

RESEARCH PAPER

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Renal protection by Okra (*Abelmoschus esculentus*) seed oil against cadmium toxicity in male rats

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ABSTRACT

Cadmium is a persistent environmental contaminant that induces oxidative stress and disrupts renal function, posing serious risks to human and animal health. This study evaluated the protective effects of okra seed oil against cadmium-induced nephrotoxicity in male Wistar rats. The rats were divided into four groups: a healthy control group, a cadmium-exposed group, a group receiving okra seed oil alone, and a co-administrated cadmium and okra seed oil group. Biochemical analysis revealed that cadmium exposure significantly impaired renal function, disrupted protein and glucose regulation, and increased oxidative stress markers, indicating severe metabolic and functional disturbances. In contrast, rats treated with okra seed oil alone maintained normal biochemical profiles, demonstrating the safety of the oil. The co-administration of okra seed oil with cadmium significantly mitigated these negative effects, restoring several biochemical parameters toward control levels. Histopathological examination supported these findings. Kidneys from cadmium-exposed rats exhibited glomerular shrinkage, widening of Bowman's spaces, tubular degeneration, medullary disorganization, and congestion. Conversely, kidneys from rats treated with okra seed oil alone maintained their normal architecture. Notably, co-administration of okra seed oil with cadmium markedly reduced these lesions, with partial preservation of glomerular and tubular morphology, improved cortical organization, and decreased medullary congestion. Overall, these results indicate that okra seed oil provides both functional and structural protection against cadmium-induced renal injury. Its antioxidant and anti-inflammatory properties serves as important biochemical and histological markers, highlighting okra seed oil as a promising natural intervention for heavy metal-induced nephrotoxicity.

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INTRODUCTION

A major worldwide issue, environmental pollution from diverse sources, harms human health and natural ecosystems. Because of their toxicity and persistence, heavy metals are one of the most dangerous environmental contaminants. Heavy metals are built up in soil, water, and air because they are insoluble and do not break down like organic contamination. Heavy metals are highly poisonous and detrimental to health because of this contamination, affecting plants and animals (Korish and Attia, 2020; Witkowska *et al.*, 2021).

One of the most mobile heavy metals among them is cadmium (Cd), which may easily enter the food chain and become highly accessible in runoff sediments. Because of its persistence and mobility in the environment, cadmium presents significant dangers even at low concentrations (Devi and Bhattacharyya, 2018).

Earth's crust naturally contains 0.1 to 0.2 parts per million of cadmium, which Friedrich Stromeyer found in 1817 (Peana *et al.*, 2022). Although it naturally occurs due to erosion and volcanic activity, human activities such as smoking, fuel combustion and industrial discharges have significantly increased the amount of cadmium in the environment. This anthropogenic increase seriously threatens human and animal health (Briffa *et al.*, 2020).

Cadmium is a non-essential metal that is toxic even at low quantities and has no biological value. It causes oxidative stress and serious harm when it accumulates in vital organs, such as the kidneys, liver, brain, bones, and testes. Studies have shown that sub-chronic exposure causes renal damage, including protein buildup and structural changes in the renal cortex and tubules, making the kidneys more susceptible to poisoning (Siddiqui, 2010). Furthermore, the accumulation of cadmium in the kidney leads to a disturbance in glomerular filtration, causing protein and glucose to be excreted in the urine, resulting in elevated levels (Satarug, 2024; Prozialeck and Edwards, 2012). Cadmium also

interacts with biological molecules, interfering with physiological processes and enzyme activity, which leads to hypertension and cardiovascular disorders (Atlam and Wills, 2020; Bonfiglio *et al.*, 2024; Genchi *et al.*, 2020; Lin *et al.*, 2021).

The effects of cadmium go beyond cardiovascular and renal health. There is evidence that exposure to cadmium can be harmful to reproduction, as evidenced by changes in anti-Mullerian hormone levels in premenopausal women and reduced ovarian function. In animal studies, cadmium exposure has also been linked to lower birth weight, preterm birth, and hormone-dependent and developmental malignancies (Huff *et al.*, 2007; Qu *et al.*, 2023; Şensoy, 2023).

These effects on development and reproduction highlight how extremely hazardous Cd is, even at comparatively low levels.

Furthermore, cadmium is an immunotoxic substance that causes oxidative stress, changes cytokine release, and accumulates in immune cells. Concerns regarding cadmium's wider consequences on human health are raised by these effects, which interfere with both innate and adaptive immune responses (Wang *et al.*, 2021).

Natural antioxidants have drawn attention as a possible way to lessen cadmium toxicity due to its adverse effects (Fan *et al.*, 2024). Okra seed oil has become well-known because of its abundant bioactive content, which includes vitamins A, E, and C, carotenoids, and phenolic compounds.

Because of these ingredients' high antioxidant activity, okra seed oil can combat free radicals, lessen oxidative stress, and shield cells from harm. Proteins, unsaturated fats (such as oleic and linoleic acids), carbs, and energy are all abundant in okra seed oil. Furthermore, okra seed oil has anti-inflammatory qualities due to phenolic components like procyanidin and catechin, which increase its capacity to treat disorders linked to oxidative stress, including

diabetes, cardiovascular diseases, and chronic inflammation (Al-Kanani *et al.*, 2019). According to research, okra seed oil is a viable option for preventing cadmium poisoning, as it alters lipid metabolism, boosts antioxidant defenses, and reduces oxidative stress indicators (Zhanga *et al.*, 2019).

Thus, this study aims to assess the protective ability of okra seed oil against cadmium-induced toxicity while examining the precise biochemical and histological effects of cadmium on the tissues of the kidneys in male Wistar rats. To better understand the processes of cadmium toxicity and determine the possible protective benefits of okra seed oil, this study compares the results of four groups: a control group, a group exposed to cadmium, a group receiving okra seed oil, and a group cadmium-induced treated with okra seed oil.

MATERIALS AND METHODS

Cadmium chloride (CdCl₂)

Cadmium chloride simulates exposure to cadmium in the environment due to its high bioavailability. It is dissolved at a rate of 5 mg/kg per day in 10 milliliters per kilogram of deionized water. This dosage complies with recognized toxicity procedures and was carefully selected to avoid realistic exposure levels.

Okra seed oil

Using a pure cold-pressed okra seed oil obtained from RV Essential Company, India. Giving a daily gavage dose of 800 mg/kg/day.

Ethics and animals

The study was conducted in compliance with institutional guidelines for the ethical use of animals and used 8-week-old male Wistar rats weighing an average of 120 grams. The rats were obtained from the King Fahad Medical Research Center (KFMRC) and approved by the Unit of Biochemical Ethics, King Abdulaziz University (kau).

Experimental design and sample collection

Rats were kept in a 12-hour light/dark cycle at room temperature in pathogen-free conditions prior to the

experiment, allowing them to acclimate to the environment for 1 week. They were given water and a standard rodent diet. Dividing it into four groups:

Control group: daily oral administration of deionized water.

Cadmium group: receiving 5 mg/kg/day of CdCl₂ diluted in deionized water.

Okra seed oil group: given 800 mg/kg/day of okra seed oil.

Okra seed oil + Cadmium group: treated with 800 mg/kg/day of okra seed oil and 5 mg/kg/day of CdCl₂ orally after three hours.

At the end of the treatment period, rats are euthanized for sample collection. Organ tissues from the kidneys are harvested for biochemical analysis. Biochemical Tests: Focuses on kidney function (BUN, uric acid, and creatinine), and blood tests, including glucose and protein.

Statistical analysis

One-way analysis of variance (ANOVA) is used to compare the control, cadmium-exposed, okra seed oil treatment, and cadmium-exposed treated groups; post hoc tests are then used to pinpoint individual group differences. A *p*-value of less than 0.05 indicates that the difference is statistically significant. These results underline the promise of okra seed oil as a natural remedy for toxicity caused by cadmium, which calls for more investigation into its processes and uses.

Histological examination

Kidney tissue samples were collected immediately after sacrifice from experimental groups. Small portions of the renal cortex and medulla were carefully excised, rinsed in cold saline to remove blood, and fixed in 10% neutral buffered formalin for at least 24 h.

Fixed tissues were then processed by routine paraffin-embedding techniques. Sections of 4-5 µm thickness were cut using a rotary microtome and mounted on glass slides. The sections were stained with

hematoxylin and Eison (HandE) for general histological evaluation. Prepared slides were examined under a light microscope at x100 magnifications, and representative photomicrographs were captured. Structural changes in the glomeruli, Bowman's space, convoluted tubules, and medullary region (including the loop of Henle) were qualitatively assessed and compared among the experimental groups.

RESULTS AND DISCUSSION

Biochemical parameters for kidney function

Blood urea nitrogen (BUN)

Commonly used as a marker to evaluate renal excretory function. It reflects the serum concentration of nitrogen derived from urea, a waste product of protein metabolism, which is normally filtered and excreted by the kidneys. Elevated BUN levels may indicate reduced glomerular filtration, dehydration, or increased protein catabolism, though nonrenal factors also influence BUN (Amir, 2010).

Administration of cadmium resulted in a significant increase in blood urea nitrogen (BUN) levels compared to the control group, indicating impaired renal function. In contrast, rats treated with okra seed oil alongside cadmium exhibited markedly lower BUN levels, approaching normal values typically ranging from 10 to 21 mg/dL (Fauzi *et al.*, 2020). Additionally, rats that received okra seed oil alone maintained BUN levels similar to those of the control group, confirming that the oil does not adversely affect renal function (Fig. 1).

Uric acid (UA)

Uric acid is the end product of purine metabolism and is excreted primarily via the kidneys. Serum uric acid levels increase when renal excretory function declines, making it an indirect marker of renal function (Bobulescu and Moe, 2012).

Serum uric acid (UA) levels increased significantly in rats intoxicated with cadmium, which suggests a decrease in renal excretory capacity. However, co-treatment with okra seed oil effectively reduced this

elevation, bringing UA concentrations back towards the baseline range of 1.7 to 3 mg/dL (Nugrahaningsih *et al.*, 2021). Notably, the group treated with okra seed oil showed no significant difference in UA levels compared to the control group (Fig. 1).

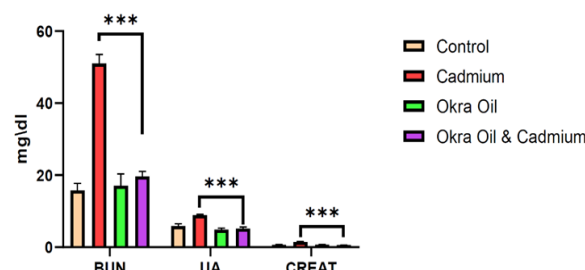


Fig. 1. Effect of cadmium and okra seed oil on kidney levels in mg/dl (BUN, UA, and creatinine) for different experimental groups

The results are expressed as mean $\pm$ SEM.

Creatinine (CREAT)

Creatinine is generated at a relatively constant rate through muscle metabolism and is commonly used to estimate glomerular filtration rate (eGFR). However, several factors, including age, gender, muscle mass, and tubular secretion, can influence creatinine levels, which may delay the detection of actual changes in kidney function (Peake and Whiting, 2006).

Creatinine levels were significantly elevated in rats exposed to cadmium, indicating renal dysfunction and decreased glomerular filtration. However, the administration of okra seed oil in combination with cadmium significantly reduced creatinine levels compared to the cadmium-only group. In contrast, the group that received only the oil maintained normal creatinine levels, which ranged between 0.2 and 0.8 mg/dL (Fauzi *et al.*, 2020), thus reinforcing the nephroprotective role of the oil (Fig. 1).

Glucose

Kidney function is essential for regulating blood glucose levels, mainly through its influence on gluconeogenesis and the renal threshold for glucose (Fernandes, 2021). As illustrated in (Fig. 2), exposure to cadmium led to a significant increase in blood glucose levels; several mechanisms may underline this effect:

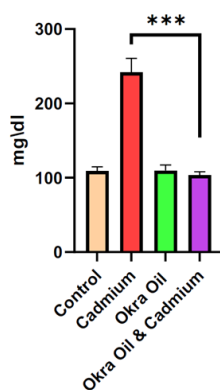


Fig. 2. Effect of cadmium and okra seed oil on glucose level in mg/dl for different experimental groups

The results are expressed as mean $\pm$ SEM.

Renal damage: High blood sugar levels may indicate that kidney function is impaired due to cadmium poisoning. Damage to the kidneys can lead to improper glucose reabsorption and result in hyperglycemia, as the kidneys play a key role in filtering and reabsorbing glucose (Sędzikowska and Szablewski, 2021).

Stress response: Cadmium exposure may trigger a stress reaction that increases blood glucose levels by releasing stress hormones, such as catecholamines and glucocorticoids (Sharma *et al.*, 2022).

Insulin resistance: Heavy metals, such as cadmium, can disrupt regular insulin signaling pathways, leading to insulin resistance (Haidar *et al.*, 2023). This condition causes the body to struggle to utilize insulin, resulting in elevated blood glucose levels.

In contrast, the co-administration of okra seed oil alone maintained stable glucose levels, similar to those seen in untreated rats. This finding indicates that okra seed oil does not produce any adverse metabolic effects.

Total protein

Renal filtration plays a crucial role in maintaining overall body homeostasis and preventing excessive protein from being excreted in the urine, which is reflected in normal blood protein levels (Murray and Paolini, 2020). In this study, Cadmium-

intoxicated rats show an increase in total protein levels, which may lead to several possible kidney-related problems:

Proteinuria: Elevated serum protein levels can sometimes indicate overflow proteinuria, a condition where the kidneys struggle to filter excess proteins due to cadmium poisoning. Typically, however, higher levels of protein in the urine, rather than in serum, are used to diagnose proteinuria (Kamińska *et al.*, 2020).

Acute phase reaction: Increased levels of blood proteins may also signify an acute phase reaction, wherein the body produces more specific proteins in response to damage or inflammation. This reaction can indicate a systemic response to cadmium toxicity (Mantovani and Garlanda, 2023).

Glomerular damage: Cadmium exposure can lead to changes in glomerular permeability, resulting in glomerular damage. The increased synthesis of specific proteins, such as acute phase proteins, can raise serum protein levels; however, this often affects protein filtration, resulting in proteinuria (Satarug *et al.*, 2023).

Administration of okra seed oil significantly reduced the increase in protein levels observed in cadmium-treated rats. In contrast, protein concentrations in the oil-only group were comparable to those of the control group (Fig. 3).

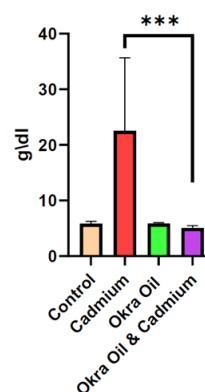


Fig. 3. Effect of cadmium and okra seed oil on protein levels in different experimental groups
The results are expressed as mean $\pm$ SEM.

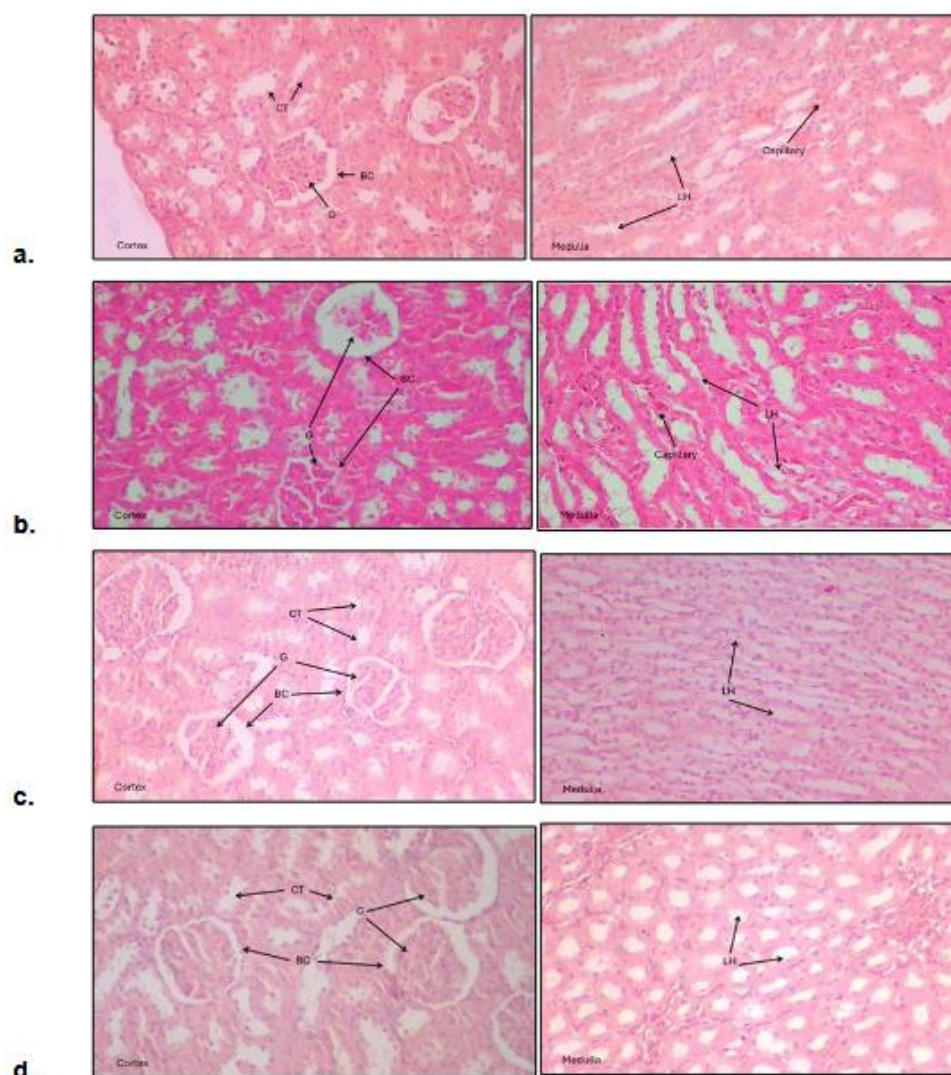


Fig. 4. Representative microscopic of renal sections stained by H&E and observed under light microscopic and photographed at x100 magnification to show cortex and medulla regions

A. Control group. B. Cadmium-intoxicated group. C. Okra seed oil only. D. Co-administration of cadmium and okra seed oil.

Overall, these findings demonstrate that cadmium exposure disrupts renal and metabolic functions, 176 while okra seed oil offers substantial protection by reducing biochemical markers of nephrotoxicity and 177 helping to restore homeostasis.

Microscopic evaluation of kidney tissue

Control Group (Fig. 4a): Cortex shows normal renal architecture observed. Glomeruli (G) appear intact with well-defined Bowman's capsules (BC). Convoluted tubules (CT) are regularly arranged and exhibit standard epithelial lining without signs of degeneration. In medulla the Loop of Henle (LH) tubules are evenly spaced and display clear lumens.

Interstitial capillaries are not congested. Overall, no histopathological alterations are noted, conforming to healthy renal tissue.

Cadmium Group (Fig. 4b): cortex region marked histopathological alterations are evident. Glomeruli (G) appear shrunken or distorted with widened Bowman's spaces. Convoluted tubules (CT) show sign of degeneration, including epithelial desquamation and cytoplasmic vacuolation. In medulla, Loop of Henle (LH) tubules display disorganization and irregularity. Interstitial capillaries exhibit signs of congestion. These findings indicate cadmium-induced nephrotoxicity, likely mediated by oxidative stress and tubular injury.

Okra seed oil group (Fig. 4c): Renal cortex reveals preserved architecture. Glomeruli (G) and Bowman's capsules (BC) retain normal appearance. Convoluted tubules (CT) maintain healthy morphology with no signs of cytoplasmic or nuclear abnormalities. In medulla, Loop of Henle (LH) and surrounding tubules appear normal with intact epithelial lining and no evidence of congestion or inflammation. Okra seed oil alone does not induce any detectable damage.

Okra seed oil + cadmium group (Fig. 4d): Partial preservation of renal cortex architecture noted. Glomeruli (G) and Bowman's capsules (BC) largely retain normal morphology with only minor changes. Convoluted tubules (CT) display improved organization compared to the cadmium-only group, with reduced degeneration and vacuolation. In medulla, Loop of Henle (LH) tubules are well-preserved and exhibit less structural damage than cadmium-only kidneys, with minimal signs of congestion. The findings suggest that okra seed oil protects against cadmium-induced renal injury.

DISCUSSION

This study demonstrated that okra seed oil offers significant protection against nephrotoxicity induced by cadmium in male Wistar rats. The oil's rich antioxidant composition—especially carotenoids, phenolic compounds, and vitamins A, C, and E—helped maintain renal function by keeping key biochemical markers within normal ranges, even in the presence of cadmium exposure. These findings align with those of Al-kanani *et al.* (2019), who reported similar antioxidant effects in reducing oxidative stress.

Cadmium exposure led to notable increases in BUN, creatinine, and UA levels, indicating considerable renal impairment. These results support observations from Sotomayor *et al.* (2021), who connected cadmium toxicity to oxidative stress and diminished renal filtration, and from Fujishiro *et al.* (2018), who demonstrated that cadmium-induced glomerular injury results in proteinuria. In line with this, the heightened protein levels observed in cadmium-

intoxicated rats in our study suggest glomerular dysfunction. The administration of okra seed oil effectively reversed these changes, confirming its nephroprotective properties. Similar protective effects were noted by Elkhailifa *et al.* (2021), who showed that okra seed oil mitigates oxidative damage and enhances renal performance.

Furthermore, cadmium toxicity disrupted glucose regulation, likely due to oxidative stress and impaired insulin signaling, which fosters insulin resistance. These findings are consistent with Sarmiento-Ortega *et al.* (2022), who documented how cadmium interferes with insulin pathways, and Satarug (2024), who emphasized its negative impact on glucose homeostasis. Treatment with okra seed oil restored glucose levels to near-normal values, corroborating the work of Tavakolizadeh *et al.* (2023), who found that okra seed intake lowers fasting blood glucose.

Additional evidence from Mokgalaboni *et al.* (2023) attributes the antidiabetic and antioxidant properties of okra to its flavonoid and polysaccharide content.

The current study confirms that exposure to cadmium causes significant histopathological changes in the kidney, particularly impacting the glomeruli and tubular structures. These changes are marked by glomerular shrinkage, widening of Bowman's spaces, tubular degeneration, and medullary disorganization. Similar microscopic findings have been reported previously by Badawy *et al.* (2024), where cadmium chloride administration caused vascular lesions, hemorrhage, edema, fibrotic changes, mononuclear cell infiltration, and marked glomerular and tubular degeneration and necrosis in rat kidneys. Previous studies have linked these lesions to increased oxidative stress, lipid peroxidation, and loss of integrity in the tubular epithelium (Al-Gebaly, 2017; Scott *et al.*, 1977).

Abdel-Moneim *et al.* (2020) reported that exposure to lead acetate in mice resulted in significant increases in oxidative stress and considerable renal tissue damage. However, administration extract from *Abelmoschus esculentus* improved the biochemical and histological

changes, helping to restore the structure of both glomeruli and tubules. In line with these observations, our data indicates that rats treated with okra seed oil alone maintained their normal renal histological architecture, confirming the safety of the oil itself. In contrast, co-administration of okra seed oil with cadmium significantly reduced the structural damage observed in the cadmium-only group.

This partial preservation of glomerular and tubular structure, along with reduced congestion in the medulla, reflects the antioxidant and anti-inflammatory properties of okra seed oil. These properties may help mitigate cadmium-induced oxidative stress and protect the integrity of tubular epithelial cells.

In conclusion, cadmium exposure severely compromises renal function, protein regulation, and glucose metabolism. Okra seed oil, due to its bioactive components, mitigates these toxic effects and supports metabolic balance. These findings contribute to the growing body of evidence supporting the therapeutic potential of natural antioxidants in protecting against heavy metal-induced toxicity.

CONCLUSION

Collectively, the present findings demonstrate that okra seed oil exerts both functional and structural protection against cadmium-induced nephrotoxicity. By mitigating oxidative stress and preserving the integrity of glomerular and tubular structures, the oil markedly attenuated biochemical disturbances and histopathological lesions in the kidneys. These results highlight okra seed oil as a promising natural antioxidant capable of alleviating cadmium-related renal and metabolic damage and providing a basis for future studies to further clarify its mechanisms and therapeutic potential in clinical settings.

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