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# Attenuation of inflammation, oxidative stress and TGF-β1/Smad3 signaling and upregulation of Nrf2/HO-1 signaling mediate the protective effect of diallyl disulfide against cadmium nephrotoxicity

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**Abstract:**

Heavy metals are toxic environmental pollutants with serious health effects on humans and animals. Cadmium (Cd) is known for its serious nephrotoxic effect and its toxicity involves oxidative stress (OS) and inflammation. Diallyl disulfide (DADS), a main constituent of garlic, exhibited cytoprotective and antioxidant activities. This study investigated the effect of DADS on OS, inflammation, and fibrosis induced by Cd in rat kidney, pointing to the involvement of transforming growth factor-β (TGF-β)/Smad3 and nuclear factor erythroid 2–related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling, and peroxisome proliferator-activated receptor gamma (PPARγ). Rats received DADS for 14 days and Cd on day 7 and blood and kidney samples were collected. Cd elevated serum creatinine, urea and uric acid, provoked kidney histopathological alterations and collagen deposition, increased kidney malondialdehyde (MDA) level, and decreased glutathione (GSH) and antioxidant enzymes. Nuclear factor-kappaB (NF-κB) p65, interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, and CD68 were upregulated in Cd-administered rat kidney. DADS prevented kidney injury, mitigated OS, suppressed NF-κB, CD68 and pro-inflammatory mediators, and boosted antioxidants. DADS downregulated TGF-β1, Smad3 phosphorylation and Kelch-like ECH-associated protein-1 (Keap1), and increased Nrf2, HO-1, cytoglobin, and PPARγ. In conclusion, DADS protects the kidney against Cd toxicity by attenuating OS, inflammation, and TGF-β1/Smad3 signaling, and enhancement of Nrf2/HO-1 signaling, antioxidants, and PPARγ.

**Keywords:** Heavy metals; Cadmium; Nephrotoxicity; Garlic; Oxidative stress; Inflammation.

## 1. Introduction

Cadmium (Cd) is a common environmental heavy metal (HM) contaminant in developing countries, posing a significant risk to both humans and animals [1]. The primary sources of Cd exposure include food, cigarette smoke, contaminated water, and occupational exposure [2, 3]. Cd accumulation, particularly in the kidneys, can lead to organ and systemic damage over time.

Given its extended biological half-life, Cd tends to accumulate in the liver, kidney, and bone, which in turn promotes the development of chronic diseases such as renal disease, cardiovascular disease, cancer, and others [4]. Kidney damage is one of the toxic consequences of exposure to Cd and the association between occupational and environmental exposure and kidney injury is well-acknowledged [5]. Several factors, such as duration of exposure, dose, age, sex and route of exposure are involved in Cd nephrotoxicity [6]. Following its pulmonary or intestinal cells-mediated absorption, Cd is mainly deposited in the liver and kidney following its entry to the systemic circulation [7, 8], making the kidneys a target of toxicity [9]. Cd accumulation predominantly occurs in the kidney, particularly in the proximal convoluted tubules (PCT), and has been related to renal dysfunction and damage, culminating in polyuria and proteinuria [10]. This is attributed to proximal tubular epithelial cell hypertrophy and the subsequent accumulation and destruction of Cd at the site [11].

The toxicity of HMs, including Cd is associated with oxidative stress (OS), a status results from the excess levels of reactive oxygen species (ROS) that damage cell macromolecules and suppress the cell intrinsic ability to counteract xenobiotic exposure [12-14]. This condition leads to lipid peroxidation (LPO), damage to membrane proteins, modifications in the antioxidant system, DNA damage, and apoptosis [15, 16]. Cd causes renal injury through diverse mechanisms, including the generation of ROS, inflammation, and apoptosis [11, 17]. It is widely acknowledged that Cd induces OS in animal tissues by altering the balance between pro-oxidants and antioxidants. Cd can cause OS through its ability to diminish intracellular reduced glutathione (GSH) levels or inhibit antioxidant enzymes such as glutathione peroxidase (GPx) through thiol group interaction [18]. Excess ROS can activate inflammatory pathways, resulting in inflammation and together can provoke cell death. In accordance, exposure to Cd was associated with antioxidant depletion and inflammation [19-21]. Fibrosis could also be a consequence of OS and inflammation [22]. ROS can activate transforming

growth factor-β1 (TGF-β1) that activates Smads and subsequently increase the production of extracellular matrix (ECM). TGF-β1 promotes renal fibrosis by provoking epithelial-mesenchymal transition (EMT) [23].

Nuclear factor erythroid 2–related factor 2 (Nrf-2), a cytosolic transcription factor negatively regulated by the Kelch-like ECH-associated protein-1 (Keap1), plays a key role in counteracting OS [24]. In response to electrophiles or increased ROS, Nrf2 dissociates from Keap1, enters the nucleus, and transactivates a variety of cytoprotective genes [24]. Nrf2 regulates the production of antioxidant and cytoprotective genes, therefore minimizing both inflammation and oxidative damage in the kidney [25]. Heme oxygenase-1 (HO-1) is a Nrf2-controlled enzyme that is widely recognized for its antioxidative characteristics, which help to regulate OS [24]. Hence, Nrf2/HO-1 signaling activation and suppression of OS and inflammation represent an effective strategy for the prevention or attenuation of Cd nephrotoxicity.

Previous studies have proven epidemiologically, clinically, and in laboratory that garlic possesses important biological and pharmacological properties against several diseases as it contains essential compounds such as diallyl disulfide (DADS) [26]. Given its rich content of organic sulfur compounds, garlic exhibits beneficial effects against different toxic agents and diseases associated with OS [26, 27]. DADS is a main constituent of garlic known for its cytoprotective effects against carcinogenesis and chemically induced cellular toxicity [28, 29]. Increasing number of studies have shown that DADS exhibits anticancer properties against different tumors including lung and breast cancers [29]. The anticancer properties of DADS involve activation of antioxidant enzymes, attenuation of ROS generation, and suppression of DNA damage [30]. In addition to these benefits, DADS attenuated inflammation in murine pancreatitis and lung injury [31], and lipopolysaccharide (LPS)-challenged microglia [32] and macrophages [33]. However, the mechanisms by which DADS confers protection against Cd-

induced OS, inflammation and nephrotoxicity are not elucidated. The present study was performed to evaluate the potential protective effects of DADS on Cd nephrotoxicity, pointing to its effect on OS, inflammation, and TGF-β1/Smad3 and Nrf2/HO-1 signaling.

## 2. Materials and Methods

### 2.1. Animals and treatments

Male Wistar rats (*Rattus norvegicus*) (190 ± 10 g) were included to investigate the effect of DADS against Cd nephrotoxicity. The animals were kept under standard temperature (22 ± 1°C) and humidity (50-60%) on a 12 h dark light cycle and given water and food *ad libitum*. Four experimental groups ($n$ = 6, randomly allocated) were assigned to this study. Cadmium chloride ($CdCl_2$; 1.2 mg/kg) [34] (Sigma, USA; Cat. no. 202908) was administered via intraperitoneal route to groups III and IV whereas groups I and II received 0.9% saline. Ten mg/kg DADS (Sigma, USA; Cat. no. SMB00378) was supplemented to groups II and IV via oral gavage [35]. DADS was supplemented for 14 days and $CdCl_2$ was injected on day 7. No animals were excluded from the study.

DADS and $CdCl_2$ were dissolved in 0.5% carboxymethyl cellulose (CMC) and 0.9% saline, respectively. Following treatments, blood was collected under ketamine/xylazine anesthesia, and the animals were then sacrificed. Samples from the liver were collected on 10% neutral buffered formalin (NBF) and others were kept at -80°C. Another set of samples was homogenized in cold Tris-HCl buffer (10 mM, pH = 7.4) and the supernatant was collected following centrifugation and stored at -80°C.

### 2.2. Biochemical assays

Serum levels of creatinine, urea and uric acid, and kidney GSH, catalase (CAT), MDA, and superoxide dismutase (SOD) were assayed using Bio-diagnostic (Egypt) kits (Cat. no.: CR1250; UR2110, UA2120, TA2511, CA2517, MD2528, and SD2521, respectively). Kidney

IL-1β, IL-6, and TNF-α were measured using ELabscience (China) ELISA kits (Cat. no.: E-EL-R0012, E-EL-R0015, and E-EL-R2856, respectively).

2.3. Histopathology and immunohistochemistry

Kidney samples were fixed in 10% NBF for 24 h and then dehydrated in ethanol series, cleared in xylene, and embedded in paraffin. Using a microtome, 5-µm sections were prepared and stained with hematoxylin and eosin (H&E) and Sirius red. Histopathological quantitative scoring was evaluated in kidney tissue for the number of degenerated renal corpuscles, number of degenerated renal tubules with cellular necrosis, number of inflammatory cells, amount of collagen fibers, number of congested blood vessels and area of interstitial hemorrhage per cross-sectional area using the image analysis system Leica QWin DW3000 (LEICA Imaging Systems Ltd., Cambridge, England). The most representative six fields were assessed for each section in all groups using 100x magnification via light microscopy transferred to the screen. Other sections were processed for staining with antibodies against PPARγ (Cat. no. YPA2204), inducible nitric oxide synthase (iNOS) (Cat. no. YPA1072) (Biospes, China), NF-κB p65, CD68, and cytoglobin (Santa Cruz Biotechnology, USA; Cat. no.: sc-8008, and sc-20060, respectively). The slides were dewaxed, rehydrated and then treated with 0.05 M citrate buffer (pH 6.8) and 0.3% hydrogen peroxide ($H_2O_2$). The sections were blocked and incubated overnight at 4°C with the primary antibodies, washed and then incubated with secondary antibodies (Biospes, China), followed by 3,3'-diaminobenzidine (DAB) in $H_2O_2$. Hematoxylin was employed for counterstaining, and ImageJ (NIH, USA) was used to measure color intensity (6/rat).

2.4. Western blotting

Frozen samples were homogenized in RIPA buffer with proteinase-phosphatase inhibitors and protein was assayed using Bradford reagent. Forty µg protein was electrophoresed on SDS-PAGE followed by transfer onto PVDF membranes (Millipore, Merck, Germany). 5% bovine

serum albumin (BSA) was added for blocking the membranes followed by antibodies for p-Smad3, Smad3, HO-1, Keap1 (Santa Cruz Biotechnology, USA; Cat. no.: sc-517575, sc-101154, sc-390991, and sc-514914, respectively), TGF-β1, Nrf2, and β-actin (Biospes, China; Cat. no.: YPA1196, YPA1865, and BPA1012, respectively). Following overnight incubation at 4 °C, secondary antibodies were added, and the bands were developed. The band intensity was determined using ImageJ (NIH, USA).

2.5. Statistical analysis

The results are expressed as mean ± standard deviation (SD). Comparisons between groups were performed using one-way ANOVA and Tukey's tests on GraphPad 8. A P value $<0.05$ was considered significant.

**3. Results**

3.1. DADS prevents kidney injury induced by Cd

Cd caused dysfunction and injury of the kidney as shown by the significant increase in serum creatinine (Fig. 1A), urea (Fig. 1B), and uric acid (Fig. 1C) in rats ($P<0.001$). DADS remarkably decreased the levels of these parameters in Cd-intoxicated rats ($P<0.001$). Examination of tissue section showed normal renal corpuscles and tubules in groups I and II (Fig. 1D). Cd provoked vacuolations of the glomeruli, dilation of interglomerular space, tubular degeneration with desquamation and pyknotic nuclei, noticeable increase in fibers along with infiltration of inflammatory cells, vascular congestion, and interstitial hemorrhage (Fig. 1D and Table 1). DADS significantly prevented tissue injury as revealed by nearly normal corpuscles with mild vacuolations, renal tubules with intact lining epithelium, obvious decline in fiber deposition and inflammatory cells (Fig. 1D and Table 1). However, slight interstitial edema was noticed (Fig. 1D).

3.2. DADS mitigates kidney OS and inflammation induced by Cd

Cd exposure increased MDA (Fig. 2A) and suppressed GSH, SOD, and CAT (Fig. 2B-D) in rat kidney ($P<0.001$). These effects were reversed by DADS where MDA was decreased, and antioxidants were enhanced ($P<0.001$). NF-κB p65, iNOS, CD68 and pro-inflammatory cytokines were assayed to determine the beneficial role of DADS on Cd-induced inflammation. Data represented in Figure 3A-C revealed upregulated NF-κB p65 and iNOS in Cd-challenged rats ($P<0.001$). Likewise, TNF-α (Fig. 4A), IL-1β (Fig. 4B), IL-6 (Fig. 4C), and CD68 immunostaining (Fig. 4D-E) were increased following Cd exposure. DADS effectively downregulated NF-κB p65, iNOS, cytokines, and CD68 in Cd-administered rats.

3.3. DADS suppresses TGF-β1/Smad3 and fibrosis in Cd-administered rats

TGF-β1 and Smad3 phosphorylation (Fig. 5A-C) were elevated and increased collagen disposition was observed in the kidney of Cd-intoxicated animals (Fig. 5D). Both control and DADS-treated animals exhibited normal collagen amounts (Fig. 5D). DADS suppressed TGF-β1 and Smad3 phosphorylation, an effect that was associated with attenuated collagen deposition.

3.4. DADS upregulates kidney and Nrf2/HO-1 signaling, cytoglobin and PPARγ in Cd-administered rats

Keap1 (Fig. 6A-B) was upregulated whereas Nrf2 and HO-1 (Fig. 6A, C, D) were declined in Cd-treated rat kidney ($P<0.001$) that also showed suppressed cytoglobin (Fig. 7A) and PPARγ (Fig. 7B). DADS upregulated kidney Nrf2, HO-1, PPARγ and cytoglobin and decreased Keap1 in Cd-intoxicated rats. Of note, DADS had no effect on normal rats.

## 4. Discussion

Exposure to chemical agents and pollutants that endanger human health represents a global concern. Cd is a HM with serious health consequences that affect different organs, including the kidneys [5]. Humans and animals exposure to Cd occurs via ingestion of contaminated water or food, smoking, inhalation of polluted air, and different industries such as oil extraction,

mining, and stone quarrying [36-38]. The role of OS in Cd nephrotoxicity has been reported to be central in the toxicity mechanism which is not fully understood [39]. Given its promising cytoprotective properties, this study investigated the effect of DADS on kidney OS, inflammation and fibrogenesis associated with Cd exposure in rats, pointing to the involvement of TGF-β1/Smad3 and Nrf2/HO-1 signaling and PPARγ.

In current study, administration of Cd resulted in remarkable increase in serum creatinine, urea, and uric acid, demonstrating kidney dysfunction and injury. Increased creatinine indicates disruption of glomerular filtration which can result in accumulation of various toxicants and xenobiotics in the kidneys [40]. Elevated blood levels of these biochemical parameters has been previously reported [41]. These data were supported by microscopic findings which revealed severe renal damage comprising vacuolated glomeruli, dilated interglomerular space, tubular degeneration and pyknosis, noticeable increase in fibers quantity along with infiltration of inflammatory cells, vascular congestion, and interstitial hemorrhage. The reported kidney injury is a direct result of Cd accumulation because approximately 50% of Cd found in the body accumulates in the PCTs resulting in dysregulated reabsorption and subsequently proteinuria [10, 11]. Cd enters the PCT cells following reabsorption via several transporters and channels, including organic cation transporter and voltage-dependent $Ca^{2+}$ channels [39]. In addition, Cd absorbed by the intestinal and pulmonary cells enters the circulation and induces metallothionein (MT) synthesis within hepatocytes and Cd-MT complexes generation [42]. The formed complexes reach the kidney and the release of Cd facilitates the formation of additional Cd-MT complexes and Cd accumulation within the PCTs [42]. In alignment with these findings, PCTs in Cd-administered animals in this study revealed tubular degeneration with desquamation. Moreover, Cd-mediated inhibition of the proteins responsible for elimination of cationic drugs and toxins contributes to Cd accumulation and kidney dysfunction and injury [43]. DADS prevented tissue damage and ameliorated circulating creatinine, urea

and uric acid. These findings pinpointed its protective efficacy against Cd nephrotoxicity and added support to previous studies revealing its nephroprotective effects. In this context, Sharma *et al* [44] reported the efficacy of DADS in preventing kidney tissue injury induced by glycerol in rats and Ko *et al* [45] reported similar findings in the kidney of rats challenged with acetaminophen (APAP). Moreover, in animal models of nephrotoxicity induced by cisplatin (CIS) [46], gentamicin (GM) [47], and trichloromethane ($CHCl_3$) [48], DADS showed protective effects against tissue injury. Our study provided new information that DADS can protect the kidney against damage induced by Cd.

There is a growing evidence on the role of OS and inflammation in mediating the toxic mechanism of Cd [11]. Therefore, it is noteworthy assuming that the observed nephroprotective efficacy of DADS in this study is a result of its antioxidant and anti-inflammatory efficacies. Exposure to Cd can increase ROS generation, but indirectly through Fenton-type reactions and other reactions provoked via free iron [49]. Mitochondrial dysfunction and enhanced ROS generation are also effects of Cd [11]. Cd-mediated ROS generation includes $H_2O_2$, and hydroxyl and superoxide radicals. Superoxide can react with NO to produce peroxynitrite that increases ROS and damage DNA [50]. ROS can damage cellular lipids via peroxidation, and oxidative damage of proteins and DNA. LPO is one of the main indicators of tissue damage and OS and has been clearly implicated in Cd toxicity. We showed that Cd intoxication significantly increased LPO indicated by elevated MDA levels and decreased GSH, SOD, and CAT. These findings align with our previous research as well as other literature on Cd-induced OS in different tissues [19, 41, 51, 52]. The diminished kidney antioxidants is attributed to Cd binding to the sulfhydryl groups on GSH and other proteins [53], disruption of the catalytic function of SOD [54] and interaction of Cd with the catalytic center of CAT [55]. Another consequence of ROS in the promotion of inflammatory responses via activation of NF-κB and expression of pro-inflammatory mediators.

Cytokines and other inflammatory mediators are essential tissue markers of inflammatory responses inflicted by Cd and other environmental toxicant [56, 57]. Here, administration of Cd significantly upregulated kidney NF-κB p65, IL-6, TNF-α, IL-1β, and iNOS, demonstrating an inflammatory response. Upon its ROS- and tissue damage-mediated activation, NF-κB promotes inflammation in different tissues by controlling large number of important pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and iNOS [58]. TNF-α, IL-6, and IL-1β promote inflammatory responses and in combination with ROS inhibit mitochondrial function and initiate cellular apoptosis [59]. Our findings are in consistent with previous studies on Cd-induced inflammatory responses in murine hepatocytes [56], human hepatoma cell line HepG2 [60], and rat kidney [41, 61]. Additionally, the kidney of Cd-administered rats showed high levels of CD68, the most reliable marker for macrophages [62] and this aligned with the infiltration of inflammatory cells observed in the microscopic examination. Within the inflammatory environment, cytokines could promote the polarization of macrophage towards a profibrotic phenotype [63]. This polarization contributes to the secretion of fibrogenesis mediators and lead to renal fibrosis [63]. The association between CD68 and TGF-β1 as diagnostic markers for renal disease has been demonstrated [64]. Accordingly, the current study revealed significant upregulation of TGF-β1 and Smad3 phosphorylation associated with collagen deposition and interstitial fibrosis. TGF-β1 is a pleiotropic cytokine that promotes renal fibrosis by provoking EMT, activating Smads and increasing the production of ECM [23]. The binding of TGF-β1 to its receptor promotes the phosphorylation of Smad2 and Smad3 and form a complex with Smad4. Upon nuclear translocation, this complex promotes the transcription of several genes involved in fibrogenesis [65]. TGF-β1 could be activated by ROS and Smads act as signal integrators that interact with NF-κB signaling [65]. Therefore, OS, inflammation and fibrogenesis are interconnected pathological processes. While chronic Cd

exposure for 24 weeks is associated with renal fibrosis in mice [66], our study provided evidence that fibrosis in the kidney could be a consequence of acute exposure.

DADS prevented OS, inflammatory mediators and fibrosis in the kidney of animals subjected to Cd. DADS suppressed LPO, NF-κB, iNOS, inflammatory cytokines, leukocyte infiltration and TGF-β1/Smad3 signaling, and enhanced antioxidants. These findings demonstrated the protective efficacy of DADS against Cd nephrotoxicity and added support to previous studies revealing its beneficial effect against kidney injury induced by different chemicals and drugs. For instance, DADS ameliorated LPO and enhanced GSH in the kidney of rats challenged with glycerol [44]. In APAP-induced rats, DADS decreased kidney MDA, suppressed NF-κB and TNF-α, and enhanced GSH, SOD and CAT [45]. In a rat model of CIS nephrotoxicity [46], high doses of DADS decreased MDA and pro-inflammatory and boosted antioxidant enzymes. In addition, DADS attenuated kidney OS and inflammation associated with GM [47] and $CHCl_3$ [48] in experimental animals. These investigations along with the findings of this study demonstrated the involvement of OS and inflammation suppression in the nephroprotective mechanism of DADS. Owing to the role of OS and inflammation in provoking fibrosis via activation of TGF-β1/Smad3 signaling, the anti-fibrosis efficacy of DADS reported in this study is directly connected to its dual antioxidant and anti-inflammatory effect.

To further explore the underlying mechanism(s), the effects of Cd and/or DADS on Nrf2/HO-1 signaling, cytoglobin and PPARγ were investigated. Kidney injury induced by Cd was associated with upregulated Keap1 and suppression of Nrf2, HO-1, cytoglobin, and PPARγ. Interestingly, DADS showed a preventive effect on the negative impact of Cd on Nrf2/HO-1 signaling, cytoglobin and PPARγ in rat kidney. DADS downregulated kidney Keap1 and increased Nrf2 and HO-1 in Cd-administered rats. Nrf2 activation elicits the expression of cytodefensive genes, including HO-1, SOD and CAT to counteract elevated ROS levels and OS [24]. This explained, at least in part, the suppressed OS and enhanced antioxidant enzymes

following treatment with DADS. Upregulation of Nrf2 can suppress inflammation by binding to the proximity of IL-6 and IL-1β genes and preventing their transcription. Additionally, upregulation of antioxidant defenses eliminates ROS and contributes to the anti-inflammatory consequence of Nrf2 activation [67]. Upregulation of Nrf2 participated in the anti-fibrosis efficacy of DADS. Accordingly, Che et al [66] demonstrated that mice lacking Nrf2 were more prone to develop kidney fibrosis following chronic exposure to Cd. This enhanced susceptibility was attributed to suppressed antioxidant and detoxification capacities [66]. The antioxidant efficacy of DADS was associated with increased cytoglobin in rat kidney. The cytodefensive function of cytoglobin is mediated via its ROS-scavenging ability and maintaining redox balance [68]. The suppression of cytoglobin leads to cell damage mediated via oxidative DNA damage, and cells and organs lacking cytoglobin are more prone to radiation-induced fibrogenesis and inflammation [68]. In contrast, upregulation of cytoglobin suppressed ROS and cell death [69], and ability to suppress superoxide and peroxynitrite generation was reported by Zweier et al [70]. Besides Nrf2/HO-1 signaling and cytoglobin, the antioxidant, anti-inflammatory and anti-fibrosis efficacies of DADS could be connected to PPARγ upregulation. PPARγ belongs to the nuclear receptor superfamily that prevents inflammation, OS and fibrosis upon activation [71]. PPARγ activation attenuates inflammation by suppressing NF-κB and upregulates antioxidant enzymes. It inhibits IκBα degradation and p65 nuclear translocation, leading to suppression of NF-κB transcriptional activity [72, 73]. Furthermore, PPARγ activation mitigated fibrosis in different organs by suppressing TGF-β/Smad signaling [74]. Previous studies have shown the positive effect of DADS on PPARγ in a mouse model of acute pancreatitis [75]. The role of PPARγ in DADS-mediated protection against lung cancer [76] and the upregulation of PPARγ gene in mice with hepatic steatosis following treatment with DADS [77] have been reported. The role of PPARγ in the nephroprotective mechanism of DADS was supported by the study of Sharma et al [44] where

pretreatment of the rats with PPARγ antagonist abolished DADS renoprotection. Our findings supported the beneficial effect of DADS on PPARγ and pinpointed its involvement in the conferred protection against Cd nephrotoxicity. However, the lack of data showing the use PPARγ and Nrf2 agonists/antagonists and different doses of DADS could be considered as limitations of this study.

## 5. Conclusion

These findings introduced new information that DADS prevented Cd nephrotoxicity and the possible underlying mechanism. Cd caused kidney dysfunction, injury, inflammation, OS, and fibrosis. This nephrotoxic effect was accompanied with upregulated TGF-β1/Smad3 signaling, and suppressed Nrf2/HO-1 pathway, PPARγ and cytoglobin. DADS mitigated kidney damage by preventing OS, inflammation, and TGF-β1/Smad3 signaling, and enhancement Nrf2/HO-1 pathway, PPARγ and antioxidants. Therefore, DADS can afford protection against Cd toxicity by modulating redox homeostasis and inflammatory response. DADS could be valuable to protect the kidney of individuals at risk of Cd exposure and toxicity. However, further investigations to elucidate other underlying mechanism(s) and clinical trials are needed.

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Declaration of Competing Interest

All authors declare no conflict of interests in relation to the manuscript.

Ethics declarations:

All animal experiments comply with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8523, revised 1996). The study was approved by the ethics committee of Al-Azhar University (AZ-AS/PH-REC/42/2024).

Availability of data and materials

The manuscript contains all data supporting the reported results.

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**Tables:**

Table 1. Histopathological lesions scoring.

| | Control | DADS | Cd | DADS + Cd |
|---|---|---|---|---|
| Number of degenerated renal corpuscles | 0.50 ± 0.34 | 0.33 ± 0.21 | 11.50 ± 1.87*** | 3.16 ± 1.17**### |
| Number of inflammatory cells | 5.50 ± 1.88 | 7.00 ± 141 | 104.5 ± 8.57*** | 47.50 ± 6.44***### |
| Number of degenerated renal tubules with cellular necrosis | 2.50 ± 1.04 | 3.16 ± 1.74 | 56.67 ± 4.46*** | 15.00 ± 4.69***### |
| Number of congested blood vessels | 0.83 ± 0.71 | 1.00 ± 0.63 | 9.84 ± 2.48*** | 2.00 ± 1.41### |
| Amount of collagen fibers (area %) | 1.62 ± 0.68 | 1.35 ± 0.54 | 13.32 ± 2.19*** | 2.57 ± 0.65### |
| Area of interstitial hemorrhage (%) | 1.87 ± 0.91 | 1.77 ± 0.80 | 20.87 ± 2.80*** | 7.27 ± 1.75***### |

Data are mean ± SD. **P<0.01 and ***P<0.001 versus Control. ###P<0.001 versus Cd.

**Figures:**

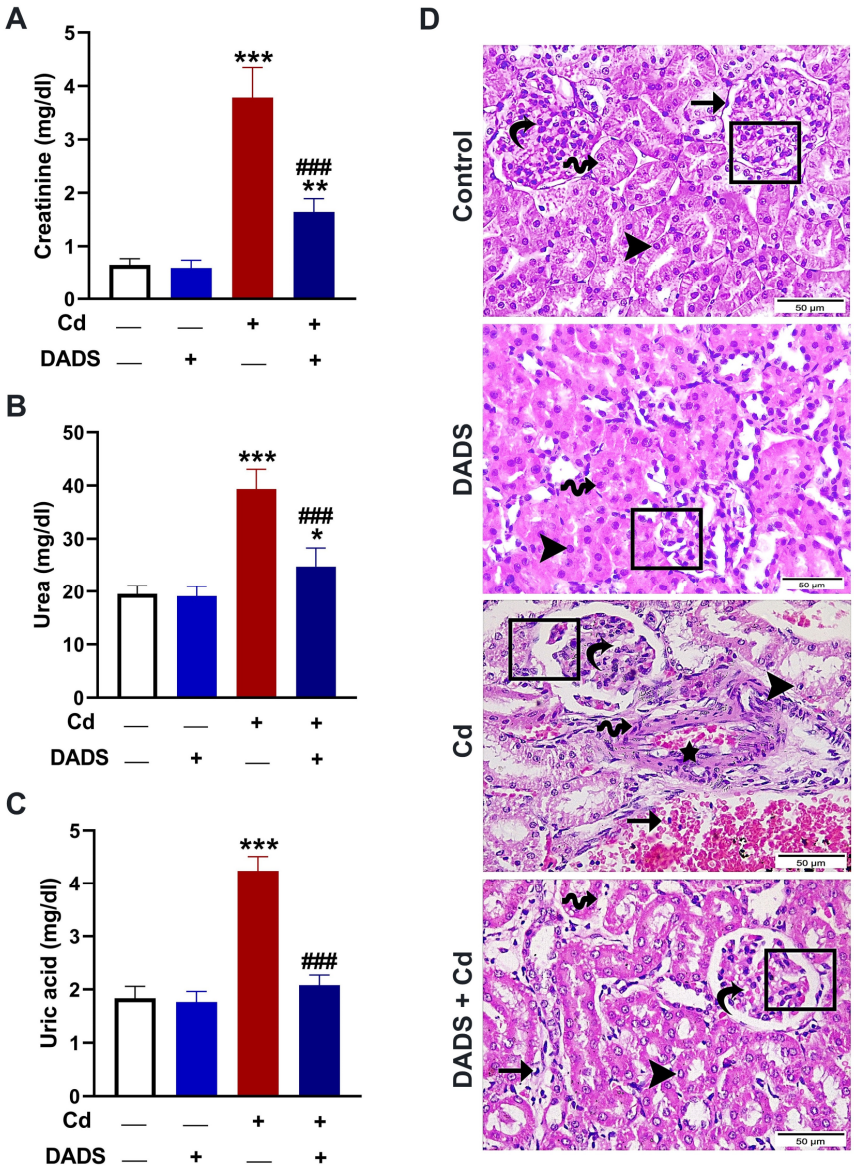


Figure 1. DADS attenuated Cd-induced kidney injury. DADS ameliorated serum creatinine (A), urea (B), and uric acid (C) in Cd-administered rats. Data are mean ± SD, ($n$ = 6). *P<0.05, **P<0.01, and ***P<0.001 versus Control. ###P<0.001 versus Cd. (D) Photomicrographs of H&E-stained kidney sections showing the protective effect of DADS against histopathological alterations induced by Cd. Control and DADS-treated rats showing normal renal corpuscles (rectangle), Bowman's capsule (arrow), glomeruli (curve arrow), and proximal (wave arrow) as well as distal (arrowhead) convoluted tubules; Cd-administered group showing vacuolated glomerulus (curvy arrow), dilated interglomerular space (rectangle), tubular degeneration with desquamation of epithelial lining and pyknotic nuclei of lining epithelium (arrowhead), fiber deposition, infiltration of inflammatory cells (wave arrow), vascular congestion (star) and interstitial hemorrhage (arrow); and Cd-administered rats treated with DADS showing normal renal corpuscle (rectangle) with mild vacuolations (circle), renal tubules with intact lining epithelium (arrowhead), decrease in fibers amount and inflammatory cells (arrow), and slight interstitial edema (wave arrow). (x400, Scale bar= 50 µm).

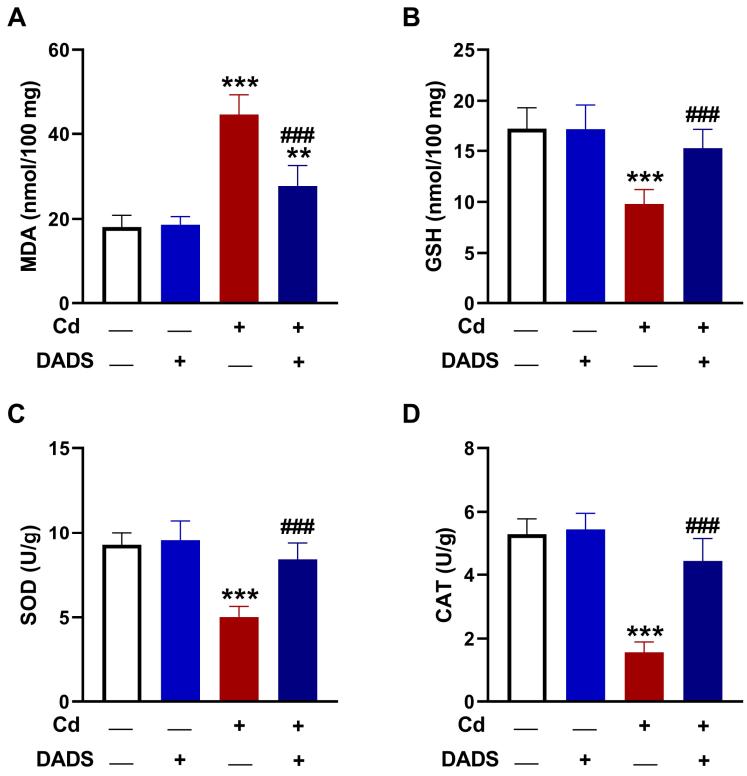


Figure 2. DADS suppressed kidney MDA (A), and increased GSH (B), SOD (C), and CAT (D) in Cd-administered rats. Data are mean ± SD, (*n* = 6). **P<0.01, and ***P<0.001 versus Control. ###P<0.001 versus Cd.

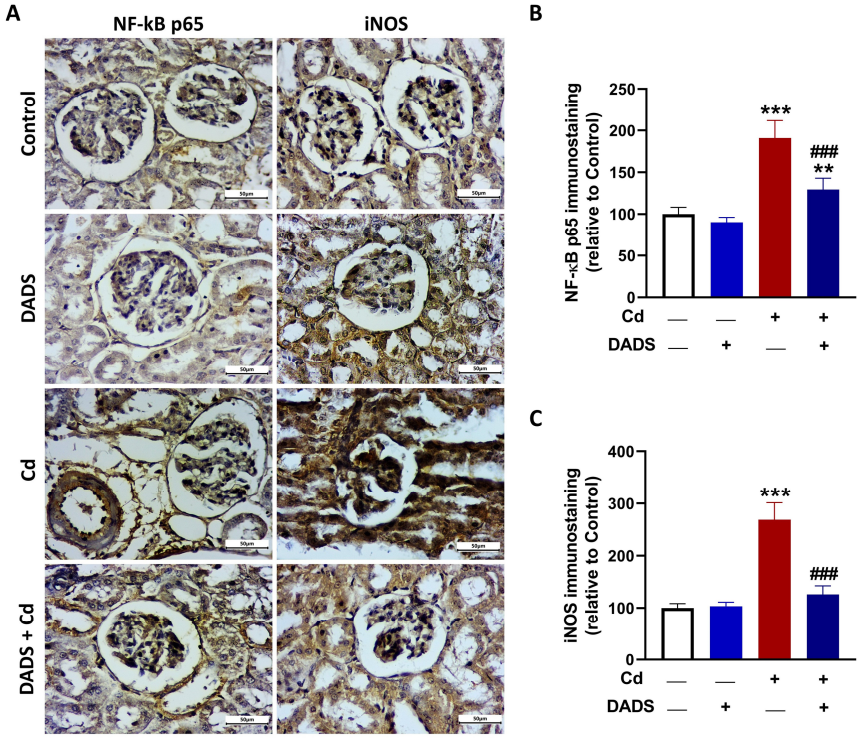


Figure 3. DADS downregulated NF-κB p65 (A,B) and iNOS (A,C) in kidney of Cd-administered rats. Data are mean ± SD, (*n* = 6). **P<0.01 and ***P<0.001 versus Control. ###P<0.001 versus Cd.

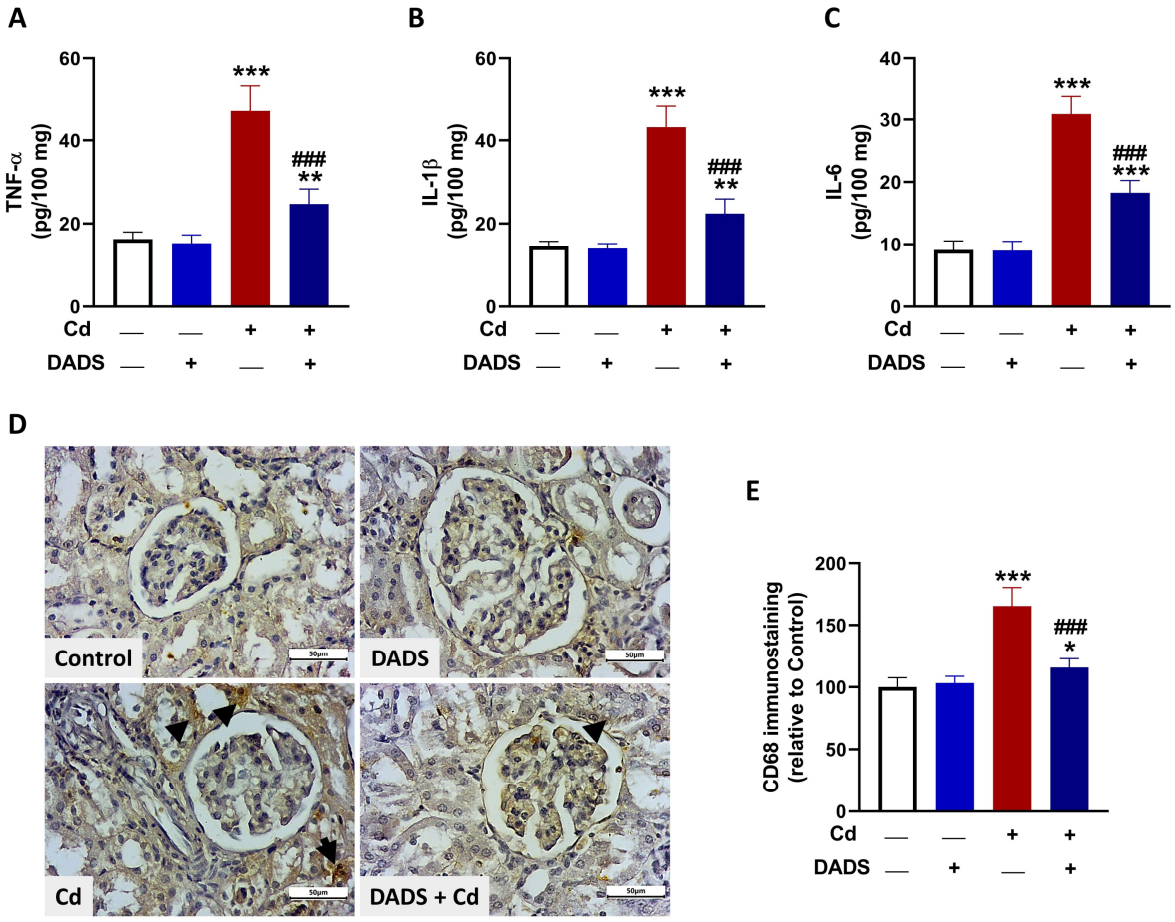


Figure 4. DADS decreased kidney TNF-α (A), IL-1β (B), IL-6 (C), and CD68 (D-E) in Cd-administered rats. Data are mean ± SD, ($n$ = 6). *P<0.05 and ***P<0.001 versus Control. ###P<0.001 versus Cd.

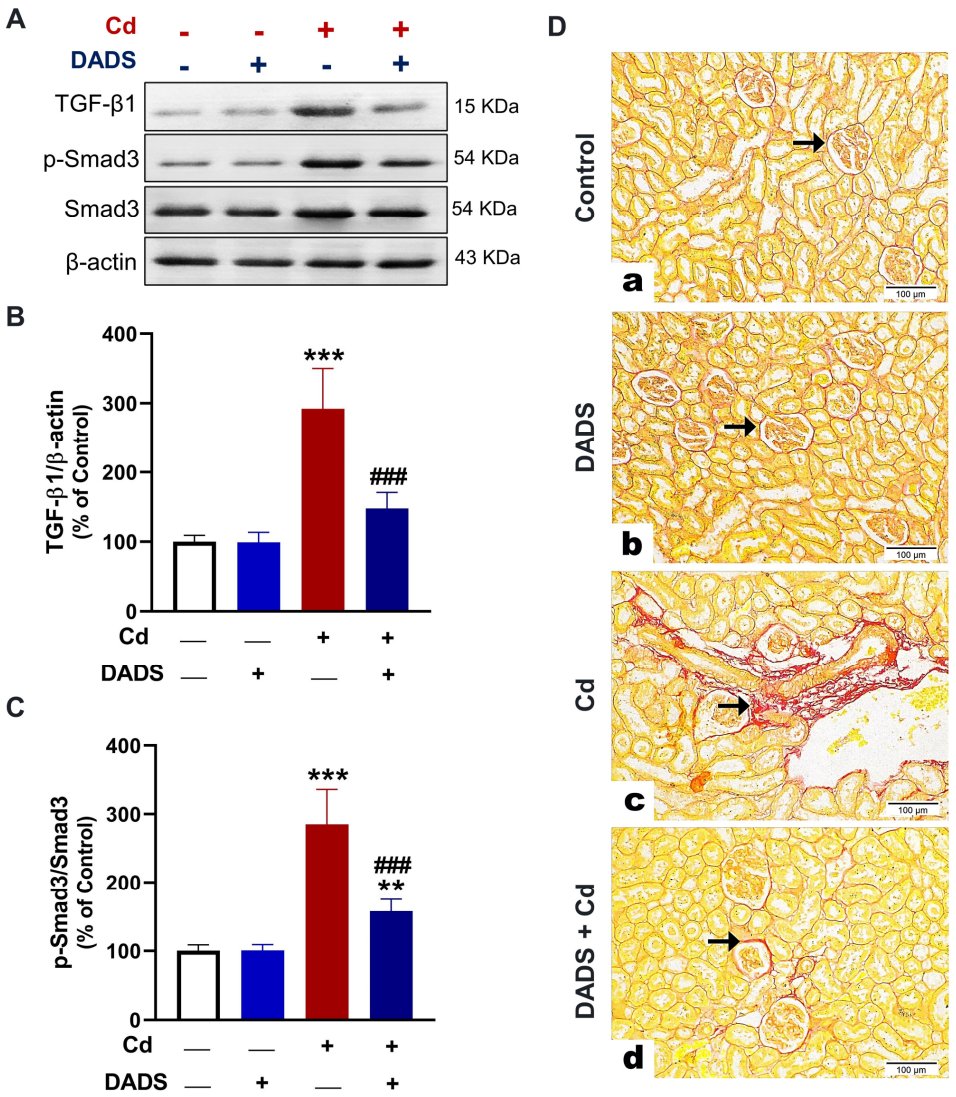


Figure 5. DADS suppressed TGF-β1/Smad3 and fibrosis in Cd-induced rats. (A-C) DADS downregulated kidney TGF-β1 and Smad3 phosphorylation in Cd-administered rats. Data are mean ± SD, ($n$ = 6). **P<0.01 and ***P<0.001 versus Control. ###P<0.001 versus Cd. (D) Sirius red-stained kidney sections from control and DADS-treated rats showing little collagen fibers between renal tubules and encircling renal corpuscle (arrows); Cd-challenged group showing high amount of collagen (arrow) and interstitial fibrosis; and Cd-administered rats treated with DADS showing noticeable decline in collagen fibers (arrow). (x200, Scale bar= 100 µm).

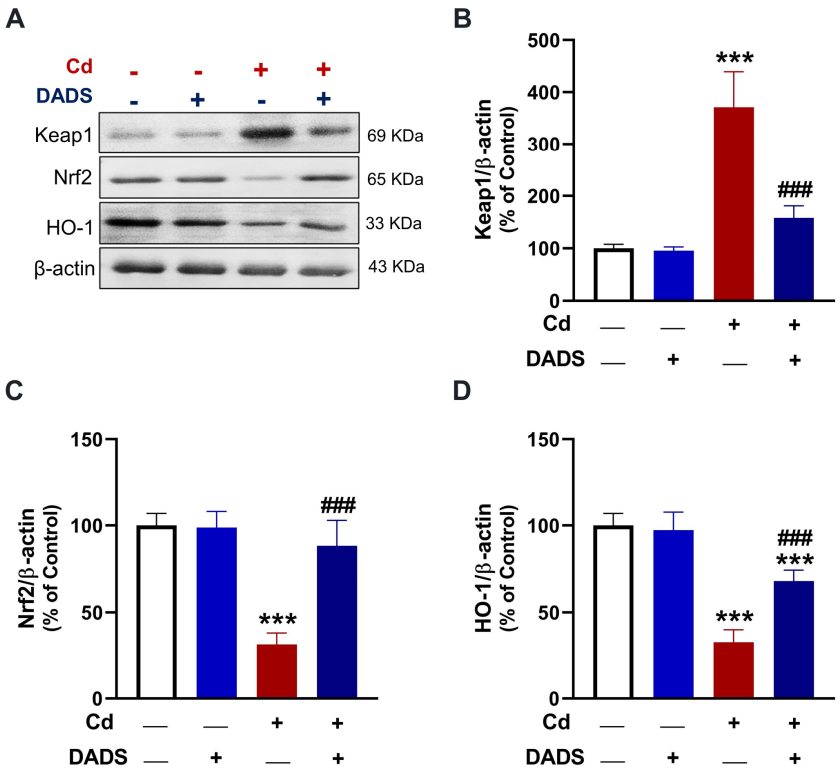


Figure 6. DADS decreased kidney Keap1 (A,B), and upregulated Nrf2 and HO-1 (A, C-D) in Cd-administered rats. Data are mean ± SD, (*n* = 6). ***P<0.001 versus Control. ###P<0.001 versus Cd.

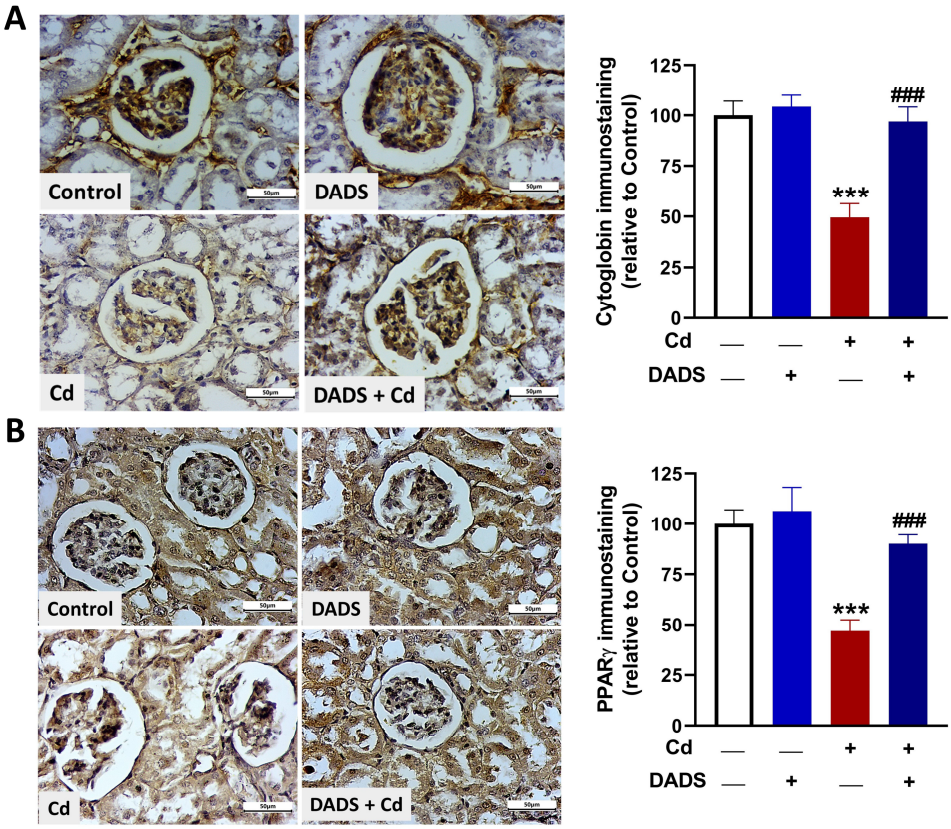


Figure 7. DADS increased kidney cytoglobin (A) and PPARγ (B) in Cd-administered rats. Data are mean ± SD, (*n* = 6). ***P<0.001 versus Control. ###P<0.001 versus Cd.