Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2024**; 29(10): 369
https://doi.org/10.31083/j.fbl2910369

Original Research

Diallyl Disulfide Mitigates Cadmium Hepatotoxicity by Attenuating Oxidative Stress and TLR-4/NF-κB Signaling and Upregulating PPARγ

Reem S. Alruhaimi[1], Emad H.M. Hassanein[2], Mohammed F. Alotaibi[3], Mohammed A. Alzoghaibi[3], Omnia A.M. Abd El-Ghafar[4], Mostafa K. Mohammad[5], Sulaiman M. Alnasser[6], Ayman M. Mahmoud[7,8,*]

[1]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, 11671 Riyadh, Saudi Arabia
[2]Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University-Assiut Branch, 71524 Assiut, Egypt
[3]Physiology Department, College of Medicine, King Saud University, 11461 Riyadh, Saudi Arabia
[4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Nahda University, 62764 Beni-Suef, Egypt
[5]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Badr University in Assiut, 71523 Assiut, Egypt
[6]Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Qassim, Saudi Arabia
[7]Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, M1 5GD Manchester, UK
[8]Molecular Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, 62514 Beni-Suef, Egypt
*Correspondence: a.mahmoud@mmu.ac.uk; ayman.mahmoud@science.bsu.edu.eg (Ayman M. Mahmoud)

Academic Editor: Viviana di Giacomo
Submitted: 2 July 2024 Revised: 23 August 2024 Accepted: 28 August 2024 Published: 23 October 2024

Abstract

Background: Heavy metals can cause serious health problems that affect different organs. Cadmium (Cd) is an environmental contaminant known for its toxicological consequences on different organs. Hepatotoxicity is a serious effect of exposure to Cd with oxidative stress (OS) and inflammation playing a central role. Diallyl disulfide (DADS), an organo-sulfur compound found in garlic, is known for its cytoprotective and antioxidant effects. In this study, the effect of DADS on Cd-induced inflammation, oxidative stress and liver injury was investigated. **Methods**: DADS was supplemented for 14 days via oral gavage, and a single intraperitoneal dose of Cd (1.2 mg/kg body weight) was administered to rats on day 7. Blood and liver samples were collected at the end of the experiment for analyses. **Results**: Cd administration resulted in remarkable hepatic dysfunction, degenerative changes, necrosis, infiltration of inflammatory cells, collagen deposition and other histopathological alterations. Cd increased liver malondialdehyde (MDA) and nitric oxide (NO) ($p < 0.001$), upregulated toll-like receptor (TLR)-4, nuclear factor-kappaB (NF-κB), pro-inflammatory mediators, and caspase-3 ($p < 0.001$) whereas decreased glutathione (GSH) and antioxidant enzymes ($p < 0.001$). Cd downregulated peroxisome proliferator activated receptor gamma (PPARγ), a transcription factor involved in inflammation and OS suppression ($p < 0.001$). DADS ameliorated liver injury and tissue alterations, attenuated OS and apoptosis, suppressed TLR-4/NF-κB signaling, and enhanced antioxidants. In addition, DADS upregulated PPARγ in the liver of Cd-administered rats. **Conclusions**: DADS is effective against Cd-induced hepatotoxicity and its beneficial effects are linked to suppression of inflammation, OS and apoptosis and upregulation of PPARγ. DADS could be valuable to protect the liver in individuals at risk of Cd exposure, pending further studies to elucidate other underlying mechanism(s).

Keywords: heavy metals; garlic; diallyl disulfide; hepatotoxicity; oxidative stress; inflammation

1. Introduction

Exposure of humans to heavy metals (HMs) can cause serious health problems that affect the liver, kidney, nervous system, heart, and other main organs. Given the non-biodegradable nature of HMs, they accumulate within the body and disrupt normal function of the cells, leading to serious disorders that deteriorate over time [1,2]. Cadmium (Cd) is one of the HMs that can pose serious health issues if reached the body in levels exceeding the permissible limits. It is a non-essential element known as an environmental pollutant that can reach the human body via multiple sources. Food, water, cigarette smoke and industrial activities such as mining, plastics, petroleum, stone quarrying, and batteries are sources of Cd [3–7]. Exposure to Cd is on increasing trajectory in developing countries and this is associated with adverse effects on animals and human health [8]. It has been estimated that 60% of the absorbed Cd deposited in the liver and kidney and approximately 0.007–0.009% is excreted in feces and urine [9]. Cd has no specific channels and enters the cells via calcium (Ca) and zinc (Zn) channels where it accumulates and binds to proteins including metallothionein (MT), ultimately leading to cell death [10,11]. Exposure to Cd for either short or long periods of time results in its accumulation in the liver which acts as the main site of HMs deposition. Studies on humans and animals revealed Cd accumulation in the liver, kidney, and many other organs [12–14], demonstrating its serious health consequences.

Hepatotoxicity represents one of the hazardous consequences of exposure to Cd with oxidative stress (OS) play-

ing a central role in the mechanism of toxicity [1]. In humans exposed to Cd, a strong positive correlation between soil Cd concentrations and fatty liver disease [15] and other metabolic alterations such as type 2 diabetes [16] has been reported. High blood Cd levels were associated with liver steatosis and fibrogenesis in both male and female human subjects [17], and liver cirrhotic/cancer patients exhibited increased levels of serum Cd [18]. Excess levels of reactive oxygen species (ROS) production and consequently OS provoked by Cd disrupt the cellular redox balance and activate inflammatory responses and cell death [19]. ROS can activate many signaling molecules such as toll-like receptor (TLR)-4 and subsequently nuclear factor-kappaB (NF-κB) and the release of multiple mediators of inflammatory response [20,21]. Along with ROS, the released mediators provoke mitochondrial dysfunction and cell death via apoptosis. In human hepatocytes, Cd causes apoptotic cell death mediated via mitochondrial damage [22]. Thus, attenuation of inflammation and OS can confer protection for hepatocytes against Cd-induced injury.

Plants are valuable sources of numerous components with beneficial pharmacological properties. Garlic is a functional food that has beneficial effects in preventing several disorders and toxicities [23]. The organic sulfur compounds are believed to mediate the beneficial biological and health-promoting activities of garlic [24]. Diallyl disulfide (DADS) is a major bioactive organosulfur compound of garlic. DADS showed promising pharmacological activities, including antioxidant, anti-inflammatory and protective efficacy against infections, cancer and other disorders affecting different organs [25]. The effect of DADS on inflammatory response in different disorders has been well-acknowledged. In murine pancreatitis and lung injury, DADS was effective in attenuating inflammation via suppressing NF-κB [26]. In microglia [27] and macrophages [28] challenged with lipopolysaccharide (LPS), DADS prevented the release of inflammatory mediators, demonstrating its potent anti-inflammatory activity. Moreover, DADS suppressed ROS generation in Barrett's epithelial cells challenged with deoxycholic acid [29] and mesenchymal stem cells treated with interleukin (IL)-1β [30]. By suppressing inflammation and OS, DADS conferred protection against liver steatosis induced by ethanol in mice [31]. In an *in vitro* study, pre-treatment with DADS protected rat hepatocytes against injury induced by Cd [32]. Despite the reported beneficial efficacies of DADS, its protective effect against inflammation and OS associated with Cd-induced liver injury hasn't been elucidated yet. Therefore, this study investigated the effect of DADS on liver injury, OS, inflammation, and fibrosis induced by Cd, pointing to the possible involvement of TLR-4/NF-κB signaling and the nuclear receptor peroxisome proliferator activated receptor gamma (PPARγ).

2. Materials and Methods

2.1 Animals and Treatments

Twenty-four male Wistar rats (180–200 g) were kept on a 12 h dark light cycle under standard temperature (22 $\pm$ 1 °C) and humidity (50–60%) with *ad libitum* standard food (62% carbohydrates, 19% protein, 6% fibers, 3.5% fats, 1% vitamin mix, 6.5% ash, and 2% minerals) and water. 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 experimental protocol was approved by the ethics committee at Al Azhar University (Assiut - Egypt) (AZ-AS/PH-REC/28/24). The rats were allocated into four groups (n = 6). $CdCl_2$ (1.2 mg/kg) [33] (Sigma, St. Louis, MO, USA) was administered via intraperitoneal route to groups III and IV whereas groups I and II received 0.9% saline. Ten mg/kg DADS (Sigma, St. Louis, MO, USA) was supplemented to groups II and IV via oral gavage [34]. DADS was supplemented for 14 days and $CdCl_2$ (1.2 mg/kg dissolved in 5 mL) was injected on day 7. Following treatments, blood was collected under ketamine anesthesia and the animals were then sacrificed via cervical dislocation. 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 (10% *w*/*v*) in cold Tris-HCl buffer (10 mM, pH = 7.4) and the supernatant was collected following centrifugation at 8000 rpm for 10 min and stored at –80 °C.

2.2 Biochemical Assays

Serum transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and albumin were measured using Bio-diagnostic (Giza, Egypt) kits. To determine the content of liver malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide (NO), and activities of superoxide dismutase (SOD), and catalase (CAT), specific kits from Bio-diagnostic (Giza, Egypt) were used. Tumor necrosis factor (TNF)-α, IL-1β, and IL-6 were assayed using ELabscience (Wuhan, China) ELISA kits.

2.3 Histopathology and Immunohistochemical Investigations

Liver samples were fixed in a 10% NBF for 24 h and then dehydrated in ethanol and cleared in xylene. The samples were infiltrated in pure soft paraffin followed by embedding in paraffin and 5-µm sections were cut. The sections were stained with hematoxylin & eosin (H&E) [35], periodic acid-Schiff (PAS) [35], and Sirius red [36]. Another set of sections were dewaxed, rehydrated, and immersed in 0.05 M citrate buffer (pH 6.8) and then 0.3% hydrogen peroxide (H_2O_2) and protein block. The sections were probed with anti-inducible NO synthase (iNOS), anti-cleaved caspase-3, and anti-PPARγ (Biospes, Chongqing,

China) overnight at 4 °C, followed by the secondary antibody for 1 h at room temperature. 3,3′-diaminobenzidine (DAB) in H_2O_2 was employed for color development and hematoxylin was used for counterstaining [37]. The color intensity was measured (6/rat) using ImageJ 1.52 (NIH, Bethesda, MD, USA).

2.4 Western Blotting

The effect of Cd and/or DADS on TLR-4 and NF-κB was conducted as we previously reported [38]. Liver samples were homogenized in RIPA buffer supplemented with phosphatase/proteinase inhibitors, centrifuged at 10,000 rpm for 10 min and the supernatant was separated for protein assay using Bradford reagent. Forty µg protein was subjected to SDS-PAGE followed by transfer onto PVDF membranes and blocking with 5% bovine serum albumin (BSA; Sigma, St. Louis, MO, USA). The membranes were propped with anti-NF-κB p65, anti-pNF-κB p65, anti-TLR-4, and anti-β-actin (Biospes, Chongqing, China) overnight at 4 °C, followed by washing and incubation with the secondary antibody for 1 h at room temperature. After washing, the bands were developed, and the band intensity was determined using ImageJ 1.52 (NIH, Bethesda, MD, USA).

2.5 Statistical Analysis

The findings are shown as mean ± standard error of mean (SEM). Statistical analysis and multiple comparisons were accomplished using one-way analysis of variance (ANOVA) with subsequent Tukey's post-hoc analysis. The statistical analysis was conducted using GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA). A p value < 0.05 was considered significant.

3. Results

3.1 DADS Mitigates Cd-induced Liver Injury

The biochemical findings revealed significant elevation in circulating ALT, AST, ALP, and LDH (Fig. 1A–D) and declined albumin (Fig. 1E) in Cd-treated rats ($p < 0.001$). DADS effectively decreased the activities of the assayed enzymes and increased albumin in serum of rats that received Cd ($p < 0.001$). These data were supported with microscopic examinations using three different stains as depicted in Fig. 2. H&E, PAS and Sirius red staining showed normal hepatocytes, sinusoids, and collagen fibers in control (Fig. 2a) and DADS-supplemented rats (Fig. 2b). Cd induced severe alterations, including loss of hepatic cord regularity, hydropic degeneration with cytoplasmic vacuolation, congestion, necrosis, inflammatory cell infiltration, and high amount of collagen deposition (Fig. 2c). Treatment of Cd-challenged rats with DADS resulted in marked recovery represented by intact central vein, less congestion, regular hepatic cords, mostly normal hepatocytes, and noticeable decline in collagen fiber deposition (Fig. 2d).

3.2 DADS Suppresses Cd-induced Liver Oxidative Stress

Administration of Cd increased liver levels of MDA (Fig. 3A), and decreased GSH (Fig. 3B), SOD (Fig. 3C), and CAT (Fig. 3D) significantly ($p < 0.001$) as compared to the control rats. While showed no effect in normal rats, DADS decreased MDA and increased GSH, SOD, and CAT in Cd-challenged rats.

3.3 DADS Attenuates Cd-induced Liver Inflammation

The effect of DADS on Cd-induced inflammation was evaluated by assessing TLR-4/NF-κB signaling and inflammatory mediators (Figs. 4,5). Cd upregulated TLR-4 expression and NF-κB p65 phosphorylation in the liver of rats (Fig. 4A). Consequently, hepatic TNF-α (Fig. 4B), IL-1β (Fig. 4C), and IL-6 (Fig. 4D) showed significant increase in Cd-challenged rats ($p < 0.001$). IHC staining revealed significant upregulation of iNOS (Fig. 5A,B) and NO levels were also increased (Fig. 5C) in Cd-treated rats. DADS remarkably downregulated TLR-4, NF-κB p65, iNOS, and cytokines in Cd-administered rats.

3.4 DADS Mitigates Liver Apoptosis and Upregulates PPARγ in Cd-administered Rats

Changes in cleaved caspase-3 (Fig. 6) and PPARγ (Fig. 7) were determined in the liver of rats that received Cd and/or DADS. Cd-administered rats exhibited remarkable upregulation of cleaved caspase-3 and downregulation of PPARγ ($p < 0.001$). DADS effectively suppressed hepatic cleaved caspase-3 and upregulated PPARγ in Cd-administered rats.

4. Discussion

Hepatotoxicity and other liver disorders been reported as hazardous effects of exposure to Cd, particularly in developing and industrial countries [1,8]. Inflammation and OS are key elements in Cd hepatotoxicity and the development of strategies to mitigate or prevent these processes can protect against liver injury in vulnerable individuals. This study demonstrated the protective role of DADS against Cd-induced OS, inflammation, and liver injury in rats.

Liver injury following Cd administration was evidenced by altered biochemical parameters (ALT, AST, ALP, LDH and albumin) of hepatocyte injury and histopathological alterations. Elevation of circulating ALT, AST, LDH, and ALP has been acknowledged in animals exposed to Cd [39,40]. In adult humans, exposure to Cd resulted in elevated blood transaminases as reported by Kang *et al.* [41]. Exposure to Cd is associated with liver disorders, including steatohepatitis [15], and increased serum transaminases was closely associated with Cd levels [42]. Rats that received Cd showed declined serum albumin which along with the elevated transaminases denoted hepatocyte injury. The decrease in albumin was linked to decreased hepatic and renal Klotho-methylation as a result of Cd exposure [43]. Examination of tissue sections from Cd-

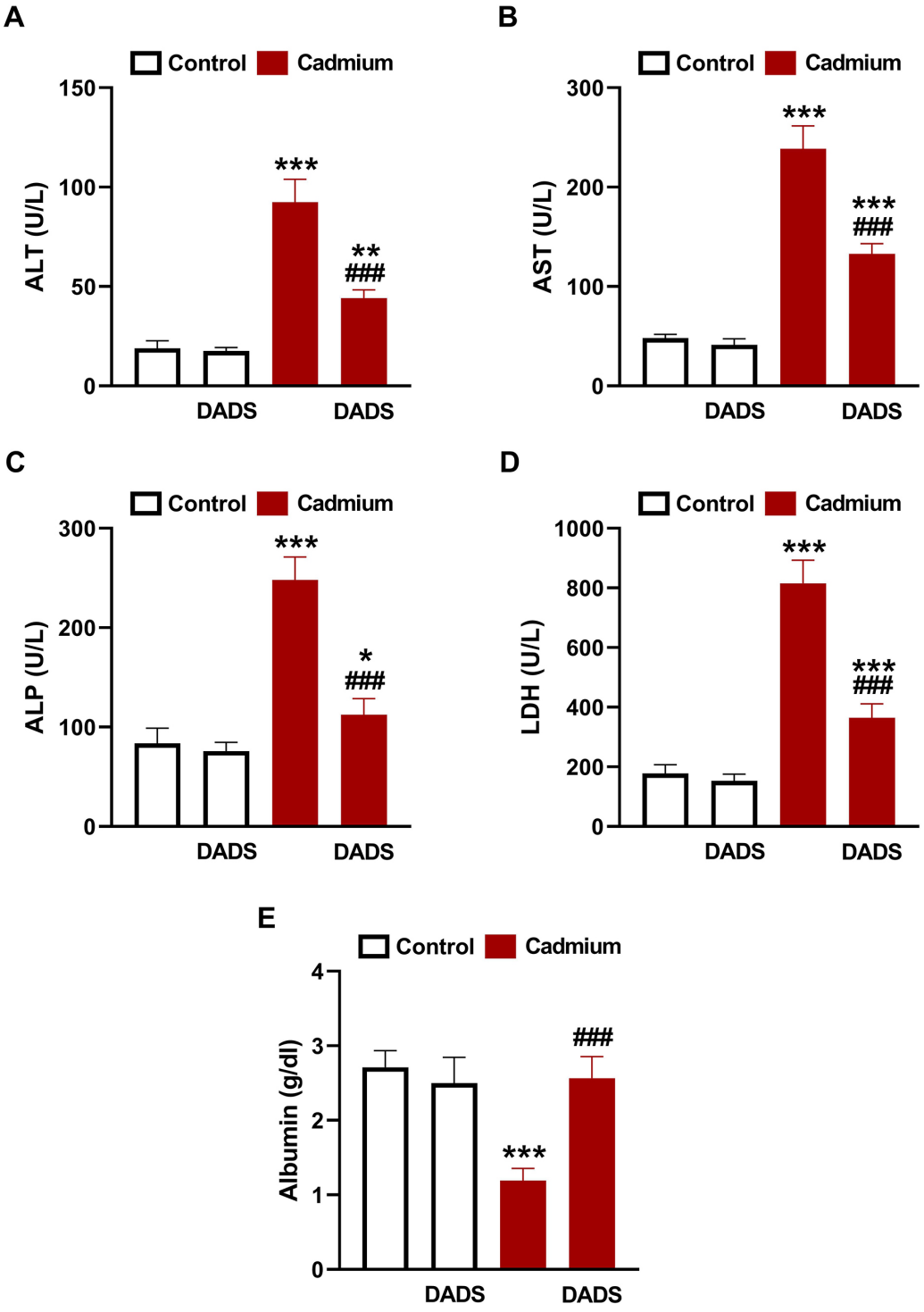

Fig. 1. Diallyl disulfide (DADS) prevented Cd-induced liver injury. DADS ameliorated serum alanine aminotransferase (ALT) (A), aspartate aminotransferase (AST) (B), alkaline phosphatase (ALP) (C), lactate dehydrogenase (LDH) (D), and albumin (E) in Cadmium (Cd)-administered rats. Data are mean ± SEM, ($n = 6$). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus Control. ###$p < 0.001$ versus Cd.

exposed rats revealed severe alterations, including necrosis, loss of hepatic cord regularity, congested central vein and sinusoids, severe hydropic degeneration, pyknosis, inflammatory and fat cells infiltration, and increased collagen deposition. Previous studies have shown hyperplasia, necrosis, inflammatory cells infiltration, and apoptosis associated with Cd hepatotoxicity [44,45]. A relationship between circulating Cd and liver steatosis and fibrogenesis in both male and female human subjects [17], and liver cirrhotic/cancer patients [18] was reported. This explained the

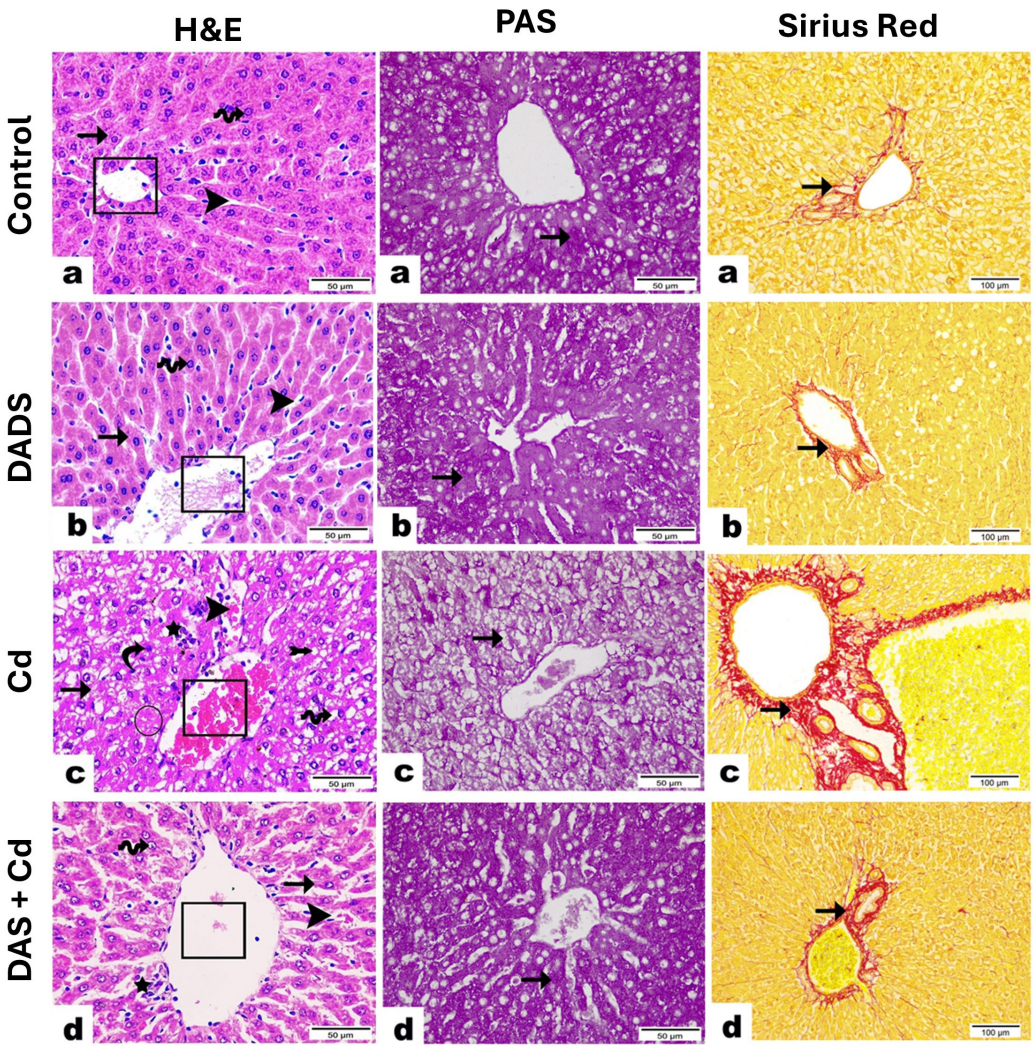

Fig. 2. Photomicrographs demonstrated the protective effect of Diallyl disulfide (DADS) on histopathological alterations induced by Cadmium (Cd) in rat liver. Hematoxylin & eosin (H&E): (a,b) Liver section from control (a) and DADS-treated (b) groups exhibiting the normal central vein (cube) and hepatocytes with central vesicular nucleus (wave arrow) arranged in regular cords (arrow) and separated by blood sinusoids (arrowhead). (c) Liver section from Cd-administered group revealing necrotic area (circle), loss of hepatic cord regularity (arrow), congestion of the central vein (cube) and blood sinusoids (arrowhead), severe hydropic degeneration with cytoplasmic vacuolation (wave arrow), deep basophilic pyknotic nuclei of hepatocytes (curvy arrow), inflammatory cell infiltration encircling central vein (star), and obvious appearance of fat cells in between hepatocytes (arrow with tail). (d) Liver section from Cd-administered rats treated with DADS showing a marked recovery represented by intact central vein (cube), less congested blood sinusoids (arrowhead), and regular hepatic cords (arrow). Most hepatocytes appear nearly normal with acidophilic cytoplasm and central vesicular nuclei (wave arrow). However, few inflammatory cells observed surrounding central vein (star). (×400, Scale bar = 50 µm). Periodic acid-Schiff (PAS): (a,b) Liver section from control (a) and DADS-treated (b) groups showing marked intense positive PAS staining of most hepatocytes (arrow) in area surrounding central vein. (c) Liver section from Cd-administered group emphasizing noticeable decline in intensity of PAS staining in addition to the number of hepatocytes (arrow) with positive PAS staining encircling central vein area. (d) Liver section from Cd-administered rats treated with DADS showing marked obvious increase in the strength of staining along with moderate number of hepatocytes (arrow) close to central vein with positive PAS staining than positive model group. (×400, Scale bar = 50 µm). Sirius red: (a,b) Liver section from control (a) and DADS-treated (b) groups showing the usual few amounts of collagen encircling portal area (arrow). (c) Liver section from Cd-administered group showing the highest quantities of collagen fibers accumulated around portal area (arrow). (d) Liver section from Cd-administered rats treated with DADS showing noticeable decline in collagen fibers (arrow). (×200, Scale bar = 100 µm).

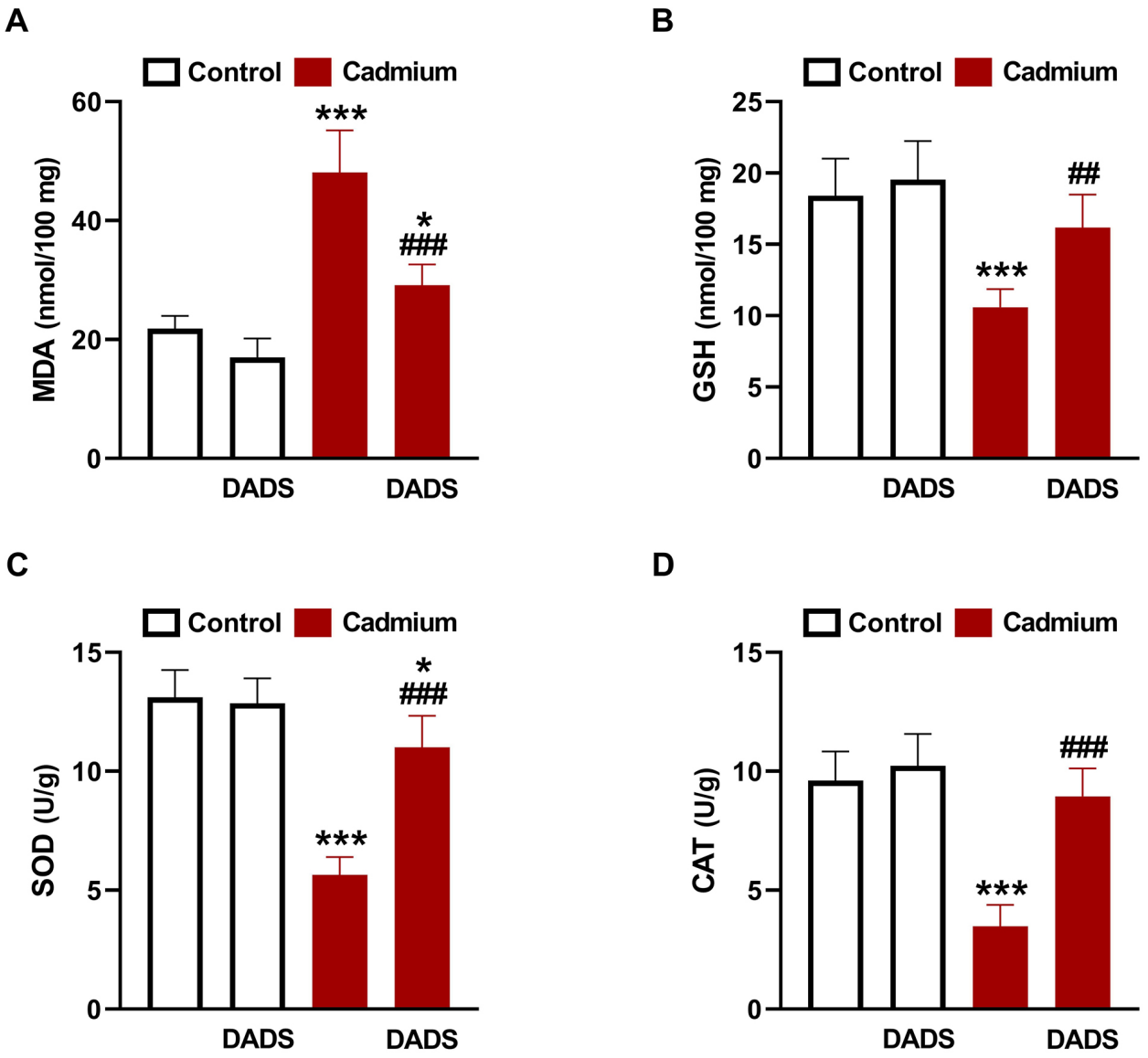

Fig. 3. Diallyl disulfide (DADS) suppressed Cadmium (Cd)-induced liver oxidative stress. DADS ameliorated liver malondialdehyde (MDA) (A), and increased glutathione (GSH) (B), superoxide dismutase (SOD) (C), and catalase (CAT) (D) in Cd-administered rats. Data are mean ± SEM, ($n = 6$). *$p < 0.05$, and ***$p < 0.001$ versus Control. ##$p < 0.01$ and ###$p < 0.001$ versus Cd.

increase in collagen deposition and fat cells in the liver of Cd-administered rats. In support of our findings, Li *et al.* [45] demonstrated a higher collagen peak in liver samples exposed to Cd by using Raman confocal imaging. DADS effectively mitigated liver injury in Cd-administered rats, supporting its previously reported hepatoprotective activity [31]. Treatment of ethanol-administered mice with DADS ameliorated circulating transaminases and prevented hepatic lipid deposition and tissue injury [31]. This protective efficacy of DADS was also established in rats challenged with carbon tetrachloride (CCl_4) [46] where it effectively prevented tissue injury and ameliorated transaminases. These studies along with our findings demonstrated the efficacy of DADS to confer protection against different hepatotoxic chemicals.

The mechanism of Cd hepatotoxicity involves significant contribution of OS and inflammation [1]. Given the reported antioxidant and anti-inflammatory properties of DADS [25,28,29], it is noteworthy assuming that these efficacies contributed to its protection against Cd hepatotoxicity. Here, rats exposed to Cd showed increased hepatic MDA, NO, NF-κB p65, iNOS, and pro-inflammatory cytokines, and decreased GSH and enzymatic antioxidants, demonstrating OS and inflammation. Ionic Cd can enter hepatocytes through voltage-gated Ca^{2+} channels or via binding to Fe^{2+} and Zn^{2+} transporters. Cd can also form complexes with MT which enter hepatocytes via receptor-mediated endocytosis and Cd is then released through the action of lysosomes [10,11]. Owing to its presence in the +2 oxidation state, Cd doesn't generate ROS via redox reac-

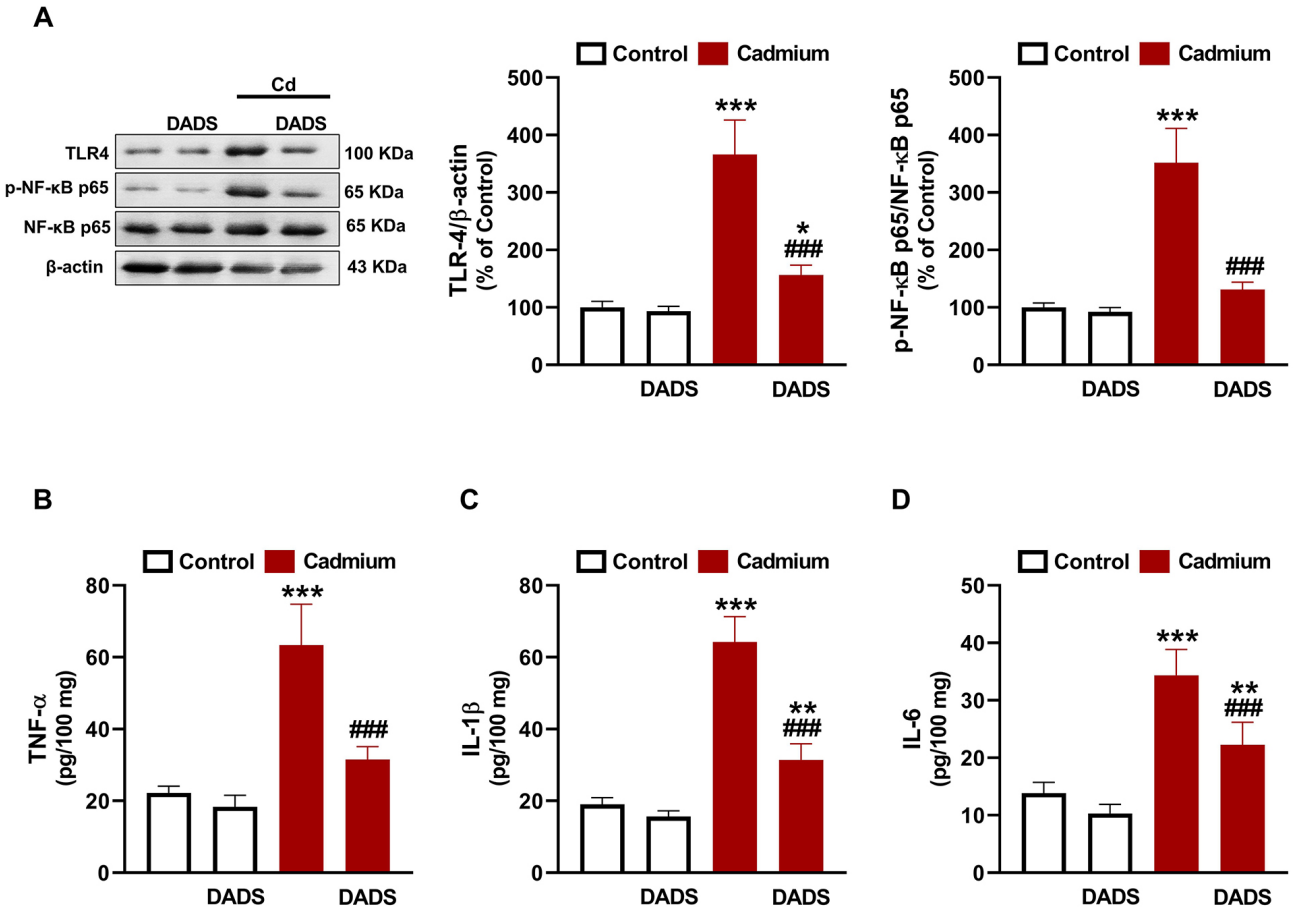

Fig. 4. Diallyl disulfide (DADS) attenuated Cadmium (Cd)-induced liver inflammation. DADS downregulated toll-like recptor (TLR)-4 and nuclear factor-kappaB (NF-κB) p65 (A) expression, and decreased Tumor necrosis factor (TNF)-α (B), interleukin (IL)-1β (C), and IL-6 (D) in Cadmium (Cd)-administered rats. Data are mean $\pm$ SEM, ($n = 6$). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus Control. ###$p < 0.001$ versus Cd.

tions but produces H_2O_2, NO, and superoxide and hydroxyl radicals indirectly via other reactions, including Fenton-type reactions mediated via Cd-induced liberation of unbound iron [47–49]. Excess ROS oxidize cellular macromolecules and produce peroxynitrite by interacting with NO, thereby increasing ROS further and oxidize DNA [50]. Within hepatocytes, Cd directly binds the sulfhydryl groups on GSH and other proteins [1], resulting in GSH depletion and thereby fostering ROS generation and OS. In addition, Cd can accumulate within the organelles, in particular the mitochondria [51] and the resultant dysfunction and injury can result in more ROS generation. Mitochondrial damage was reported to mediate Cd-induced apoptotic cell death in human hepatocytes [22]. Cd-triggered ROS provoke inflammatory reactions by activating TLR-4/NF-κB axis and subsequent production of pro-inflammatory mediators which in concert with ROS promote cell death [52]. ROS and inflammatory mediators elicit mitochondrial dysfunction and apoptotic cell death. Excess ROS disrupts mitochondrial membrane potential and increases its permeability with subsequent release of cytochrome c. Within the cytosol, cytochrome c combines with caspase-9 and Apaf-1 and the produced apoptosome activates caspase-3 which initiates apoptotic cascade [53]. In human hepatocytes, Cd caused apoptotic cell death mediated via mitochondrial damage [22]. Cd-administered rats in this study exhibited upregulation of hepatic caspase-3, demonstrating apoptotic cell death.

DADS effectively mitigated OS and prevented inflammation and cell death in the liver of Cd-administered rats. DADS attenuated lipid peroxidation (LPO), suppressed TLR-4 and NF-κB and the subsequent release of cytokines and caspase-3, and enhanced antioxidant defenses. Several *in vitro* and *in vivo* investigations demonstrated the antioxidant and anti-inflammatory activities of DADS [25]. The protective effect of DADS against ethanol hepatotoxicity was associated with its ability to attenuate OS and inflammation [31]. The effect of DADS on inflammatory response in different disorders has been well-acknowledged. It reduced colonic mucosal and submucosal edema in a murine model of colitis [54] and prevented acute inflammation and OS in mouse paw model [55]. In microglia [27] and

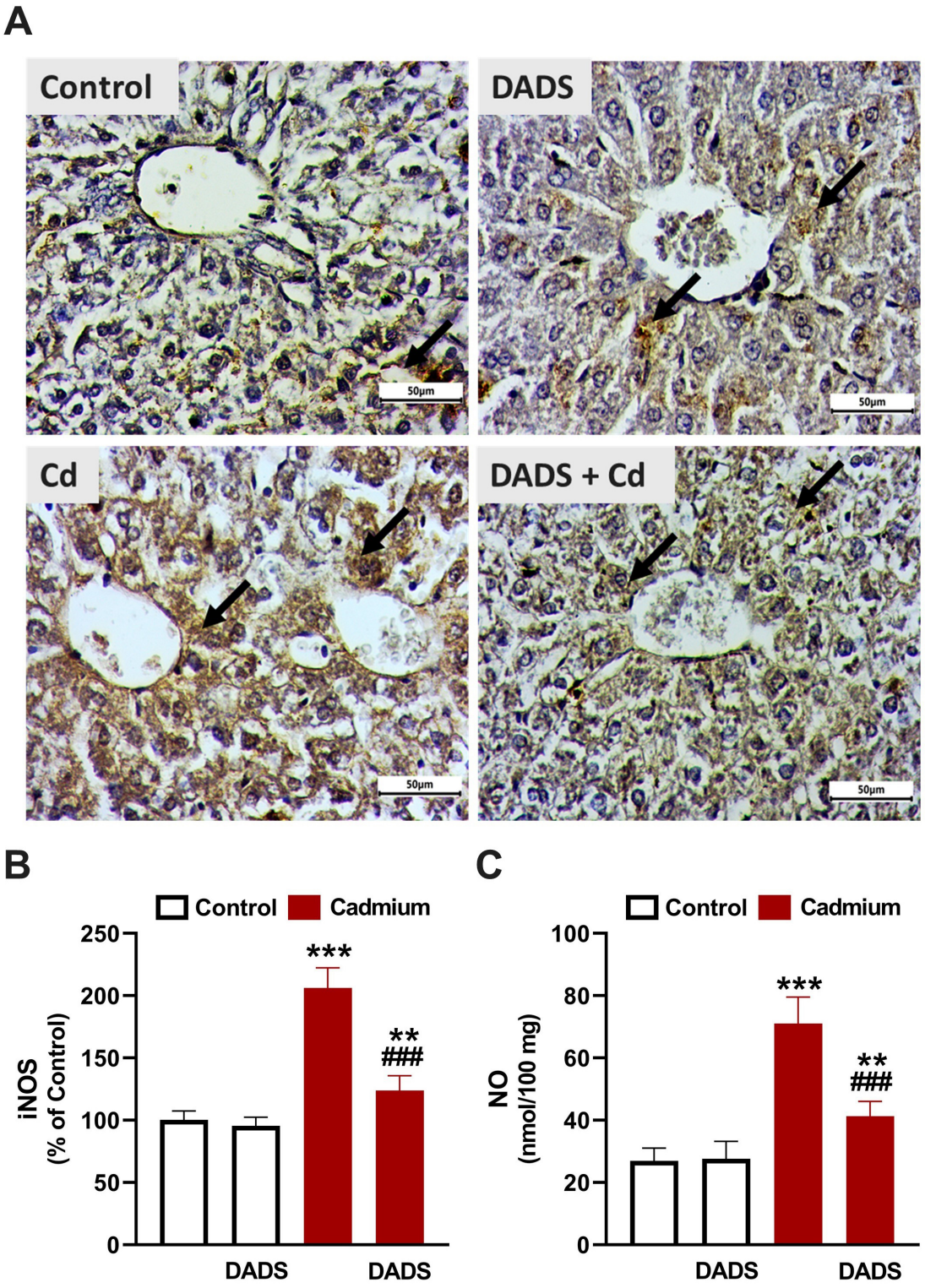

Fig. 5. Diallyl disulfide (DADS) downregulated inducible NO synthase (iNOS) in liver of Cadmium (Cd)-administered rats. (A) Photomicrographs showing upregulated iNOS in the liver of Cd-administered rats and the ameliorative effect of DADS. (×400, Scale bar = 50 µm). (B) Image analysis of iNOS immunostaining. (C) DADS decrease liver NO levels in Cd-administered rats. Data are mean ± SEM, ($n = 6$). **$p < 0.01$ and ***$p < 0.001$ versus Control. ###$p < 0.001$ versus Cd.

macrophages [28] challenged with LPS, DADS prevented the release of inflammatory mediators, demonstrating its potent anti-inflammatory activity. The anti-inflammatory role of DADS was linked to its efficacy to modulate circulating immune cells and the activation of NF-κB [34]. In mice with pancreatitis, DADS suppressed the transcrip-

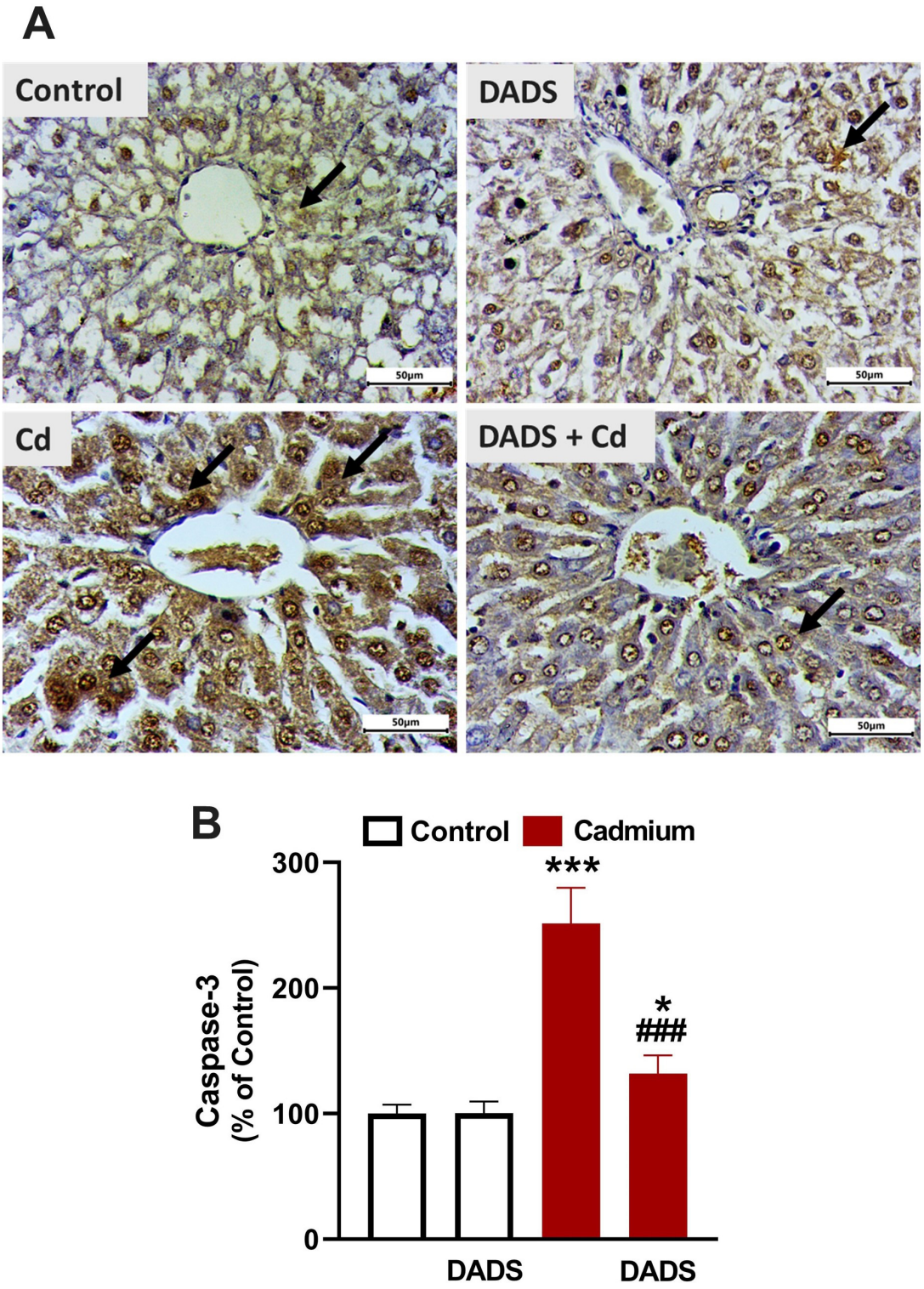

Fig. 6. DADS downregulated cleaved caspase-3 in liver of Cadmium (Cd)-administered rats. (A) Photomicrographs showing upregulated caspase-3 in the liver of Cd-administered rats and the ameliorative effect of DADS. (×400, Scale bar = 50 μm). (B) Image analysis of caspase-3 immunostaining. Data are mean ± SEM, (n = 6). *$p < 0.05$ and ***$p < 0.001$ versus Control. ###$p < 0.001$ versus Cd.

tional activity of NF-κB [26], effects that were demonstrated in a rat model of hepatotoxicity by Lee *et al.* [46]. Moreover, DADS suppressed ROS generation in Barrett's epithelial cells challenged with deoxycholic acid [29] and mesenchymal stem cells treated with IL-1β [30]. By suppressing inflammation and OS, DADS conferred protection

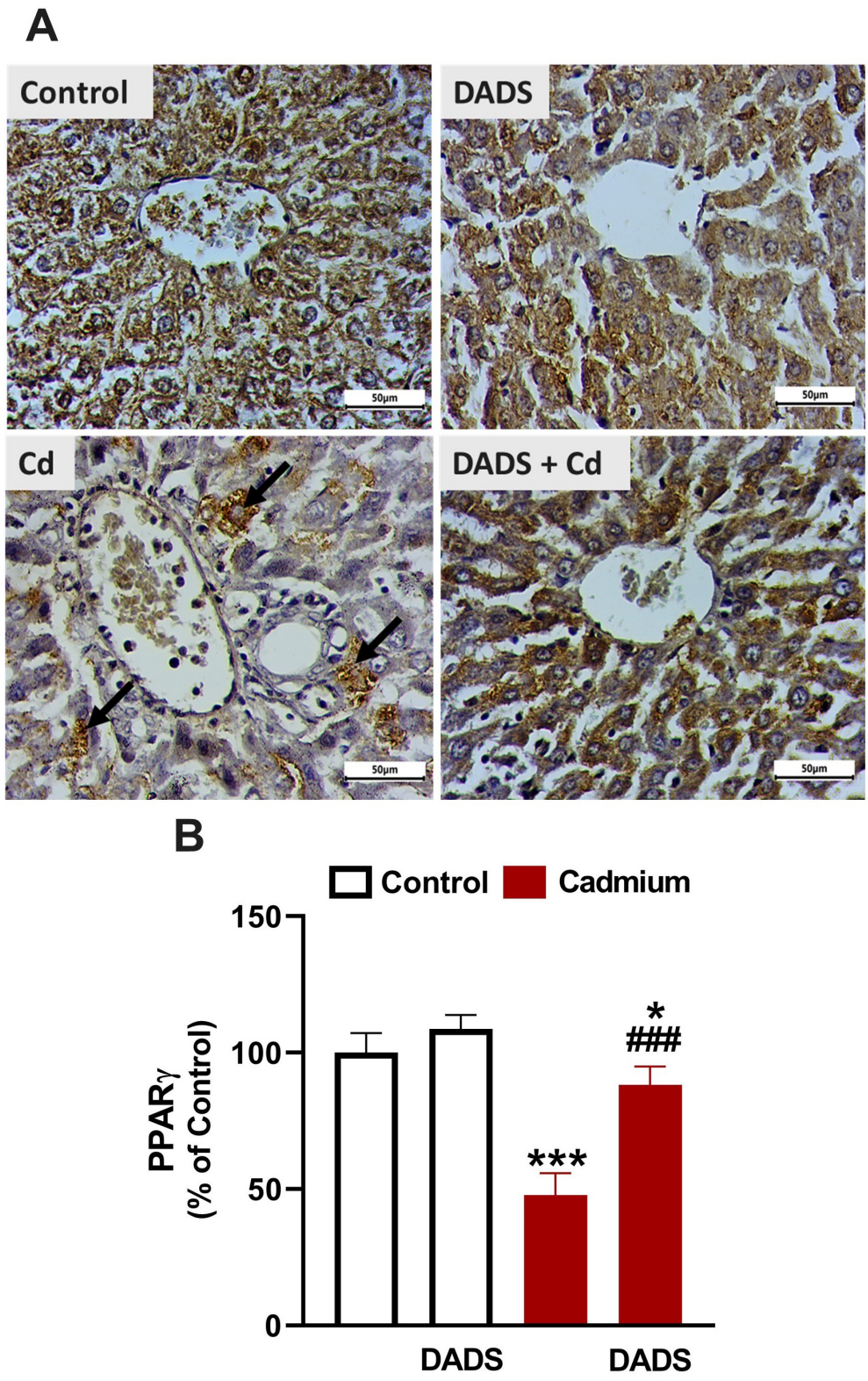

Fig. 7. Diallyl disulfide (DADS) upregulated peroxisome proliferator activated receptor gamma (PPARγ) in liver of Cadmium (Cd)-administered rats. (A) Photomicrographs showing downregulated PPARγ in the liver of Cd-administered rats and the ameliorative effect of DADS. (×400, Scale bar = 50 µm). (B) Image analysis of PPARγ immunostaining. Data are mean ± SEM, ($n = 6$). *$p < 0.05$ and ***$p < 0.001$ versus Control. ###$p < 0.001$ versus Cd.

against liver steatosis induced by ethanol in mice [31]. The antioxidant properties of DADS were demonstrated via activation of enzymes such as SOD, CAT, and HO-1 and suppression of ROS [29]. In ethanol-challenged mice and hu-

man hepatocytes [56] and LPS-treated macrophages [57], DADS upregulated antioxidants via activation of Nrf2, and restored CAT activity in H_2O_2-treated epithelial cells [58].

The beneficial role of DADS on Cd-induced OS and inflammation could be associated with PPARγ activation. PPARγ directly promotes the expression of enzymatic antioxidants and suppresses ROS generation via NADPH oxidase inhibition [59,60]. Its activation is linked to attenuation of inflammation via its suppressive effect on NF-κB. PPARγ controls NF-κB transcriptional activity, reduces p65 nuclear translocation and inhibits the degradation of IκBα [61,62]. The activation of PPARγ can mitigate fibrogenesis in different organs through suppression of TGF-β/Smad signaling [63]. In this study, Cd downregulated PPARγ whereas DADS increased its expression in the liver. The role of PPARγ upregulation in protecting the liver against toxicity of drugs and chemicals was demonstrated in several studies [64–66]. In support of our findings, activation of PPARγ by DADS suppressed NF-κB in a mouse model of pancreatitis and lung injury as reported recently by Marimuthu *et al*. [67]. In mice with hepatic steatosis, treatment with DADS upregulated the gene expression of PPARγ [68]. It activated PPARγ coactivator 1 alpha and potentiated the effect of green tea in experimental obesity [69]. The role of PPARγ in the protective mechanism of DADS against nephrotoxicity induced by glycerol was supported by the study of Sharma *et al*. [70] where pretreatment of the rats with PPARγ antagonist abolished DADS renoprotection. Very recently, Qu *et al*. [71] demonstrated the involvement of PPARγ in mediating the beneficial role of DADS against lung cancer. However, the lack of data showing the dose-response and the use of PPARγ agonists/antagonists could be considered limitations of this study.

5. Conclusions

This study shows new information on the protective role of DADS against Cd-induced liver injury and the involvement of PPARγ. DADS prevented liver tissue injury, and suppressed MDA, NO, TLR-4/NF-κB pathway, caspase-3, inflammatory mediators, and enhanced PPARγ and enzymatic antioxidants in Cd-administered rats. This study may have significant clinical implications and underscore the protective role of Cd against Cd hepatotoxicity. DADS could be valuable to confer hepatic protection against Cd toxicity in vulnerable individuals. However, further research is needed to explore other mechanism(s) of action od DADS.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Conceptualization, EHMH, and AMM; methodology, RSA, SMA, MAA, OAMA, MKM, EHMH, MFA, and AMM; formal analysis, RSA, EHMH, and AMM; investigation, RSA, OAMA, MKM, EHMH, and AMM; resources, SMA, MAA, and MFA; data curation, RSA, EHMH, and AMM; writing—original draft preparation, AMM; writing—review and editing, MFA and AMM; supervision, EHMH, and AMM; project administration, RSA, and AMM; funding acquisition, RSA. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental protocol was approved by the ethics committee at Al Azhar University (Assiut - Egypt) (AZ-AS/PH-REC/28/24).

Acknowledgment

Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2024R381), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Funding

This research was funded by Princess Nourah bint Abdulrahman University, Researchers Supporting Project Number (PNURSP2024R381).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Renu K, Chakraborty R, Myakala H, Koti R, Famurewa AC, Madhyastha H, *et al*. Molecular mechanism of heavy metals (Lead, Chromium, Arsenic, Mercury, Nickel and Cadmium) - induced hepatotoxicity - A review. Chemosphere. 2021; 271: 129735.

[2] Alruhaimi RS, Hassanein EHM, Bin-Jumah MN, Mahmoud AM. Cadmium cardiotoxicity is associated with oxidative stress and upregulated TLR-4/NF-kB pathway in rats; protective role of agomelatine. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association. 2023; 180: 114055.

[3] Friberg LT, Elinder GG, Kjellstrom T, Nordberg GF. Cadmium and health: A toxicological and epidemiological appraisal: Volume 2: Effects and response. CRC Press: Boca Raton, FL, USA. 2019.

[4] Järup L. Hazards of heavy metal contamination. British Medical Bulletin. 2003; 68: 167–182.

[5] Al-Otaibi FS, Ajarem JS, Abdel-Maksoud MA, Maodaa S, Allam AA, Al-Basher GI, *et al*. Stone quarrying induces organ dysfunction and oxidative stress in Meriones libycus. Toxicology and Industrial Health. 2018; 34: 679–692.

[6] Ajarem JS, Hegazy AK, Allam GA, Allam AA, Maodaa SN, Mahmoud AM. Heavy Metal Accumulation, Tissue Injury, Ox-

idative Stress, and Inflammation in Dromedary Camels Living near Petroleum Industry Sites in Saudi Arabia. Animals: an Open Access Journal from MDPI. 2022; 12: 707.
[7] Almalki AM, Ajarem J, Allam AA, El-Serehy HA, Maodaa SN, Mahmoud AM. Use of *Spilopelia senegalensis* as a Biomonitor of Heavy Metal Contamination from Mining Activities in Riyadh (Saudi Arabia). Animals: an Open Access Journal from MDPI. 2019; 9: 1046.
[8] Anyanwu BO, Ezejiofor AN, Igweze ZN, Orisakwe OE. Heavy Metal Mixture Exposure and Effects in Developing Nations: An Update. Toxics. 2018; 6: 65.
[9] Bernhoft RA. Cadmium toxicity and treatment. The Scientific World Journal. 2013; 2013: 394652.
[10] Abouhamed M, Wolff NA, Lee WK, Smith CP, Thévenod F. Knockdown of endosomal/lysosomal divalent metal transporter 1 by RNA interference prevents cadmium-metallothionein-1 cytotoxicity in renal proximal tubule cells. American Journal of Physiology. Renal Physiology. 2007; 293: F705–F712.
[11] Wolff NA, Abouhamed M, Verroust PJ, Thévenod F. Megalin-dependent internalization of cadmium-metallothionein and cytotoxicity in cultured renal proximal tubule cells. The Journal of Pharmacology and Experimental Therapeutics. 2006; 318: 782–791.
[12] Egger AE, Grabmann G, Gollmann-Tepeköylü C, Pechriggl EJ, Artner C, Türkcan A, *et al*. Chemical imaging and assessment of cadmium distribution in the human body. Metallomics: Integrated Biometal Science. 2019; 11: 2010–2019.
[13] Young JL, Yan X, Xu J, Yin X, Zhang X, Arteel GE, *et al*. Cadmium and High-Fat Diet Disrupt Renal, Cardiac and Hepatic Essential Metals. Scientific Reports. 2019; 9: 14675.
[14] Karmakar R, Bhattacharya R, Chatterjee M. Biochemical, haematological and histopathological study in relation to time-related cadmium-induced hepatotoxicity in mice. Biometals: an International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine. 2000; 13: 231–239.
[15] Lin YC, Lian IB, Kor CT, Chang CC, Su PY, Chang WT, *et al*. Association between soil heavy metals and fatty liver disease in men in Taiwan: a cross sectional study. BMJ Open. 2017; 7: e014215.
[16] Hildebrand J, Thakar S, Watts TL, Banfield L, Thabane L, Macri J, *et al*. The impact of environmental cadmium exposure on type 2 diabetes risk: a protocol for an overview of systematic reviews. Systematic Reviews. 2019; 8: 309.
[17] Chung SM, Moon JS, Yoon JS, Won KC, Lee HW. The sex-specific effects of blood lead, mercury, and cadmium levels on hepatic steatosis and fibrosis: Korean nationwide cross-sectional study. Journal of Trace Elements in Medicine and Biology: Organ of the Society for Minerals and Trace Elements (GMS). 2020; 62: 126601.
[18] Kazi TG, Kolachi NF, Afridi HI, Kazi NG, Sirajuddin, Naeemullah, *et al*. Effects of mineral supplementation on liver cirrhotic/cancer male patients. Biological Trace Element Research. 2012; 150: 81–90.
[19] Liu J, Qu W, Kadiiska MB. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicology and Applied Pharmacology. 2009; 238: 209–214.
[20] Baeuerle PA, Baichwal VR. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. Advances in Immunology. 1997; 65: 111–137.
[21] Asehnoune K, Strassheim D, Mitra S, Kim JY, Abraham E. Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-kappa B. Journal of Immunology (Baltimore, Md.: 1950). 2004; 172: 2522–2529.
[22] Lasfer M, Vadrot N, Aoudjehane L, Conti F, Bringuier AF, Feldmann G, *et al*. Cadmium induces mitochondria-dependent apoptosis of normal human hepatocytes. Cell Biology and Toxicology. 2008; 24: 55–62.
[23] Dorrigiv M, Zareiyan A, Hosseinzadeh H. Garlic (Allium sativum) as an antidote or a protective agent against natural or chemical toxicities: A comprehensive update review. Phytotherapy Research. 2020; 34: 1770–1797.
[24] Trio PZ, You S, He X, He J, Sakao K, Hou DX. Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. Food & Function. 2014; 5: 833–844.
[25] Song X, Yue Z, Nie L, Zhao P, Zhu K, Wang Q. Biological Functions of Diallyl Disulfide, a Garlic-Derived Natural Organic Sulfur Compound. Evidence-based Complementary and Alternative Medicine: ECAM. 2021; 2021: 5103626.
[26] Mathan Kumar M, Tamizhselvi R. Protective effect of diallyl disulfide against cerulein-induced acute pancreatitis and associated lung injury in mice. International Immunopharmacology. 2020; 80: 106136.
[27] Park HY, Kim ND, Kim GY, Hwang HJ, Kim BW, Kim WJ, *et al*. Inhibitory effects of diallyl disulfide on the production of inflammatory mediators and cytokines in lipopolysaccharide-activated BV2 microglia. Toxicology and Applied Pharmacology. 2012; 262: 177–184.
[28] Chu CC, Wu WS, Shieh JP, Chu HL, Lee CP, Duh PD. The Anti-Inflammatory and Vasodilating Effects of Three Selected Dietary Organic Sulfur Compounds from Allium Species. Journal of Functional Biomaterials. 2017; 8: 5.
[29] Feng C, Luo Y, Nian Y, Liu D, Yin X, Wu J, *et al*. Diallyl Disulfide Suppresses the Inflammation and Apoptosis Resistance Induced by DCA Through ROS and the NF-κB Signaling Pathway in Human Barrett's Epithelial Cells. Inflammation. 2017; 40: 818–831.
[30] Bahrampour Juybari K, Kamarul T, Najafi M, Jafari D, Sharifi AM. Restoring the IL-1β/NF-κB-induced impaired chondrogenesis by diallyl disulfide in human adipose-derived mesenchymal stem cells via attenuation of reactive oxygen species and elevation of antioxidant enzymes. Cell and Tissue Research. 2018; 373: 407–419.
[31] Liu SX, Liu H, Wang S, Zhang CL, Guo FF, Zeng T. Diallyl disulfide ameliorates ethanol-induced liver steatosis and inflammation by maintaining the fatty acid catabolism and regulating the gut-liver axis. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association. 2022; 164: 113108.
[32] Cobb-Abdullah A, Lyles LR, 2nd, Odewumi CO, Latinwo LM, Badisa VL, Abazinge M. Diallyl disulfide attenuation effect on transcriptome in rat liver cells against cadmium chloride toxicity. Environmental Toxicology. 2019; 34: 950–957.
[33] de Lima EC, de Moura CFG, Silva MJD, Vilegas W, Santamarina AB, Pisani LP, *et al*. Therapeutical properties of Mimosa caesalpiniifolia in rat liver intoxicated with cadmium. Environmental Science and Pollution Research International. 2020; 27: 10981–10989.
[34] Hashizume Y, Shirato K, Abe I, Kobayashi A, Mitsuhashi R, Shiono C, *et al*. Diallyl disulfide reduced dose-dependently the number of lymphocyte subsets and monocytes in rats. Journal of Nutritional Science and Vitaminology. 2012; 58: 292–296.
[35] Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier Health Sciences: Netherlands. 2008.
[36] Malatesta M. Histological and Histochemical Methods - Theory and practice. European Journal of Histochemistry. 2016; 60: 2639.
[37] Hasan IH, Shaheen SY, Alhusaini AM, Mahmoud AM. Simvastatin mitigates diabetic nephropathy by upregulating farnesoid X receptor and Nrf2/HO-1 signaling and attenuating oxidative stress and inflammation in rats. Life Sciences. 2024; 340: 122445.
[38] Atwa AM, Abd El-Ghafar OAM, Hassanein EHM, Mahdi

SE, Sayed GA, Alruhaimi RS, *et al*. Candesartan Attenuates Cisplatin-Induced Lung Injury by Modulating Oxidative Stress, Inflammation, and TLR-4/NF-κB, JAK1/STAT3, and Nrf2/HO-1 Signaling. Pharmaceuticals (Basel, Switzerland). 2022; 15: 1222.

[39] Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Experimental and Toxicologic Pathology: Official Journal of the Gesellschaft Fur Toxikologische Pathologie. 2010; 62: 171–181.

[40] Abu-El-Zahab HSH, Hamza RZ, Montaser MM, El-Mahdi MM, Al-Harthi WA. Antioxidant, antiapoptotic, antigenotoxic, and hepatic ameliorative effects of L-carnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure in male mice. Ecotoxicology and Environmental Safety. 2019; 173: 419–428.

[41] Kang MY, Cho SH, Lim YH, Seo JC, Hong YC. Effects of environmental cadmium exposure on liver function in adults. Occupational and Environmental Medicine. 2013; 70: 268–273.

[42] Hyder O, Chung M, Cosgrove D, Herman JM, Li Z, Firoozmand A, *et al*. Cadmium exposure and liver disease among US adults. Journal of Gastrointestinal Surgery: Official Journal of the Society for Surgery of the Alimentary Tract. 2013; 17: 1265–1273.

[43] Yu D, Zhang L, Yu G, Nong C, Lei M, Tang J, *et al*. Association of liver and kidney functions with Klotho gene methylation in a population environment exposed to cadmium in China. International Journal of Environmental Health Research. 2020; 30: 38–48.

[44] Tzirogiannis KN, Panoutsopoulos GI, Demonakou MD, Papadimas GK, Kondyli VG, Kourentzi KT, *et al*. The hepatoprotective effect of putrescine against cadmium-induced acute liver injury. Archives of Toxicology. 2004; 78: 321–329.

[45] Li Y, Shen R, Wu H, Yu L, Wang Z, Wang D. Liver changes induced by cadmium poisoning distinguished by confocal Raman imaging. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy. 2020; 225: 117483.

[46] Lee IC, Kim SH, Baek HS, Moon C, Kang SS, Kim SH, *et al*. The involvement of Nrf2 in the protective effects of diallyl disulfide on carbon tetrachloride-induced hepatic oxidative damage and inflammatory response in rats. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association. 2014; 63: 174–185.

[47] Ikediobi CO, Badisa VL, Ayuk-Takem LT, Latinwo LM, West J. Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. International Journal of Molecular Medicine. 2004; 14: 87–92.

[48] Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, *et al*. Cadmium stress: an oxidative challenge. Biometals: an International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine. 2010; 23: 927–940.

[49] Casalino E, Sblano C, Landriscina C. Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. Archives of Biochemistry and Biophysics. 1997; 346: 171–179.

[50] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews. 2007; 87: 315–424.

[51] Qi K, Ren L, Bai Z, Yan J, Deng X, Zhang J, *et al*. Detecting cadmium during ultrastructural characterization of hepatotoxicity. Journal of Trace Elements in Medicine and Biology: Organ of the Society for Minerals and Trace Elements (GMS). 2020; 62: 126644.

[52] Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harbor Perspectives in Biology. 2009; 1: a001651.

[53] Chen P, Hu YF, Wang L, Xiao WF, Bao XY, Pan C, *et al*. Mitochondrial Apoptotic Pathway Is Activated by H2O2-Mediated Oxidative Stress in BmN-SWU1 Cells from Bombyx mori Ovary. PloS One. 2015; 10: e0134694.

[54] Fasolino I, Izzo AA, Clavel T, Romano B, Haller D, Borrelli F. Orally administered allyl sulfides from garlic ameliorate murine colitis. Molecular Nutrition & Food Research. 2015; 59: 434–442.

[55] Zhang H, Shang C, Tian Z, Amin HK, Kassab RB, Abdel Moneim AE, *et al*. Diallyl Disulfide Suppresses Inflammatory and Oxidative Machineries following Carrageenan Injection-Induced Paw Edema in Mice. Mediators of Inflammation. 2020; 2020: 8508906.

[56] Zeng T, Zhang CL, Song FY, Zhao XL, Yu LH, Zhu ZP, *et al*. The activation of HO-1/Nrf-2 contributes to the protective effects of diallyl disulfide (DADS) against ethanol-induced oxidative stress. Biochimica et Biophysica Acta. 2013; 1830: 4848–4859.

[57] Shin IS, Hong J, Jeon CM, Shin NR, Kwon OK, Kim HS, *et al*. Diallyl-disulfide, an organosulfur compound of garlic, attenuates airway inflammation via activation of the Nrf-2/HO-1 pathway and NF-kappaB suppression. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association. 2013; 62: 506–513.

[58] Jin W, Wang H, Yan W, Xu L, Wang X, Zhao X, *et al*. Disruption of Nrf2 enhances upregulation of nuclear factor-kappaB activity, proinflammatory cytokines, and intercellular adhesion molecule-1 in the brain after traumatic brain injury. Mediators of Inflammation. 2008; 2008: 725174.

[59] Hwang J, Kleinhenz DJ, Lassègue B, Griendling KK, Dikalov S, Hart CM. Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. American Journal of Physiology. Cell Physiology. 2005; 288: C899–C905.

[60] Okuno Y, Matsuda M, Miyata Y, Fukuhara A, Komuro R, Shimabukuro M, *et al*. Human catalase gene is regulated by peroxisome proliferator activated receptor-gamma through a response element distinct from that of mouse. Endocrine Journal. 2010; 57: 303–309.

[61] Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature. 2000; 405: 421–424.

[62] Remels AHV, Langen RCJ, Gosker HR, Russell AP, Spaapen F, Voncken JW, *et al*. PPARgamma inhibits NF-kappaB-dependent transcriptional activation in skeletal muscle. American Journal of Physiology. Endocrinology and Metabolism. 2009; 297: E174–E183.

[63] Mahmoud AM, Hozayen WG, Hasan IH, Shaban E, Bin-Jumah M. Umbelliferone Ameliorates CCl_4-Induced Liver Fibrosis in Rats by Upregulating PPARγ and Attenuating Oxidative Stress, Inflammation, and TGF-β1/Smad3 Signaling. Inflammation. 2019; 42: 1103–1116.

[64] Mahmoud AM, Al Dera HS. 18β-Glycyrrhetinic acid exerts protective effects against cyclophosphamide-induced hepatotoxicity: potential role of PPARγ and Nrf2 upregulation. Genes & Nutrition. 2015; 10: 41.

[65] Mahmoud AM, Hussein OE, Hozayen WG, Abd El-Twab SM. Methotrexate hepatotoxicity is associated with oxidative stress, and down-regulation of PPARγ and Nrf2: Protective effect of 18β-Glycyrrhetinic acid. Chemico-biological Interactions. 2017; 270: 59–72.

[66] El-Sheikh AAK, Rifaai RA. Peroxisome Proliferator Activator Receptor (PPAR)-γ Ligand, but Not PPAR-α, Ameliorates Cyclophosphamide-Induced Oxidative Stress and Inflammation in Rat Liver. PPAR Research. 2014; 2014: 626319.

[67] Marimuthu MK, Moorthy A, Ramasamy T. Diallyl Disulfide Attenuates STAT3 and NF-κB Pathway Through PPAR-γ Activation in Cerulein-Induced Acute Pancreatitis and Associated

Lung Injury in Mice. Inflammation. 2022; 45: 45–58.
[68] Yang Y, Yang F, Huang M, Wu H, Yang C, Zhang X, *et al*. Fatty liver and alteration of the gut microbiome induced by diallyl disulfide. International Journal of Molecular Medicine. 2019; 44: 1908–1920.
[69] Bae J, Kumazoe M, Fujimura Y, Tachibana H. Diallyl disulfide potentiates anti-obesity effect of green tea in high-fat/high-sucrose diet-induced obesity. The Journal of Nutritional Biochemistry. 2019; 64: 152–161.
[70] Sharma AK, Kaur A, Kaur J, Kaur G, Chawla A, Khanna M, *et al*. Ameliorative Role of Diallyl Disulfide Against Glycerol-induced Nephrotoxicity in Rats. Journal of Pharmacy & Bioallied Sciences. 2021; 13: 129–135.
[71] Qu Z, Tian J, Sun J, Shi Y, Yu J, Zhang W, *et al*. Diallyl trisulfide inhibits 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung cancer *via* modulating gut microbiota and the PPARγ/NF-κB pathway. Food & Function. 2024; 15: 158–171.