lesion categories (***Table 3***). Cadmium exposure induced profound structural degradation, with tubular injury scores rising sharply from baseline control levels ($p<0.001$). Similarly, marked elevations were observed in both glomerular injury and interstitial inflammation scores ($p<0.001$ for all comparisons), underscores the widespread nature of cadmium-induced renal remodeling.

**Table 3**
Mean histopathological scores for tubular, glomerular, and interstitial alterations across experimental groups (n=10)

| Parameter | Control | Cd | Cd + NAC | *p*-value (ANOVA) |
|---|---|---|---|---|
| Tubular damage score | 0.2±0.1 | 3.4±0.3 | 1.1±0.2 | <0.001*** |
| Glomerular change score | 0.1±0.1 | 2.3±0.2 | 0.8±0.2 | <0.001*** |
| Interstitial inflammation | 0.0±0.0 | 2.5±0.3 | 0.9±0.2 | <0.001*** |

*Note:* All histopathological parameters were significantly worsened by cadmium exposure and significantly improved by NAC treatment (Tukey post hoc, $p<0.01$). ***$p<0.001$.

N-acetylcysteine treatment significantly improved renal histology. Tubular epithelial integrity was largely preserved, inflammatory infiltration was markedly reduced, and glomerular morphology approached normal

appearance. Tubular injury scores decreased substantially, indicating robust structural protection.

These findings support previous studies demonstrating that oxidative stress represents a principal mechanism of cadmium-induced nephrotoxicity [11, 13]. Cadmium promotes excessive generation of reactive oxygen species, resulting in mitochondrial dysfunction, membrane lipid peroxidation, and activation of inflammatory pathways [4, 14].

The protective effects of N-acetylcysteine observed in the present study are likely related to its antioxidant properties, enhancement of glutathione synthesis, and suppression of oxidative tissue damage [2, 8, 17]. Furthermore, the reduction of inflammatory infiltration observed in the N-acetylcysteine-treated group suggests inhibition of pro-inflammatory signaling pathways, including NF-κB activation, which has been implicated in chronic cadmium nephropathy [11, 18].

***Morphometric analysis***

Quantitative morphometric assessment revealed significant structural deterioration of renal tissue following chronic cadmium exposure (***Figures 3–5***). Measurement of glomerular diameter demonstrated a marked reduction in the cadmium-treated group (82.4 µm) compared with the control group (105.6 µm; p=0.004), representing a decrease of approximately 22 % (***Figure 3***)

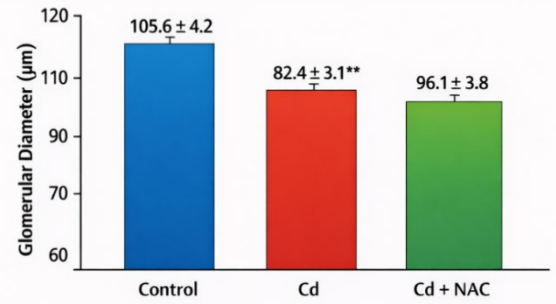


**Figure 3.** Mean glomerular diameter (µm) in the renal cortex of experimental rats following chronic cadmium exposure and N-acetylcysteine treatment (n=10):
Bar graph demonstrates a significant reduction in glomerular diameter in the Cd group, indicating glomerular collapse, and its partial restoration following NAC co-administration. Values are expressed as mean ± SEM. **p<0.01

Reduction in glomerular diameter is indicative of glomerular tuft collapse, mesangial contraction, and loss of functional filtration surface area. Such alterations are recognized hallmarks of chronic cadmium nephropathy and are frequently associated with impaired glomerular filtration and progressive renal dysfunction [5, 9]. Similar findings were reported by Liu et al. [6], who observed significant glomerular shrinkage following prolonged low-dose cadmium exposure.

Administration of N-acetylcysteine partially restored glomerular dimensions, resulting in a mean diameter of 96.1 µm. Although complete normalization was not achieved, the substantial improvement indicates preservation of glomerular architecture and attenuation of cadmium-induced structural damage.

Assessment of tubular lumen area demonstrated a pronounced decrease in the cadmium group (42.3 %) compared with controls (68.5 %; p<0.001), corresponding to a 38 % reduction (***Figure 4***).

Tubular lumen narrowing reflects epithelial swelling, cellular degeneration, cast formation, and obstruction of tubular flow. These structural abnormalities contribute directly to impaired tubular transport and reabsorptive dysfunction, which are characteristic features of cadmium-induced proximal tubulopathy [7, 10].

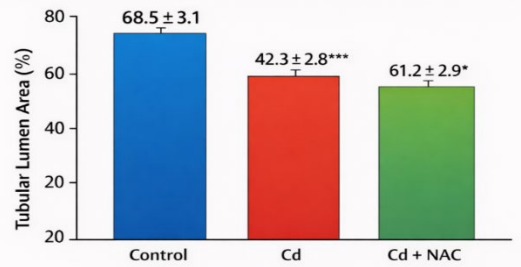


**Figure 4.** Tubular lumen area (% of total tubular cross-sectional area) in experimental groups ($n$ = 10).
Bar graph demonstrates a significant decrease in tubular lumen area following cadmium exposure, reflecting epithelial swelling or cast obstruction, and its marked improvement with N-acetylcysteine co-administration. Values are expressed as mean ± SEM. *p<0.05, ***p<0.051

N-acetylcysteine treatment significantly improved tubular lumen preservation, increasing the lumen area to 61.2 % (p=0.02 versus cadmium group). This improvement suggests that antioxidant therapy effectively mitigated epithelial injury and maintained tubular patency.

The most striking morphometric alteration was observed in interstitial fibrosis. Quantification of Masson's trichrome-stained sections demonstrated a 4.1-fold increase in fibrotic area in cadmium-treated animals (15.8 %) compared with controls (3.9 %; p= 0.001) (***Figure 5***).

Excessive collagen accumulation within the renal interstitium is considered a key pathological event in the progression of chronic kidney disease because it progressively replaces functional parenchyma with scar tissue [4, 11].

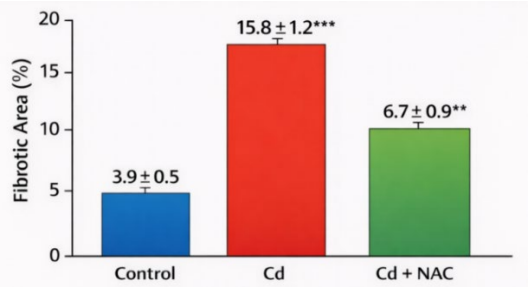


**Figure 5.** Quantitative assessment of interstitial fibrotic area (%) in renal cortex sections ($n$ = 10).
Bar graph demonstrates a sharp elevation of extracellular matrix accumulation and collagen deposition following chronic cadmium exposure, and its significant mitigation with N-acetylcysteine therapy. Values are expressed as mean ± SEM. **p<0.01, ***p<0.001

N-acetylcysteine significantly reduced collagen deposition to 6.7 % (p=0.003 versus cadmium group), representing a reduction of approximately 62 %. This finding suggests that N-acetylcysteine possesses substantial anti-fibrotic activity, potentially through suppression of oxidative stress-mediated activation of transforming growth factor-beta (TGF-β)-dependent fibrogenic pathways [4, 18].

Collectively, these morphometric findings provide quantitative evidence that chronic cadmium exposure induces severe structural remodeling of renal tissue, whereas N-acetylcysteine markedly attenuates these pathological alterations.

***Immunohistochemical findings***

To further elucidate the molecular mechanisms underlying renal injury, immunohistochemical expression of Caspase-3 and Ki-67 was evaluated (***Figures 6*** and ***7***).

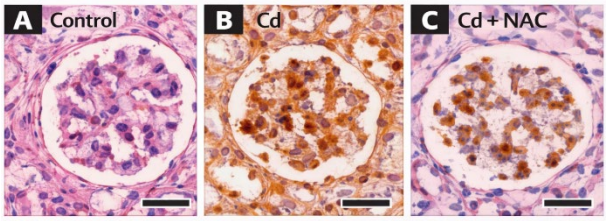


**Figure 6.** Caspase-3 immunohistochemical expression in renal glomerular structures of control, cadmium-exposed, and N-acetylcysteine-treated rats (×400): Representative photomicrographs showing Caspase-3 immunoreactivity in renal glomeruli: (A) control group showing minimal baseline staining; (B) cadmium-exposed group showing marked positive cytoplasmic immunoreactivity within the glomerular tuft; (C) cadmium + N-acetylcysteine group showing significantly reduced Caspase-3 expression. Scale bar = 50 µm

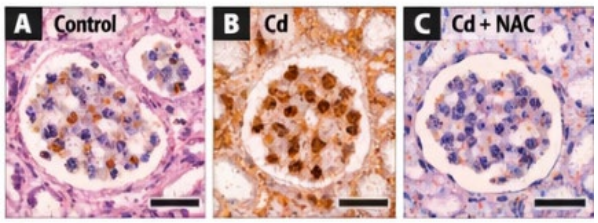


**Figure 7.** Ki-67 immunohistochemical expression in renal glomerular structures of experimental rats (×400): Representative photomicrographs showing Ki-67 immunoreactivity within renal glomeruli: (A) control group demonstrating basal physiological proliferative activity; (B) cadmium-exposed group displaying a marked reduction in positive cell nuclei, indicating suppression of regenerative capacity; (C) cadmium + N-acetylcysteine group showing notable restoration of Ki-67-indexed cellular proliferation. Scale bar = 50 µm

*Caspase-3 expression*

Control renal tissues exhibited minimal Caspase-3 immunoreactivity, with only occasional weak cytoplasmic staining observed in tubular epithelial cells. The mean labeling index was 5.6 % (***Table 4***).

In contrast, cadmium exposure induced a marked increase in Caspase-3 expression. Strong diffuse cytoplasmic staining was observed throughout the proximal tubular epithelium, and the labeling index increased to 18.2 %, representing a 3.2-fold elevation compared with controls (p=0.002).

**Table 4**

Caspase-3 immunohistochemical labeling index (%) in renal tubular epithelium across experimental groups

| Group | Caspase-3 Labeling Index (%) | Mean ± SEM | Fold Change vs. Control | p-value (vs. Control) |
|---|---|---|---|---|
| Control | 4.2–6.8 | 5.6±1.0 | 1.0× | – |
| Cd | 15.1–21.3 | 18.2±2.1 | 3.2× | 0.002 |
| Cd + NAC | 6.9–9.8 | 8.3±1.2 | 1.5× | 0.01 |

*Note:* Caspase-3 labeling index reflects the percentage of positively stained tubular epithelial cells per high-power field (×400). Elevated index in Cd group confirms activation of apoptotic pathways.

Activation of Caspase-3 is a critical indicator of apoptosis and reflects irreversible commitment of cells to programmed cell death. The observed increase confirms that apoptosis represents a major mechanism of cadmium-induced renal injury.

Previous investigations have demonstrated that cadmium disrupts mitochondrial membrane integrity, promotes cytochrome-c release, and activates downstream caspase-dependent apoptotic pathways [5, 13, 15].

Treatment with N-acetylcysteine significantly reduced Caspase-3 expression to 8.3 % (p=0.01 versus cadmium group). This reduction suggests effective suppression of oxidative stress-induced apoptosis through restoration of intracellular glutathione levels and stabilization of mitochondrial function [2, 8].

*Ki-67 expression*

Ki-67 immunostaining revealed abundant nuclear positivity in tubular epithelial cells of control animals, with a mean labeling index of 17.0 % (***Table 5***), indicating normal cellular turnover and regenerative activity.

**Table 5**

Ki-67 immunohistochemical labeling index (%) indicating cellular proliferation in renal tubules

| Group | Ki-67 Labeling Index (%) | Mean ± SEM | % Reduction vs. Control | p-value (vs. Control) |
|---|---|---|---|---|
| Control | 14.8–19.2 | 17.0±1.8 | – | – |
| Cd | 7.1–11.5 | 9.3±1.4 | 45 | 0.008 |
| Cd + NAC | 11.2–15.7 | 13.5±1.3 | 21 | 0.04 |

*Note:* Ki-67 nuclear positivity signifies active cell cycling. Suppression in the Cd group indicates impaired regenerative capacity, partially rescued by NAC.

Cadmium exposure significantly suppressed proliferative activity, reducing Ki-67 expression to 9.3 % (p=0.008), corresponding to a 45 % reduction relative to controls. This decline indicates impairment of tissue regeneration and diminished capacity for repair following toxic injury.

Suppression of proliferative signaling has been reported previously in association with cadmium-induced oxidative DNA damage, cell-cycle arrest, and mitochondrial dysfunction [6, 14]. Consequently, the simultaneous occurrence of increased apoptosis

and reduced proliferation creates a pathological environment favoring progressive tissue loss and fibrosis.

To further investigate the relationship between this cellular destruction and chronic tissue remodeling, a correlation analysis was performed (***Figure 8***). A highly significant, strong positive correlation was detected between the interstitial fibrotic area and the Caspase-3 labeling index (Pearson $r$ = 0.89, p=0.001). This robust statistical association suggests that programmed cell death is directly intertwined with extracellular matrix deposition, meaning that the acceleration of apoptotic pathways and the parallel shutdown of Ki-67-mediated proliferation act as primary driving events that trigger subsequent interstitial scar tissue deposition.

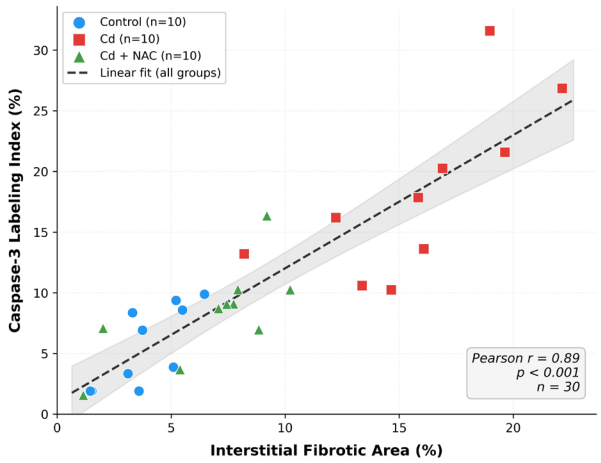


**Figure 8.** Pearson correlation between interstitial fibrotic area (%) and Caspase-3 labeling index (%) across all experimental groups ($n$ = 30):

Scatter plot demonstrates a strong positive correlation (Pearson $r$ = 0.89, p<0.001), indicating a tight mechanistic link between the execution of programmed cell death (apoptosis) and the progression of chronic renal remodeling (interstitial fibrosis). The shaded area represents the 95 % confidence interval for the linear fit line

Administration of N-acetylcysteine partially restored proliferative activity, increasing the Ki-67 labeling index to 13.5 % (p=0.04 versus cadmium group). Restoration of cellular proliferation likely contributed to the improved histological architecture and reduced fibrosis observed in treated animals.

Taken together, the immunohistochemical findings demonstrate that cadmium-induced nephrotoxicity is characterized by activation of apoptotic pathways and suppression of regenerative mechanisms, whereas N-acetylcysteine exerts significant cytoprotective effects through simultaneous inhibition of apoptosis and enhancement of tissue repair processes.

The present study demonstrated that chronic low-dose cadmium exposure (0.5 mg/kg body weight, twice weekly for 8 weeks) induced significant renal injury in Sprague-Dawley rats at both structural and molecular levels. Cadmium exposure increased the kidney-to-body weight ratio by 29.6 %, reduced mean glomerular diameter by 22 % (82.4±3.1 vs. 105.6±4.2 µm; p=0.004), decreased tubular lumen area by 38 % (42.3±2.8 % vs. 68.5±3.1 %; p<0.001), and increased interstitial fibrosis 4.1-fold (15.8±1.2 % vs. 3.9±0.5 %; p=0.001).

Immunohistochemical analysis revealed a 3.2-fold increase in Caspase-3 expression (18.2±2.1 % vs. 5.6±1.0 %; p=0.002) accompanied by a 45 % reduction in the Ki-67 labeling index (9.3±1.4 % vs. 17.0±1.8 %; p=0.008), indicating enhanced apoptosis and impaired regenerative capacity. A strong positive correlation between fibrosis and Caspase-3 expression ($r$ = 0.89, p<0.001) further supports the mechanistic association between apoptotic activity and renal fibrogenesis.

Co-administration of N-acetylcysteine (150 g/kg/day, *i.p.*) significantly attenuated all cadmium-induced renal alterations: interstitial fibrosis was reduced by 62 % (from 15.8±1.2 % to 6.7±0.9 %; p=0.003), glomerular diameter was partially restored (96.1±3.8 µm), tubular lumen area recovered to 61.2±2.9 % (p=0.02), Caspase-3 expression decreased to 8.3±1.2 % (p=0.01), and the Ki-67 labeling index increased to 13.5±1.3 % (p=0.04). These quantitative outcomes confirm that N-acetylcysteine confers substantial nephroprotection through simultaneous suppression of oxidative stress, inhibition of apoptosis, attenuation of fibrogenesis, and partial restoration of cellular proliferative capacity.

Collectively, the results identify apoptosis-driven fibrosis as a major pathological mechanism of chronic cadmium nephrotoxicity and support N-acetylcysteine as a promising therapeutic strategy for reducing renal damage associated with long-term cadmium exposure.

## Conclusions

The present study provides clear quantitative evidence that chronic low-dose cadmium exposure induces severe renal remodeling at both structural and molecular levels, which is characterized by the following outcomes:

- Parenchymal Damage: Cadmium exposure significantly alters renal biometrics and microarchitecture, causing a 29.6 % increase in the kidney-to-body weight ratio, a 22 % reduction in mean glomerular diameter (p=0.004), and a 38 % narrowing of the tubular lumen area (p<0.001).

- Fibrotic Axis: Chronic toxicity triggers extensive tissue remodeling, resulting in a 4.1-fold increase in interstitial fibrosis (p=0.001). This structural degradation is tightly linked to cellular destruction, as evidenced by a strong positive correlation between fibrogenesis and Caspase-3 expression ($r$ = 0.89, p<0.001).

- Molecular Imbalance: At the cellular level, cadmium shifts the tissue balance toward degeneration by inducing a 3.2-fold elevation in Caspase-3-mediated apoptosis (p=0.002) and a parallel 45 % suppression of Ki-67-indexed cellular proliferation (p=0.008).

- NAC Protection: Co-administration of N-acetylcysteine effectively halts this pathology. It reduces interstitial fibrosis by 62 % (p=0.003), downregulates Caspase-3 expression to 8.3±1.2 % (p=0.01), and restores cellular proliferation to 13.5±1.3 % (p=0.04), while preserving overall glomerular and tubular patency.

Collectively, these findings identify apoptosis-driven fibrosis as a central mechanism of chronic cadmium nephrotoxicity and support N-acetylcysteine as a highly

effective nephroprotective strategy capable of mitigating long-term heavy metal-induced kidney injury.

## DECLARATIONS

### Ethical Statement

All experimental procedures and animal handling protocols in this study were strictly conducted in accordance with the national legislation of Iraq, the ARRIVE 2.0 guidelines, and the ethical principles governing the use of laboratory animals in biomedical research.

The experimental design and study protocol were formally reviewed and officially approved by the Institutional Ethics Committee of the College of Veterinary Medicine, University of Wasit, Iraq (Approval No.: UOW.VET.EC.2025.232). Every possible effort was proactively made to minimize animal suffering, reduce procedural distress, and optimize the total number of animals utilized throughout the investigation.


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### Conflict of Interest

The author declares no conflict of interest.


### Acknowledgements

None.


### Declaration of AI and AI-assisted technologies

The authors declare that no artificial intelligence tools were utilized in the generation, interpretation, manipulation, or reporting of the scientific results presented in this manuscript. Artificial intelligence-assisted tools were employed solely for language editing and grammatical improvement of the manuscript text, without influencing the scientific content, data analysis, core findings, or conclusions.

### Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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