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Nephroprotective effects of (*Atriplex halimus)* against cadmium-induced renal toxicity: biochemical, antioxidant, and histopathological insights

[*Efeitos nefroprotetores do (*Atriplex halimus*) contra a toxicidade renal induzida pelo cádmio: insights bioquímicos, antioxidantes e histopatológicos*]

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ABSTRACT

This study investigates the nephroprotective potential of *Atriplex halimus* against cadmium-induced renal toxicity in Wistar rats. Cadmium, a toxic environmental pollutant, induces oxidative stress and renal damage. The research evaluates the protective effects of ethanolic extracts of *A. halimus*, which are rich in polyphenols and flavonoids, through in vivo experiments. The total polyphenol content of the extract was 20.64±1.44µg GAE/mg E, and the flavonoid content was 19.28±0.43µg QE/mg E. Forty-two rats were divided into six groups and treated with cadmium chloride (8.8 mg/kg/day), *A. halimus* (400 or 600mg/kg/day), or a combination for 40 days. Biochemical assays indicated that *A. halimus* alleviated cadmium-induced hyperglycemia and improved renal markers such as urea, creatinine, and uric acid. Antioxidant analyses showed increased glutathione levels in treated rats, suggesting a reduction in oxidative stress. Histological examination confirmed less renal damage, with preservation of glomerular and tubular structures in the treatment groups. These findings suggest that *A. halimus* has potent nephroprotective properties, offering promise as a natural remedy for mitigating the harmful effects of cadmium exposure. This study supports its potential use in dietary supplementation for populations at risk of cadmium toxicity.

Keywords: *Atriplex halimus*, nephroprotection, cadmium toxicity, antioxidant activity

RESUMO

Este estudo investiga o potencial nefroprotetor do Atriplex halimus *contra a toxicidade renal induzida pelo cádmio em ratos Wistar. O cádmio, um poluente ambiental tóxico, induz estresse oxidativo e danos renais. A pesquisa avalia os efeitos protetores dos extratos etanólicos de* A. halimus*, ricos em polifenóis e flavonóides, por meio de experimentos in vivo. O conteúdo total de polifenóis do extrato foi de 20,64 ± 1,44 µg GAE/mg E, e o teor de flavonóides foi de 19,28 ± 0,43 µg QE/mg E. Quarenta e dois ratos foram divididos em seis grupos e tratados com cloreto de cádmio (8,8 mg/kg/dia),* A. halimus *(400 ou 600 mg/kg/dia) ou uma combinação dos dois durante 40 dias. Ensaios bioquímicos indicaram que* A. halimus *aliviou a hiperglicemia induzida pelo cádmio e melhorou os marcadores renais, como ureia, creatinina e ácido úrico. Análises antioxidantes mostraram aumento dos níveis de glutationa em ratos tratados, sugerindo uma redução no estresse oxidativo. O exame histológico confirmou menos danos renais, com preservação das estruturas glomerulares e tubulares nos grupos tratados. Esses achados sugerem que* A. halimus *tem propriedades nefroprotetoras potentes, oferecendo esperança como um remédio natural para mitigar os efeitos nocivos da exposição ao cádmio. Este estudo apoia seu uso potencial em suplementos alimentares para populações em risco de toxicidade por cádmio.*

Palavras-chave: Atriplex halimus*, nefroproteção, toxicidade por cádmio, atividade antioxidante*

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INTRODUCTION

Over the past decade, phytotherapy has become more significant as it impacts both health and international trade. The return to natural products is essential as they are considered less toxic and equally effective (Missoun *et al.*, 2018). Algeria is known for its plant diversity and geographical location, which has been beneficial for the medicinal plant market (Bounouar *et al.*, 2022). Among these, *A. halimus* is an Algerian species commonly known as "Guettaf." It is native and grows across a wide range of soils (Aouissat *et al.*, 2011). *A. halimus* is known in traditional medicine (Kabbash and Shoeib, 2012, Zeghib and Boutlelis, 2020). This plant has been used as a traditional remedy for thousands of years (Walker *et al.*, 2014). In the Arab world, *A. halimus* has been used to treat heart disease, diabetes, rheumatism (Parvez *et al.*, 2018), anemia (Mohammedi, 2016), stomach pain, and to combat intestinal parasites (Slama *et al.*, 2018). This plant is also used to treat inflammation (cystitis) and urinary stones; it accompanies any regimen requiring tissue drainage and the removal of waste and toxins (Nedjimi *et al.*, 2013).

Atriplex halimus (commonly known as Mediterranean saltbush) is a halophytic shrub traditionally used in folk medicine for the treatment of various ailments, including diabetes, inflammation, and liver disorders. Its pharmacological potential has been increasingly attributed to its rich content of bioactive compounds, particularly polyphenols, flavonoids, saponins, and alkaloids. Studies have identified several constituents such as quercetin, rutin, and gallic acid within A. halimus, all of which possess strong antioxidants and anti-inflammatory properties, suggesting a potential role in mitigating renal damage caused by oxidative stress and heavy metal exposure (Roubi *et al*., 2024).

The industrial revolution and the use of heavy metals have become one of the most serious environmental problems, exposing humans and their environment to many toxic heavy metals (Abd Elnabi *et al.*, 2023). Heavy metals or trace elements are non-degradable and can take various chemical forms in the environment. They are also often considered toxic, such as lead, mercury, arsenic, chromium, and cadmium (Sable *et al.*, 2024). They are environmental contaminants that can be accumulated and transferred to higher organisms in food chains, leading to serious ecological and health issues (DeForest *et al.*, 2007). They can cause pathophysiological disturbances (Tchounwou *et al.*, 2012).

Cadmium (Cd) is one of the most toxic elements, present in nearly all compartments of the environment (water, air, and soil), It is highly toxic in all its chemical forms. However, its physicochemical properties, which are similar to those of zinc and calcium, allow it to cross biological barriers and accumulate in tissues (Kumar *et al.*, 2024). After the absorption phase, cadmium is transported by the bloodstream. It causes significant damage to vital organs by inducing oxidative stress (Filipic *et al.*, 2006). It can lead to an increase in reactive oxygen species (ROS) such as hydroxyl radicals, superoxide, and/or hydrogen peroxide (Oh *et al.*, 2006). Consequently, it disrupts the redox system's homeostasis and induces lipid peroxidation (Wang *et al.*, 2019). Despite the growing interest in plant-based therapies, there remains a significant gap in research exploring the nephroprotective effects of natural products, particularly halophytes such as *A. halimus*, against cadmium toxicity. Given these considerations, this study was conducted to examine the potential nephroprotective effect of *A. halimus* against renal toxicity induced by cadmium chloride in rats.

MATERIALS AND METHODS

The aerial parts of *A. halimus* L. were collected in March 2023 from Ouled Ayche Bitam, Barika, Batna province, Algeria. After cleaning, the plant material was air-dried for 15 days in a well-ventilated, shaded area. To facilitate efficient grinding, it was further dried in an oven at 40°C for 24 hours. The dried material was finely ground using an electric grinder and stored in a sealed glass container.

Crude extract preparation was performed using solid-liquid maceration. Thirty grams of plant material were immersed in 150 mL of ethanol (HPLC 99.9°, Sigma PROCHIMA) under magnetic stirring at room temperature and in darkness for 24 hours. The solvent was replaced every 24 hours, and the extraction was repeated

for three cycles. After each cycle, the mixture was filtered using Whatman filter paper to separate the liquid extract from the solid residue. The combined filtrates were concentrated at (40°C using a rotary evaporator. The concentrated extract was transferred to glass Petri dishes and incubated in an oven at 40°C until fully dried. The dried extract was weighed and stored at 4°C in a refrigerator until further use (Oran *et al.*, 2016).

The total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) reagent method (Wong *et al.* 2006). 200µL aliquot of the plant extract was mixed with 1 mL of 10% (v/v) Folin-Ciocalteu reagent in a test tube. The mixture was allowed to react for 4 minutes at room temperature. After the reaction, 800µL of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the mixture was thoroughly mixed. The reaction was allowed to proceed for 2 hours at room temperature in the dark to allow for the development of color. The solution's absorbance was measured at 765nm using a UV-Vis spectrophotometer (SP UV-2005). A standard curve was generated using gallic acid at concentrations of 6.25, 12.5, 25, 50, 100, and 200µg/mL, with absorbance readings taken at 765nm. The total phenolic content of the sample was determined from the standard curve and expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract (µg GAE/mg E). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

The total flavonoid content (TFC) was evaluated using the aluminum chloride colorimetric method (Djeridane *et al.*, 2006). A 1 mL aliquot of the plant extract was mixed with 1mL of 2% (w/v) aluminum chloride ($AlCl_3$) solution, and the mixture was incubated at room temperature for 10 minutes. The absorbance of the solution was recorded at 430nm using a UV-Vis spectrophotometer. A standard curve was prepared with quercetin at concentrations of 2.5, 5, 10, 20, and 40µg/mL The total flavonoid content of the sample was calculated from the standard curve and expressed as micrograms of quercetin equivalents (QE) per milligram of extract (µg QE/mg ES). All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

Cadmium chloride ($CdCl_2$) was prepared as an aqueous solution at a concentration of 8.8mg/kg/day, corresponding to 1/10th of the LD50. For the plant extract, 3g and 4.5g of *A. halimus* powder were macerated in 45mL of hot distilled water to obtain doses of 400mg/kg/day and 600mg/kg/day, respectively. The maceration process was performed for 4 hours, followed by filtration. Both the cadmium solution and fresh plant infusions were prepared daily for the experiment.

The study was conducted on 42 adult male Wistar albino rats, aged 2 months and weighing 120–190g, obtained from the Pasteur Institute of Algiers. The animals were housed in plastic cages within the animal facility at the University of 20 August 1955, Skikda. They were acclimatized for two weeks under standard housing conditions, maintained at 25±5°C, with a 12-hour light/dark photoperiod and relative humidity of 64±2 %. The rats had unrestricted access to standard rat chow (standard diet, supplied by the ‘‘ONAB, El-Harrouch’’, Skikda, Algeria) and tap water throughout the study period. To ensure cleanliness, cages were cleaned, and bedding was replaced daily.

Forty-two rats were randomly divided into six groups of seven animals each: G1 (control group) received oral tap water; G2 and G3 were treated with *A. halimus* at doses of 400mg/kg/day and 600mg/kg/day, respectively; G4 received cadmium chloride at a dose of 8.8mg/kg/day; G5 and G6 were co-treated with cadmium chloride (8.8mg/kg/day) and *A. halimus* at doses of 400mg/kg/day and 600mg/kg/day, respectively. All treatments were administered daily via oral gavage using a gastric tube for 40 days. Rats were weighed daily throughout the experiment. At the end of the treatment, animals were fasted overnight and then euthanized. Blood samples were collected in serum separation tubes and centrifuged to prepare serum for biochemical analysis. A longitudinal abdominal incision was performed to excise the kidneys, which were carefully freed from connective tissue and weighed. Each kidney was divided into two portions: one portion (1g of the kidney tissue) was immediately homogenized in 2ml of buffer solution of TBS (Tris 50mM, NaCl 150mM , pH=7.4). Homogenates were centrifuged at 9000rpm for 15 min at 4°C, and the obtained supernatant was used for the determination of

Reduced glutathione (GSH) and protein level. The second portion was fixed in 10% formalin for histological examination. All experimental procedures were conducted under anesthesia, with strict measures to minimize animal suffering. The study protocol was reviewed and approved by the Research Ethics Committee for Laboratory Animal Care, Department of Natural and Life Sciences, Faculty of Science, University of 20 August 1955, Skikda. All procedures adhered to the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

Parameters (glucose, urea, creatinine, uric acid, albumin, and total proteins) were measured by kinetic colorimetric method using commercial kits obtained from Spinreact,S.A./S.A.U Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) Spain.

The glutathione assay was performed according to the method of Ellman, (1959) as modified by Jollow *et al*. (1974). The principle of this assay is based on measuring the optical absorbance of 2-nitro-5-mercapturic acid, which results from the reduction of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) by the (-SH) groups of glutathione. A protein precipitation step is performed to retain only the (-SH) groups specific to glutathione.

In brief, 0.8ml of tissue homogenate was added to 0.2ml of 0.25% sulfosalicylic acid, left for 15min in an ice bath. and tubes were centrifuged at 1000rpm for 5min. Supernatant (0.5ml) was mixed with 0.025mL of 0.01 M DTNB and 1 ml of buffer solution of Tris (pH 9.6) was incubated for 5min at room temperature. Finally, the absorbance at 412nm was recorded. Total GSH content was expressed as nmol GSH/mg protein.

The protein content of the supernatant was spectrophotometrically estimated by the Bradford (1976) method using bovine serum albumin as standard.

Tissue samples were dehydrated through a graded ethanol series (70% to 100%), cleared in xylene, and embedded in paraffin to preserve their structure. Thin sections (4–6µm) were cut from the paraffin blocks using a rotary microtome and mounted onto clean glass slides. The sections were deparaffinized in xylene, rehydrated through a descending ethanol series, and stained with hematoxylin and eosin (H&E) for histological examination, following the standard protocol described by Martoja and Martoja (1967). Histopathological evaluation was performed under an optical microscope (OPTICA, Axiom 2000) to assess tissue morphology and potential pathological changes.

The results are presented as mean ± SD. Group differences were assessed using ANOVA, with significance set at $p \leq 0.05$. Post-hoc comparisons between groups were conducted using the Tukey test. Data analysis was carried out using SPSS software, version 20.

RESULT

The total polyphenol content of the crude *A. halimus* extract was 20.64±1.44 µg GAE/mg E, while the flavonoid content was 19.28±0.43µg QE/mg E.

The study evaluated the effect of *A. halimus* on cadmium chloride-induced toxicity in rats. The control group (G1), which received only water, exhibited stable body weight (106±1.7g) and relative kidney weight (0.34±0.001 %). Groups treated with *A. halimus* (G2:106±2.6g, G3:107±1.7g) showed significant ($p \leq 0.001$) weight gain, along with a slight, non-significant increase in relative kidney weight compared to the control group, indicating a growth-promoting effect without significant kidney enlargement. In contrast, the cadmium chloride-treated group (G4:57±1.7g) experienced substantial weight loss and a marked increase in relative kidney weight, reflecting cadmium-induced toxicity, likely due to its nephrotoxic effects. Interestingly, the combined treatment groups (G5:60.3±2.2g, G6: 75.3±2.2g), which received both cadmium chloride and *A. halimus*, demonstrated significant improvement, with reduced weight loss and a considerable decrease in relative kidney weight compared to the cadmium chloride-only group (G4) (Fig. 1).

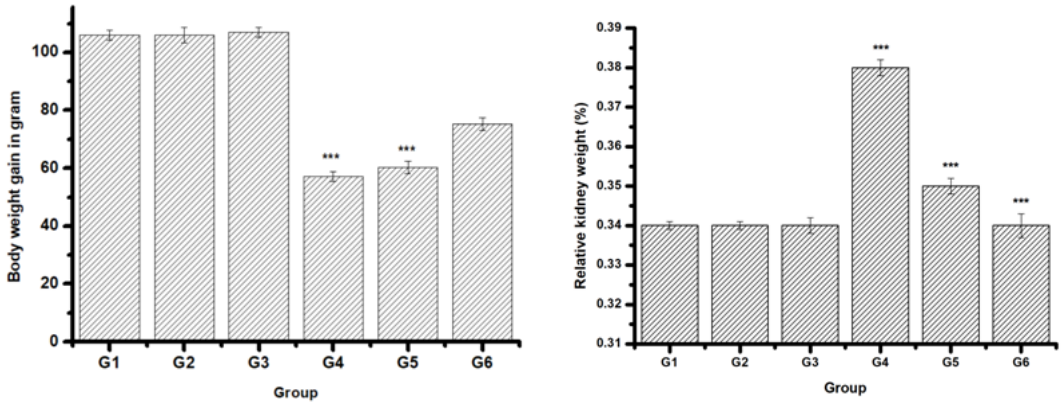

Figure 1. Body weight gain and relative kidney weight in control and experimental groups (n=7). *p≤0.05, **p≤0.01, ***p≤0.001: Significantly different between (G2, G3, G4, G5, G6) groups and the (G1) control group. ###p≤0.001: Significantly different between (G5, G6) groups and the (G4) group. Values are expressed as mean ± SD. *n* represents the number of observations.

The results shown in Fig. 2 reveal a significant increase (p≤0.001) in glucose concentration in rats exposed to cadmium (G4) compared to the control rats (G1), and a significant decrease (p≤0.01, p≤0.001) in the groups exposed to cadmium and treated with the plant *A. halimus* (G5 and G6), respectively, compared to the rats treated with cadmium (G4). However, this decrease remained significantly higher (p≤0.001) than in the control rats (G1). This decrease brought the glucose concentration in these cadmium-exposed rats, especially those treated with the 600mg/kg/day dose, close to that of the control rats. For the groups (G2 and G3) treated only with the plant, this parameter showed no significant change compared to the control group (G1).

Variation in renal function parameters was assessed by examining kidney activity (Fig. 2). Compared to the control group (G1), cadmium exposure in rats from group (G4) caused a significant increase (p≤0.001) in urea concentration, creatinine, and uric acid. However, after administering *A. halimus* in groups (G5) and (G6), these biochemical parameters improved significantly (p≤0.01). Group (G5) showed significant reductions in urea (p≤0.001), creatinine, and uric acid (p≤0.01), while group (G6) showed significant reductions in all three parameters (p≤0.001) compared to the cadmium-treated group (G4). Although these reductions were observed, the concentrations of these parameters remained significantly higher than those in the control group (G1). For groups treated only with the plant extract (G2 and G3), no significant change was observed in these parameters compared to the control group (G1).

In contrast, a significant decrease (p≤0.001) in serum levels of total proteins and albumin was noted in the cadmium-exposed group (G4) compared to the control group (G1). In rats from the cadmium-exposed groups treated with the plant (G5 and G6), these parameters returned to normal levels, with a significant increase (p≤0.001) in serum levels of total proteins and albumin in both groups compared to the cadmium-treated group (G4). Treatment with *A. halimus* alone (G2 and G3) did not affect these parameters compared to the control rats.

The results shown in Fig. 3 indicate a significant decrease (p≤0.001) in reduced glutathione (GSH) levels in the kidneys of cadmium-treated rats (G4) compared to control rats (G1). However, treatment with *A. halimus* significantly (p≤0.001) partially restored GSH levels in both groups (G5 and G6). In contrast, administration of the plant alone did not induce any change in GSH levels in groups (G2 and G3) compared to the control group (G1).

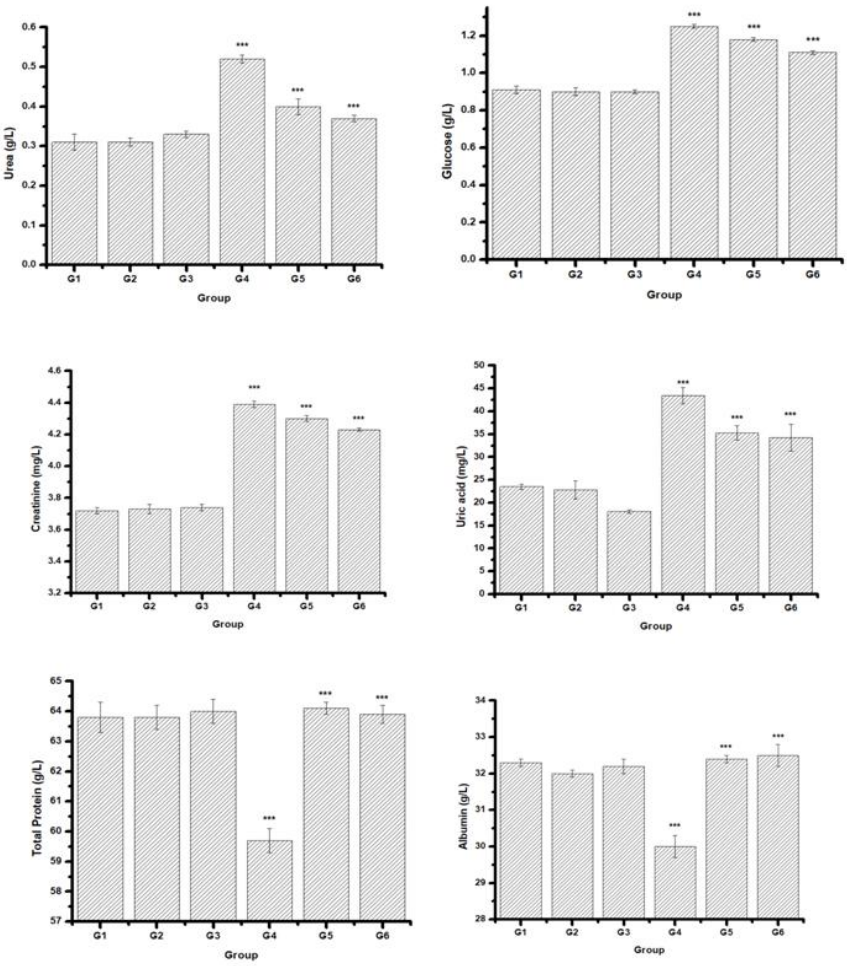

Figure 2. Variations in biochemical parameters across the control and experimental groups (*n*=7). *p≤0.05, **p≤0.01, ***p≤0.001: Significantly different between (G2, G3, G4, G5, G6) groups and the (G1) control group. #p≤0.05, ##p≤0.01, ###p≤0.001: Significantly different between (G5, G6) groups and the (G4) group. Values are presented as mean ± SD. *n* represents the number of observations.

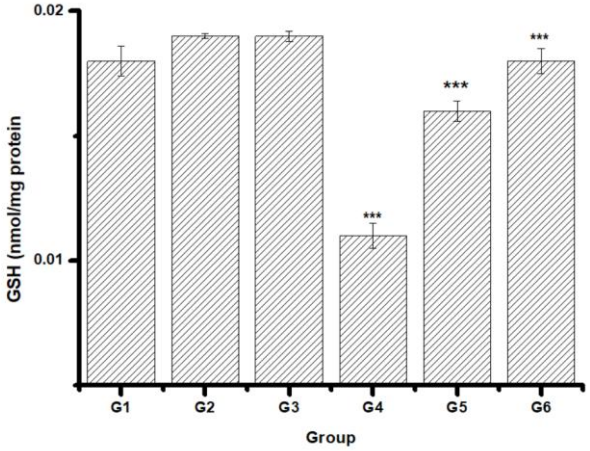

Figure 3. Variations in reduced glutathione (GSH) levels across the control and experimental groups (*n*=7). *p≤0.05, **p≤0.01, ***p≤0.001: Significantly different between (G2, G3, G4, G5, G6) groups and the (G1) control group. #p≤0.05, ##p≤0.01, ###p≤0.001: Significantly different between (G5, G6) groups and the (G4) group. Values are expressed as mean ± SD. *n* represents the number of observations.

Microscopic analysis of renal histological sections from the control rats (G1) and rats treated with A. halimus (G2 and G3) showed normal renal parenchyma, uniform glomeruli, and intact cortical and medullary regions with well-defined membranes (Fig. 4). However, the kidneys of cadmium-exposed rats (G4) exhibited severe changes in renal architecture, including degeneration, necrosis, glomerular and tubular hypertrophy, tubular dilation, and vacuolization. Additionally, areas of inflammation and infiltration of inflammatory cells were present in the renal sections of this group (Fig. 5). For cadmium-contaminated groups treated with protective *A. halimus* treatment (G5 and G6), protection against tubules and glomeruli was indicated, with the glomeruli appearing larger and not dilated. Despite these changes observed in the latter groups (Fig.6), the architecture of the renal cortex remained more intact than in the cadmium-contaminated group.

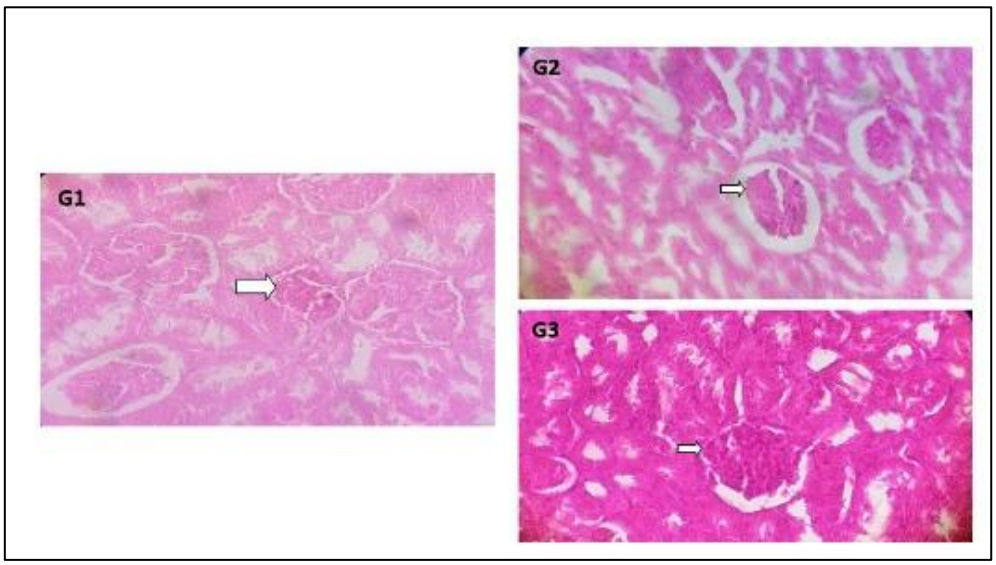

Figure 4. Histological sections of rat kidneys show the following: G1 represents the control group that received tap water orally, G2 includes rats treated with A.halimus at a dose of 400mg/kg/day, and G3 consists of rats treated with Atriplex halimus at a dose of 600mg/kg/day. Arrows point to the glomeruli.

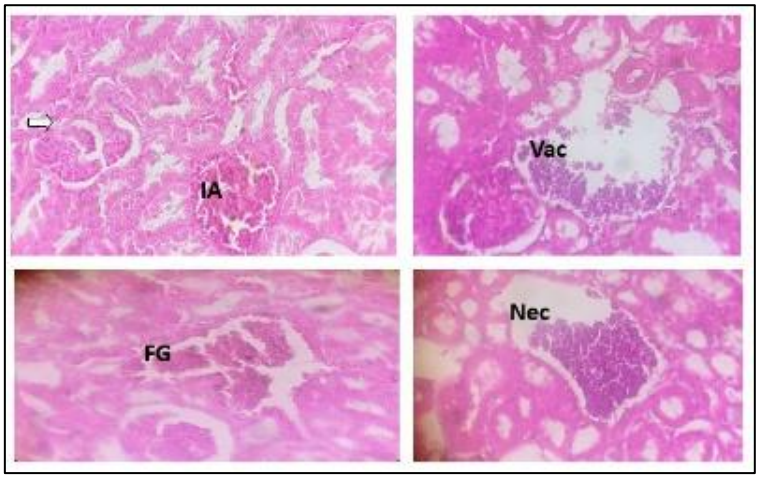

Figure 5. Histological sections of the experimental group G4 reveal notable pathological changes in the kidney tissues. These rats were treated with cadmium chloride at a dose of 8.8mg/kg/day. The sections show prominent inflammatory aspects (IA), vacuolization (Vac), and fragmentation of glomeruli (FG). Additionally, necrosis (Nec). Arrow point to the glomeruli.

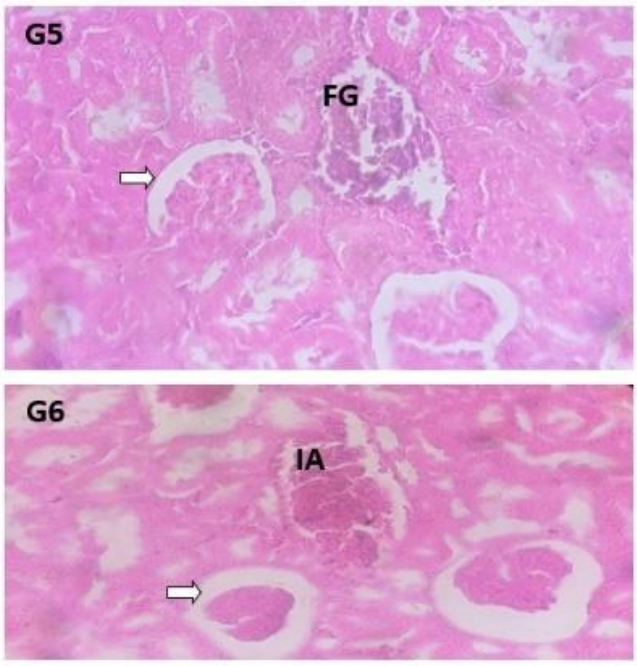

Figure 6. Histological sections of the experimental groups: G5: Rats treated with cadmium chloride (8.8mg/kg/day) and Atriplex halimus (400mg/kg/day). G6: Rats treated with cadmium chloride (8.8mg/kg/day) and Atriplex halimus (600mg/kg/day). inflammatory aspects (IA), vacuolization, and fragmentation of glomeruli (FG). Arrows indicate the glomeruli.

DISCUSSION

The aim of this study was to evaluate the effects of cadmium, a toxic pollutant, on Wistar rats and to explore the potential protective role of the medicinal plant *A. halimus* against cadmium toxicity, with a focus on both biochemical and histological mechanisms. The crude *A. halimus* extract was found to have a total polyphenol content of 20.64±1.44µg EAG/mg ES, indicating a notable concentration of polyphenols in this plant. This value surpasses those reported in earlier studies (Bounouar *et al.*, 2022, Chaouche *et al.*, 2021, Zeghib and Boutlelis, 2021, Chikhi *et al.*, 2014, Rached, 2009), where polyphenol levels ranged between 5.24mg EAG/g ES and 16.50mg EAG/g ES. However, it remains below the 27.04mg EAG/mg ES reported by Bouchoucha and Ouazeta (2018). The flavonoid content was measured at 19.28±0.43µg EQ/mg ES, which is similar to the 16.41mg EQ/g ES reported by Rached (2009) and higher than the findings of other studies (Bounouar *et al.*, 2022, Chaouche *et al.*, 2021, Zeghib and Boutlelis, 2021, Bouchoucha and Ouazeta, 2018), which ranged from 1.26 to 7.691mg EQ/g ES. Variations in phenolic content across plant species can be attributed to factors such as extract composition (Sayem *et al.*, 2024), genetic differences, and both biotic and abiotic factors (Kathuria *et al.*, 2024, Saini *et al.*, 2024, Suba *et al.*, 2024).

The findings clearly demonstrate that cadmium exposure led to weight loss in rats, consistent with observations from previous studies (Chaker, 2021, Kouadria, 2020, Layachi, 2013). This weight loss can be attributed to anorexia induced by cadmium toxicity (Zhang *et al.*, 2018). Additionally, cadmium toxicity is associated with increased lipid and protein degradation and degeneration (Mishra *et al.*, 2024), which likely contributed to the observed weight reduction due to decreased serum protein levels (El-Demerdash *et al.*, 2004). Moreover, the increase in relative kidney weight observed in cadmium-treated rats may be explained by tissue hypertrophy caused by inflammation and the accumulation of metals in target organs (Messaadia *et al.*, 2013). These results align with earlier findings (Asagba *et al.*, 2002). Treatment with *A. halimus* extract, however, appeared to counteract these effects, promoting body weight recovery and reducing relative kidney weight, indicating a potential protective effect against cadmium toxicity. These results are consistent with the findings of Zeghib and Boutlelis, (2021), who reported significant

improvements in weight gain in animals treated with *A. halimus*.

The hyperglycemia observed in cadmium-treated rats is consistent with findings from previous studies (Rana *et al.*, 1996; Massanyi *et al.*, 1995). Elevated blood glucose levels are a common consequence of heavy metal toxicity and can result from factors such as the inhibition of insulin release from pancreatic islets (Kechrid *et al.*, 2006), impaired glucose utilization by cells leading to increased insulin concentrations (Sunderman *et al.*, 1976), or disruptions in glucagon secretion, which stimulate glycogen breakdown and glucose production from non-carbohydrate sources such as proteins (Massanyi *et al.*, 1995). However, treatment with *A. halimus* improved glucose levels in cadmium-exposed rats. While the precise mechanisms underlying the antihyperglycemic effects of *A. halimus* remain unclear, the plant may exert its effects by enhancing the condition of the islets of Langerhans, as observed in the histological tissues of *A. halimus*-treated rats, or by stimulating pancreatic insulin secretion from existing beta cells (Bounouar *et al.*, 2022). This insulin-like activity could be linked to the plant's phytoconstituents (Missoun *et al.*, 2018). Several studies have reported antidiabetic activity in *Atriplex* species from desert regions (Slama *et al*., 2018, Souad *et al.*, 2019, Chikhi *et al.*, 2014). Additionally, numerous studies have demonstrated that phenolic compounds and flavonoids possess significant antidiabetic properties (Chikhi *et al.*, 2014).

The increase in urea and creatinine levels in the serum is a biomarker of renal dysfunction and kidney damage caused by cadmium exposure (Gabr *et al.*, 2019). Our results agree with studies by Sarg *et al.* (2019) and Rana *et al.* (1996). Abdelaziz *et al.* (2012) also showed that oral cadmium administration induced significant renal lesions and damage, as reflected by increased renal biomarkers. The increase in urea and creatinine is likely due to increased protein catabolism, as proteins are broken down into amino acids and subsequently into urea and creatinine. This is further confirmed by the decrease in total serum proteins. Therefore, the increase in urea and creatinine in cadmium-exposed rats reflects the nephrotoxic effects of cadmium (El-Demerdash *et al.*, 2009). The elevated serum uric acid levels in our study could be due to the degradation of genetic material (DNA and RNA) (Waisberg *et al.*, 2003). Additionally, uric acid is an important endogenous antioxidant in the body (Delattre *et al.*, 2005). Increased circulating uric acid levels may serve as an indicator of the body's defense mechanisms against free radical damage. Furthermore, analysis revealed a significant reduction in total proteins and albumin in cadmium-exposed rats, which can be attributed to the reactivity of cadmium with proteins containing sulfhydryl (-SH) and hydroxyl (-OH) groups, leading to their denaturation or fragmentation. Stress induced by cadmium exposure also impacts protein metabolism and amino acid synthesis in the liver (El-Demerdash *et al.*, 2009). Treatment with *A. halimus* significantly reduced the increases in urea, creatinine, and uric acid following cadmium exposure. Zeghib and Boutlelis, (2019) also reported the nephroprotective effects of *A. halimus* against cadmium-induced nephrotoxicity in rats. The treatment partially restored renal function, indicating that *A. halimus* possesses inhibitory properties that could provide nephroprotection likely linked to its bioactive compounds.

The reduction in levels of non-enzymatic endogenous antioxidants, such as GSH, observed in cadmium-treated rats aligns with the findings of Chaker (2021) and Layachi (2013). GSH, an intracellular thiol molecule, plays a crucial role in detoxification processes (Sairazi *et al.*, 2017). The antioxidant defense system in the body includes both enzymatic and non-enzymatic antioxidants, working together to neutralize free radicals and minimize their harmful impact on biomolecules and tissues. (Ighodaroab and Akinloye, 2018). Cadmium-induced renal toxicity is primarily driven by the abnormal generation of free radicals, which cause damage to renal cells (Kumar and Sharma, 2019). Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydrogen peroxide, and nitric oxide, are natural byproducts of cellular metabolism, primarily produced in mitochondria and lysosomes via the electron transport chain (Nohl *et al.*, 2004). However, cadmium exposure disrupts the balance between ROS production and antioxidant defenses (Ikediobi *et al.*, 2004), resulting in oxidative damage to kidney tissue (Zhang *et al.*, 2018). Treatment with *A. halimus* significantly

mitigated this oxidative damage, as evidenced by an increase in total GSH levels in the treated rats. This suggests that *A. halimus* possesses antioxidant properties that protect against oxidative damage caused by cadmium exposure. These findings are consistent with previous studies highlighting the antioxidant activity of *A. halimus* (Zeghib and Boutlelis, 2021; Bounouar *et al.*, 2022).

The histopathological examination revealed that cadmium exposure caused significant damage to the kidney, characterized by cell necrosis, inflammation. This is in line with other reports showing that cadmium exposure induces structural and functional damage to this organ (Layachi, 2013). However, *A. halimus* treatment alleviated these changes and preserved the normal architecture of kidney, indicating its potential protective effect. The Nephroprotective effects of *A. halimus* have been demonstrated in other studies (Zeghib and Boutlelis, 2019). The restoration of normal tissue architecture in the kidney suggests that *A. halimus* may protect against the structural damage induced by cadmium toxicity.

Although the current findings provide substantial evidence of the protective effects of *A. halimus* against cadmium-induced toxicity, several limitations must be addressed. First, the study focused primarily on renal toxicity. Further research is needed to assess the plant's protective role in other organs affected by cadmium, such as the liver and brain. Second, while the biochemical and histological parameters were examined, molecular mechanisms (e.g., gene expression related to oxidative stress, apoptosis, and inflammation) remain unexplored and warrant future investigation. Finally, isolation and characterization of the specific bioactive compounds responsible for the observed effects would enhance the understanding of the therapeutic potential of *A. halimus*. These future directions may lead to the development of natural therapeutic agents for managing heavy metal toxicity in both animal models and humans.

CONCLUSION

This study highlights the nephroprotective effects of *A. halimus* against cadmium-induced renal toxicity. Its high antioxidant content, particularly polyphenols and flavonoids, helps mitigate oxidative stress, reduce biochemical disruptions, and protect renal structures. These findings suggest that *A. halimus* could be a natural therapeutic agent for those exposed to cadmium, potentially reducing the risk of chronic kidney damage. Further research is needed to explore its molecular mechanisms and potential human health applications.

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