

Protective Effect of Garlic on Diazinon Induced Kidney Toxicity in Rat: A Biochemical and Histopathological Evaluation

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Introduction. Diazinon (DZN) is a widely used pesticide applied in agriculture to eliminate pests, but it can cause adverse effects on various organs. This study investigated the nephrotoxic changes and oxidative stress induced by diazinon, as well as the potential protective role of garlic in ameliorating kidney toxicity in male Wistar rats.

Methods. The animals were treated with diazinon (DZN; 70 mg/kg/day, orally) with or without garlic extract (500 mg/kg, orally) for four consecutive weeks. Biomarkers including malondialdehyde (MDA), total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD), and total thiol molecules were measured to evaluate oxidative stress. In addition, kidney histopathology was examined using the hematoxylin and eosin (H&E) staining.

Results and conclusion. As expected, DZN significantly increased MDA levels while decreased the levels of CAT, SOD, TAC, and total thiol molecules in kidney tissue. Co-treatment with garlic reversed the oxidative stress status. Moreover, garlic ameliorated the kidney histopathological injuries induced by DZN. Our findings suggest that garlic can be considered a safe and effective agent for mitigating DZN-induced nephrotoxicity.

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INTRODUCTION

Diazinon (DZN) is a synthetic organophosphate insecticide that its misuse contributes significantly to ecosystem destabilization and poses a threat to non-target species such as humans, mammals, and birds. Exposure often occurs through the consumption of contaminated vegetables, fruits, crops, and other agricultural products.^{1,2} Many organophosphate compounds, including DZN, are fat-soluble and can be readily absorbed through the skin, oral mucosa, digestive tract, and respiratory tract. DZN is widely employed for pest control;

however, it emerged as a considerable health hazard, with prolonged exposure leading to both acute and chronic toxicity in various tissues.³

The primary mechanism of DZN toxicity is the inhibition of the acetylcholinesterase enzyme; however, emerging evidence highlight the significant role of oxidative stress (OS).⁴ Oxidative stress is defined by an imbalance between high levels of reactive oxygen species (ROS) and low levels of endogenous antioxidants, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT).⁵ Research confirms that Diazinon is capable

of inducing oxidative stress in vital animal organs, including the liver, blood, kidney, and brain, by increasing the production of reactive oxygen species and promoting the oxidation of cell membrane lipids.⁶⁻⁸

The kidney is highly vulnerable to oxidative stress, triggered by radicals or inflammatory mediators (Rahbari, NAZEM *et al.* 2021). Recent studies indicate that Diazinon (DZN) can induce nephrotoxic effects, evidenced by increases in creatinine (Cr) and urea levels, and impaired renal functions such as filtration, secretion, and reabsorption— pathologies linked to lipid peroxidation and the reduction of renal antioxidant enzyme activities.⁹ Given the established evidence regarding diazinon-induced nephrotoxicity, the development of a preventative strategy is necessary.^{6,10}

Due to the inherent antioxidant properties of medicinal plants, their administration is recommended for protecting kidney tissue against DZN toxicity.⁷ Consequently, many research teams have focused on investigating the medicinal properties and antioxidant role of garlic over the past decade.^{11,12} These protective effects are attributed to its components, namely flavonoids, carbohydrates, minerals (especially selenium), and amino acids, which defend cells by scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation.^{13,14} Garlic exhibits multiple therapeutic effects, including protection against cardiovascular disorders, regulation of blood pressure, antibacterial and antifungal activities, immune system strengthening, and anti-tumor properties against various pesticides (such as lindane, chlorpyrifos, and deltamethrin), as documented by several researchers.^{10,15,16} Experimental evidence further suggests a protective effect of garlic oil against toxicity induced by organophosphate (OP) pesticides.^{8,17,18} Moreover, garlic protective efficacy, observed across diverse tissues, establishes it as a potent phytoantioxidant possessing powerful chemopreventive properties against chemically-induced oxidative stress.^{18,19}

Therefore, the current study aimed to evaluate the toxicity effects of DZN using stress oxidative markers (total thiol, MDA, TAC, CAT, SOD) and to investigate the protective effects of garlic extract on kidney tissue in a rat model.

MATERIALS AND METHODS

Chemicals

Diazinon with a high purity (99%) was obtained from the Agricultural Research, Education, and Development Organization (AREDO), a known supplier of pesticides in Tehran, Iran. Garlic extraction was prepared according to previous study. Briefly, 32 g of garlic plant was mashed in double distilled water (60 ml) and then compressed using a double cheese textile. The resulting liquid extract was stored at -20°C for subsequent use. Each ml of the extract was equivalent to approximately 500 mg of garlic.²⁰

Experimental design

A total of 20 Wistar rats weighing between 130-160 grams were obtained from the Animal Care Center, Hamadan University of Medical Sciences. The rats were kept in plastic container at a normal temperature ($21-23^{\circ}\text{C}$) with standard water and proper rodent chow for an acclimation period of 4 weeks. All the ethical standards of working with animals were in accordance with the ethical code approved by ethics committee of Hamadan University of Medical Sciences (No. 1 IR.UMSHA. REC.1401.853). Diazinon at a dose of 70 mg/kg was selected as the ideal dose that was equivalent to 1/5 of its reported LD50 (350 mg/kg) in previous studies.^{21,22} Animals were monitored daily for body weight, food/water intake, examination signs of deformity, and mortality. DZN and garlic extract were dissolved in the corn oil and H₂O respectively, and administered orally once daily for 4 weeks.

Study design

The study was conducted in a randomized and double-blind manner, wherein the experimenter and data analyst were unaware of the group allocations. Animals were allocated into four groups (n = 5 per group):

Group 1 (Control group): Received 1 ml corn oil.

Group 2 (DZN group): Received DZN (70 mg/kg/day, p.o).

Group 3 (Garlic group): Received garlic extract (500 mg/kg/day, p.o).

Group 4 (DZN + garlic): Received both garlic extract (500 mg/kg/day) and DZN (70 mg/kg/day, p.o).

Twenty-four hours after last dose, animals were anesthetized by ketamine/xylazine (8:1, i.p.).^{23,24}

Subsequently, kidney tissues were excised, then washed in cold buffer solution (PBS, pH = 7.4). Pieces of each of the tissues were kept at -80°C for oxidative stress markers assessment or fixed in formalin solution for histological evaluation.

Total protein assay

The protein level in various examples was determined by the Bradford process by Coomassie blue reagent.²⁴

Determining MDA concentration in kidney tissue

The malondialdehyde (MDA) content indicative of lipid peroxidation was determined according to a previously established method from our laboratory.²⁵ Briefly, MDA was allowed to react with thiobarbituric acid (TBA) and form a red-colored complex; whose absorbance was measured at 532 nm using a spectrophotometer. The data were expressed as nmol /mg of protein.

Assessment of total thiol molecules in kidney tissue

Total thiol groups were assessed using DTNB (2, 2 dithio nitrobenzoic acid) which reacts with the sulfhydryl (SH) groups and produces a yellow-colored complex.²⁶ Absorption was read at a wavelength of 412 nm. The results are expressed as nmol /mg of protein.

Evaluation of SOD, TAC, and CAT levels in kidney tissue

The concentration of SOD, TAC, and CAT in kidney tissues was evaluated using commercial assay kit based on the manufacturer's instructions (Carmona Pars Gene, Kerman, Iran).

Histopathology examinations

For histopathology examinations, immediately after the dissection, kidney tissue samples were fixed in 10% buffered formalin solution. Samples were dehydrated and embedded in paraffin, and 5 μm thick sections were made using a microtome. The sections were stained with hematoxylin and eosin (H&E). Images were capture with a digital camera (Nikon E800, Japan) attached to a light microscope. In each group of animals, the histological changes were estimated by reading five serial coronal sections at 400x magnification.²⁷

Statistical Analysis

The results were expressed as mean \pm standard error using the SPSS-20 software. Data analysis was performed by ANOVA and Tukey's post hoc test. A *P*-value less than .05 ($P < .05$) was considered as a criterion for statistical significance.

RESULTS

Effect of Garlic on MDA Content

According to Figure 1, there was a significant increase in the MDA concentration in the diazinon group compared to the control group ($P < .1$). Treatment with diazinon + garlic significantly reduced MDA concentration compared to the DZN group ($P < .05$).

Effect of Garlic on TAC Content

As illustrated in Figure 2, a significant decrease was observed in the TAC level in the diazinon group compared to the control group ($P < .05$). However, the TAC level in kidney tissue was found to be higher in the diazinon + garlic groups than in the diazinon group ($P < .01$). Furthermore, the TAC level in the garlic group was higher than the control group ($P < .01$).

Effect of Garlic on CAT Content

Figure 3 displays that the kidney CAT activity was significantly lower in the DZN group in comparison with the control group ($P < .05$). However, garlic remarkably increased the CAT level compared to the DZN group ($P < .01$).

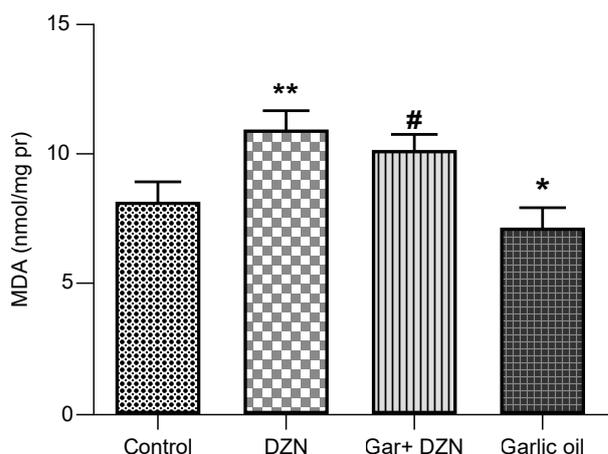


Figure 1. Effects of garlic oil on MDA levels in tissues of kidney in rats treated with diazinon (DZN). The data were reported based on Mean \pm SEM (n = 5). * $P < .05$, ** $P < .01$ and *** $P < .001$ compared to control group, # $P < .05$, ## $P < .01$ and ### $P < .001$ compared to DZN group.

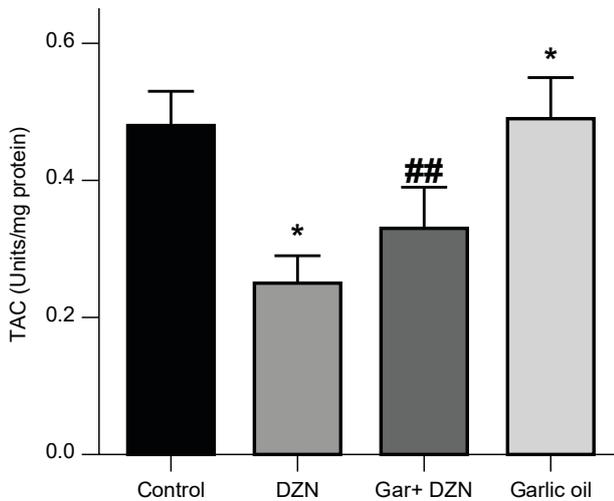


Figure 2. Effects of garlic oil on TAC levels in tissues of kidney in rats treated with diazinon (DZN). The data were reported based on Mean ± SEM (n = 5). * $P < .05$, ** $P < .01$ and *** $P < .001$ compared to control group, # $P < .05$, ## $P < .01$ and ### $P < .001$ compared to DZN group.

Evaluation of Thiol Group Content in Kidney Tissue

The content of thiol groups was evaluated in kidney tissue of animals treated with diazinon, as displayed in Figure 4. In the diazinon group, the kidney thiol concentration was lower than in the control group ($P < .01$). On the other hand, garlic administration reversed the diazinon-induced alterations of thiol groups in kidney tissue. Based on these findings, the concentration of thiol groups improved significantly ($P < .01$) in the diazinon

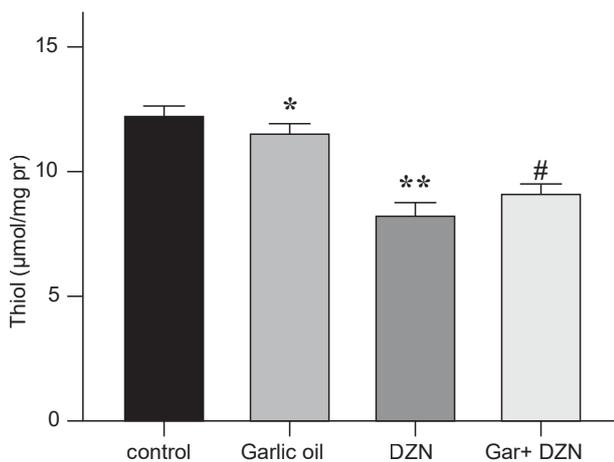


Figure 4. Effects of garlic oil on Thiol levels in kidney tissue in rats treated with diazinon (DZN). The data were reported based on Mean ± SEM (n = 5). * $P < .05$, ** $P < .01$ and *** $P < .001$ compared to control group, # $P < .05$, ## $P < .01$ and ### $P < .001$ compared to DZN group.

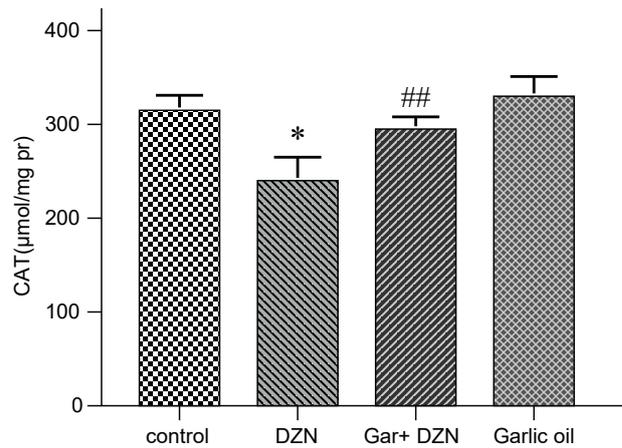


Figure 3. Effects of garlic oil on CAT levels in kidney tissue in rats treated with diazinon (DZN). The data were reported based on Mean ± SEM (n = 5). * $P < .05$, ** $P < .01$ and *** $P < .001$ compared to control group, # $P < .05$, ## $P < .01$ and ### $P < .001$ compared to DZN group.

+ garlic groups compared to the diazinon group ($P < .05$). Additionally, it was observed that the TAC level in the garlic group was higher than the control group ($P < .05$).

Effect of Garlic on SOD Activities

Consistent with Figure 5, a significant decrease in SOD activity was detected in the diazinon group compared to the control group ($P < .01$). In contrast, the SOD activity was significantly enhanced ($P < .001$) in animals that received diazinon + garlic compared to the DZN group. Furthermore, the levels of SOD activity in kidney

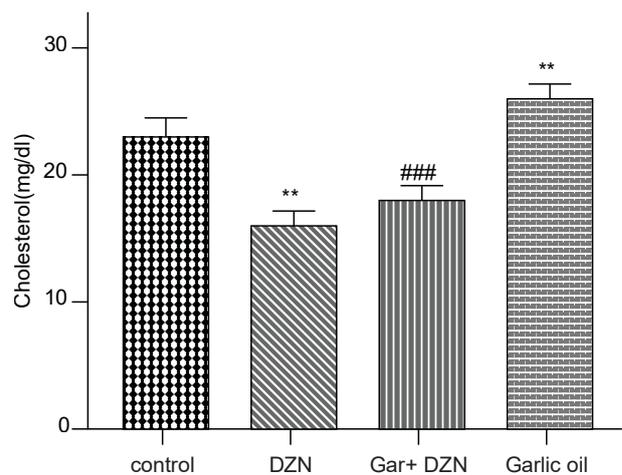


Figure 5. Effects of garlic oil on SOD levels in kidney tissue in rats treated with diazinon (DZN). The data were reported based on Mean ± SEM (n = 5). * $P < .05$, ** $P < .01$ and *** $P < .001$ compared to control group, # $P < .05$, ## $P < .01$ and ### $P < .001$ compared to DZN group.

tissue in the garlic group were higher than the control group ($P < .01$).

Results histological of kidney tissue

The results summarized in Figure 6 indicated extensive tissue damage in the diazinon-only group when compared to the control group. Observed injuries included glomerular atrophy, degenerative changes in Bowman’s capsule, tubular degeneration, cell death, disorganized tubular epithelium, and acidophilic staining of the cytoplasm. Furthermore, necrosis of tubular cells and pyknotic nuclei were observed. Conversely, in rats receiving diazinon and garlic extract, epithelial disorganization, cell death, the number of pyknotic nuclei, and the overall extent of tissue damage were all significantly reduced compared to the diazinon-only group.

DISCUSSION

Evidence from previous studies suggests that pesticides and other environmental pollutants can

disrupt antioxidant enzymes, thereby inducing oxidative stress, which may lead to various diseases in humans and animals.²⁸ Both *in vivo* and *in vitro* studies have shown that the generation of reactive oxygen species (ROS) is the initial factor responsible for oxidative stress.²⁹⁻³¹ In the present study, we investigated the nephrotoxic effects of diazinon by assessing oxidative parameters, antioxidant markers, and histopathological changes, and examined the potential protective role of garlic extract.

Our findings revealed that diazinon (DZN) impaired kidney function, evidenced by increased MDA levels and reduced activities of GSH, CAT, and SOD, along with decreased TAC in kidney tissue. Consistent with our study, Daim *et al.* found that DZN exposure can elevate oxidative stress (OS) in kidney tissue and other organs through increased reactive oxygen species (ROS) production, disturbance in the redox balance, and induction of lipid peroxidation.³¹ Similarly, other studies concluded that DZN exposure can

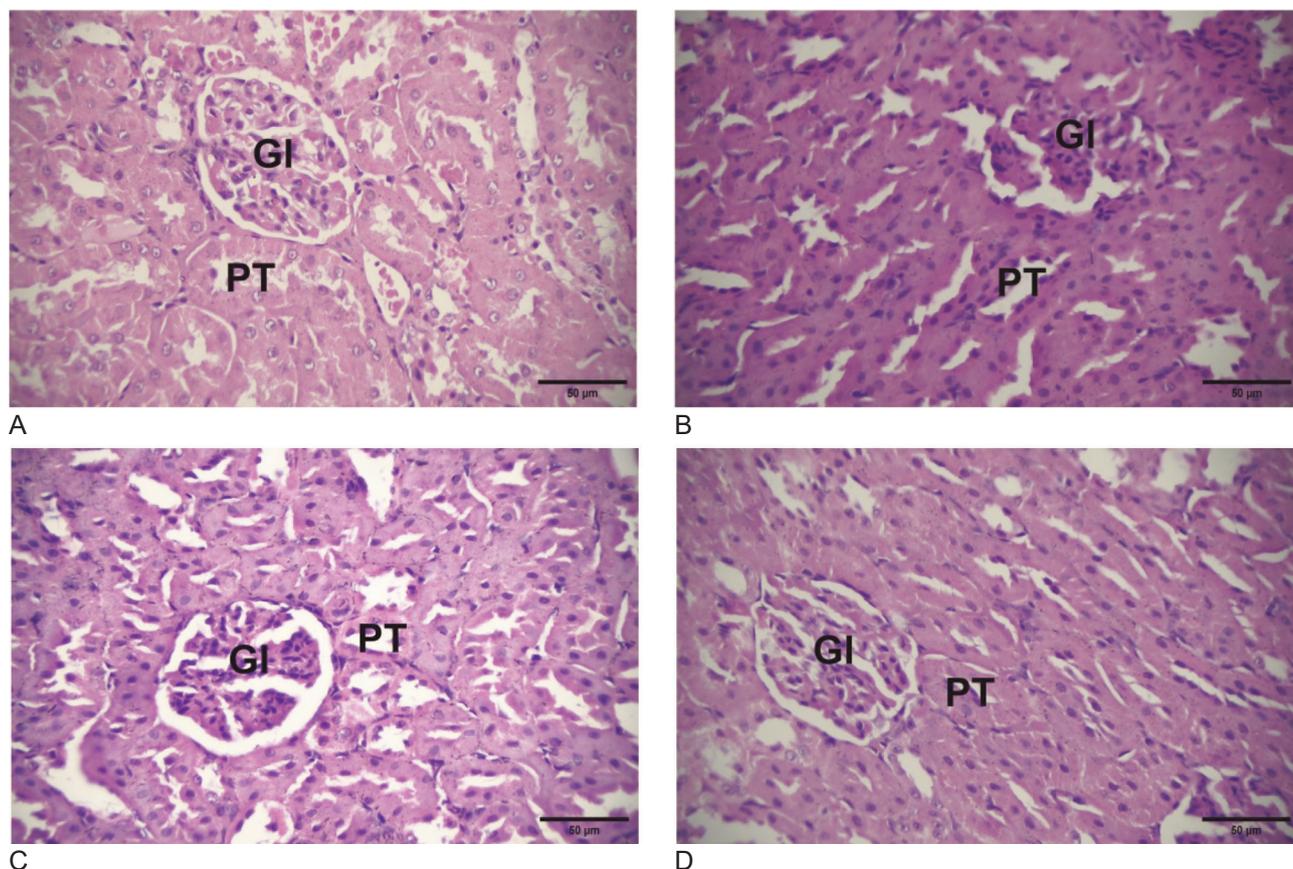


Figure 6. Effect of garlic oil on the kidney histopathological changes. Control groups (A), garlic oil (B), DZN (C), DZN+ garlic oil D. Magnification 1000 ×, scale bar = 20µm. The black arrow shows the healthy core of myofibers and the yellow arrow shows the compression of the core of myofibers.

lead to increased MDA and reduced activities of antioxidant enzymes, including CAT, GSH, and SOD, in kidney tissue.^{32,33}

Further supporting our results, Ajibade *et al.* reported that DZN induced oxidative stress (OS) in kidney tissues by increasing lipid peroxidation and reducing the antioxidant defense systems.³⁴ The pathophysiological consequences of this stress is significant; Daim *et al.* reported that OS induced by DZN exposure interrupted renal function indices, such as changes in glomerular filtration rate, reduction in sodium excretion, alterations in nephron function, and increased sodium retention.³¹

Based on reports, the nephrotoxic mechanism of DZN can be associated with a reduction in the glomerular filtration rate, an increase in mitochondrial calcium, and consequently, an increase in reactive oxygen species (ROS) and mitochondrial pathway of apoptosis in the kidney. All these factors lead to renal tubular dysfunction and an increase in renal BUN and creatinine levels.³⁵ In another section of our study, histopathological reports highlighted the biochemical changes and OS induced by DZN. Findings from animal exposure to DZN included atrophy of the glomeruli, degeneration of Bowman's capsule, disorganization in the tubular epithelium, and cytoplasmic eosinophilia.

Our results confirmed the findings of Sadiq *et al.* and Sarhan *et al.*, indicating tissue damage due to exposure to organophosphates (OPs), especially DZN.^{9,36} Antioxidant therapies can ameliorate the toxic effects of diazinon.³⁷ It is important to note that antioxidants exist in two forms: Primary antioxidants (chain-breaking) frequently act by donating a hydrogen atom, while secondary (preventive) antioxidants act by binding metal ions that can catalyze oxidative processes, blocking enzymes, absorbing UV radiation, or decomposing hydroperoxides.³⁸

Under experimental conditions, mice receiving garlic extract alongside diazinon exhibited a reduction in lipid peroxidation (LPO) levels, and an increase in SOD, TAC, CAT, and GSH in kidney tissue. Furthermore, histopathological findings demonstrated a significant reduction in tubular epithelium disorganization, cell death, and the number of pyknotic nuclei in this group. Consistent with these results, Hfaiedh *et al.* reported that garlic extract improved scavenging enzymes such as SOD,

CAT, and GSH, and decreased MDA in toxicity induced by lindane.¹⁰ Likewise, Sabah *et al.* and Ghaffar *et al.* reported the protective effect of garlic extract against OS induced by pesticides.^{8,15} In our study, a component of garlic extract effectively counteracted diazinon-induced toxicity resulting from oxidative stress and the overproduction of reactive oxygen species (ROS). The mechanism through which garlic extract improved biomarkers of kidney damage following diazinon exposure can be attributed to a variety of bioactive substances, including glutamylcysteine, phenolic, and sulfur compounds, all of which are known to be efficient radical scavengers.³⁹

Limitation study

There are some limitations in this study that should be considered in future research, such as a small sample size which limits the statistical power, potential batch effects in the garlic extract, or the lack of a dose-response analysis. It is recommended that the baseline antioxidant and oxidant profile measured which was one the shortcomings of this study. Additionally, it is required to investigate the molecular mechanisms involved in the anti-inflammatory and antioxidant effects of garlic extract against DZN-induced kidney impairment. Remarkably, such studies can help provide a new perspective on nature-based therapeutic strategies for DZN toxicity.

CONCLUSION

The current data revealed valuable evidence indicating that garlic supplementation was capable of attenuating the oxidative stress induced by DZN intoxication. The protective effects of this compound can be attributed to its ability to scavenge ROS and increase antioxidant enzyme activity. A long-term study with diverse concentrations of this compound is required to identify the specific compounds responsible for the majority of its biological effects.

DECLARATIONS

Ethical Approval

Animal study and procedures were conducted in accordance with the ethical codes of the ethics committee of Hamadan University of Medical Sciences with the code number (No. 1 IR.UMSHA.REC.1401.853).

Funding

Not applicable.

Availability of data and materials

Not applicable.

Conflict of interest

None declared.

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AUTHOR CONTRIBUTIONS

FM designed the study and interpreted the data. ES conducted the *in vitro* experiments. BA drafted the article and FM, and SA revised it critically and finally approved the version to be submitted.

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