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The effect of garlic (*Allium sativum*) on hepatic and renal functions in male rabbits exposed to zinc oxide

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Abstract

Heavy metals, including zinc, are known for their high toxicity. Although zinc is an essential trace element, excessive concentrations can lead to systemic toxicity, with adverse effects on various organs, particularly the liver and kidneys – two major target organs. This study investigated the hepatotoxicity and nephrotoxicity associated with zinc oxide (ZnO) and the protective role of *Allium sativum* (garlic) in male rabbits exposed to ZnO. A daily dose of 30 mg of ZnO and/or 5 g garlic were administered orally to 4 groups of rabbits for 60 days. The first group was used as a control, while the others were used as treatment groups. Biochemical, hepatic, renal, enzymatic, organ weights, and histological parameters were assessed. The results revealed a significant decrease in total bilirubin and triglyceride levels in the group treated with garlic. Cholesterol, uric acid, lactate dehydrogenase, and the relative weight of the liver were non significantly reduced. However, a non significant increase in urea, creatinine, alkaline phosphatase, and the relative weight of the kidney was observed in the garlic treated group. Zinc oxide administration induced a significant decrease in total bilirubin, triglycerides, and alkaline phosphatase levels. The levels of direct bilirubin, cholesterol, urea, creatinine, and lactate dehydrogenase were significantly increased in ZnO treated group compared to the control. The combination of garlic and zinc oxide led to mixed outcomes, with both amelioration and enhancement of certain parameters. Histological examination of the liver and kidney revealed severe morphological and tissue damage in ZnO treated rabbits, whereas co-treatment with garlic reduced tissue damage, indicating partial protection. Overall, these findings suggest that garlic possesses hepatoprotective and nephroprotective properties against zinc-induced toxicity, likely due to its strong antioxidant potential.

1. Introduction

Medicinal plants have been widely used throughout the history for both nutritional and therapeutic purposes. In its history of over 5,000 years, garlic (*Allium sativum*) has been most commonly used in culinary preparations (Singh et al. 2009) as well as for preventive and curative treatment of various diseases, including bacterial, viral, parasitic, and fungal infections (El-mahmood 2009; Liu et al. 2010; Pundir et al. 2010; Meriga et al. 2012; Becker et al. 2012; Lanzotti et al. 2012; Kloucek et al. 2012; Ahmed et al. 2013). Several *in vivo* and *in vitro* studies have highlighted the therapeutic properties of garlic, such as antihypertensive, cardioprotective (Harauma and Moriguchi 2006; Al-Qattan et al. 2006; Sener et al. 2007; Nwokocha et al. 2011), anticarcinogenic, antidiabetic (Sengupta et al. 2004), and anti-atherosclerotic properties (Calvo-Gómez et al. 2004). Garlic exerts these properties due to the presence of wide range of bioactive compounds,such as flavonoids, tocopherols, and sulfur-containing molecules, which act as potent antioxidants and heavy metal chelators, thereby protecting cells from oxidative stress (Gorinstein et al. 2005; Leelarungrayub et al. 2006; Cruz et al. 2007). Garlic oil and juice help relieve rheumatism, tuberculosis, and infertility (Singh et al. 2014). However, garlic contains toxic components known as alkaloids, which have adverse effects on homeostasis (Chan et al. 2007). The consumption of high quantities of garlic leads to a oxidative stress characterized by reduced antioxidant enzyme activity (superoxide dismutase, peroxidase, catalase) and elevated malondialdehyde levels (Hamlaoui-Gasmi et al 2012). High intake of garlic have also been associated with liver damage and toxicity (Rana et al. 2006).

Zinc is an essential trace element for both humans and animals (Yanagisawa 2008). It is involved in functioning of several metallo-enzymes and plays vital role in physiological processes, such as bone development, hormone sysnthesis, nucleic acid metabolism, cell growth and multiplication, neural function, wound healing, immune function, and antioxidant defense. The normal physiological levels of zinc in rabbits range from 25 - 60 mg/kg body weight (Matcos et al. 2010; Wang et al. 2012). However, excess dietary zinc leads to toxic effects in animals (Amen and Daraji 2011; Raguvaran et al. 2015). Zinc toxicity has been linked to copper deficiency due to competitive inhibition of intestinal absorption, resulting in elevated cholesterol, reduced superoxide dismutase activity, and impaired antioxidant defense (Fischer et al. 1981; Plum et al. 2010; Sandstead 1995). High zinc concentrations disrupt liver and kidney function, alter hepatocyte protein synthesis, and affect detoxification and lipid metabolism (Yang et al. 2018; Yang et al. 2024). This study aimed to explore the impact of *Allium sativum* and zinc oxide (ZnO) on hepatic (albumin, bilirubin, cholesterol, triglycerides levels) and renal (urea, creatinine, uric acid levels) parameters, the activity of plasma enzymes (alkaline phosphatase ALP, and lactate dehydrogenase LDH), liver and kidney relative weights, and hepatic and renal histological structures in male

rabbits (*Oryctolagus cuniculus*).

2. Materials and Methods

2.1 Chemical products

Zinc oxide was obtained from the Faculty of Exact Sciences and Natural and Life Sciences, University of Oum El Bouaghi, in commercial white powder form (pure zinc oxide, Labbox, France).

2.2 Plant material

The plant material used in this study was garlic cloves (*Allium sativum*). The samples were obtained in June from the Tizi Ouzou region, which is located approximately 100 kilometres from Algiers, Algeria. The cloves were administered to the animals fresh, after being washed and crushed.

2.3 Animal and rearing conditions

A total of twenty four local male rabbits (*Oryctolagus cuniculus*), aged 5-6 months, were used. The animals were obtained from the Animal Breeding Center in the Blida region, Algeria. The rabbits were housed in appropriate experimental conditions of temperature, humidity and lighting for a 15 days acclimatization period, with free access to feed and water *ad libitum*.

2.4 Experimental treatment

The experiment was conducted in accordance with ethical standards approved by the Laboratory of Animal Ecobiology and Physiology and the Faculty of Exact Sciences and Natural and Life Sciences (Approval code: AEA/LEBPA-E0612800/001/2024). The rabbits were randomly divided into four groups with six animals per group and treated with oral zinc oxide and garlic for 60 consecutive days. The treatment groups were as follows:

Group 1 (T): Control group, fed a standard diet.

Group 2 (AS): Received garlic (*Allium sativum*) in the diet once daily at a dose of 50 g/kg of feed (5%). Each animal was given 100 g of feed mixed with garlic.

Group 3 (ZnO): Received zinc oxide in the diet once daily at a dose of 300 mg/kg of feed. Each animal was given 100 g of feed mixed with zinc oxide.

Group 4 (AS–ZnO): Received a combination of zinc oxide and garlic in the diet at the same respective doses.

2.5 Sample collection

2.5.1 Blood sampling

At the end of the treatment period, all the rabbits were sacrificed and blood was collected in heparinised polyethylene tubes. After centrifugation at 4000 rpm for 15 minutes, the plasma was collected in Eppendorf tubes for biochemical and enzymatic analyses.

2.5.2 Biochemical and enzymatic analyses

Biochemical and enzymatic parameterswere measured according to the established methods: albumin (Gendler 1984), bilirubin (Kaplan et al. 1984), cholesterol (Naito 1984), triglycerides (Fossati and Prencipe 1982), plasma alkaline phosphatase (ALP) (Rosalki et al. 1993), urea (Searcy et al. 1967), creatinine (Murray et al. 1984), uric acid (Fossati et al. 1980), and lactate dehydrogenase (LDH) (Pesce 1984). All the reagents involved in the assays were purchased from Spinreact S.A./S.A.U. (Ctra. Santa Coloma, Sant Esteve De Bas, Spain).

2.5.3 Organ collection

After the dissection of the animals, the liver and kidneys were carefully removed, cleaned with physiological solution, and weighed using an analytical balance to determine their relative weights. Representative tissue samples from each organ were fixed in alcoholic Bouin's solution for histological processing.

2.5.4 Histopathological examination

Histological analysis was conducted in the pathological anatomy laboratory according to the classic technique of Martoja and Martoja (1967), with some minor changes. After 48 hours of fixation, samples were removed from Bouin's solution and carefully washed with distilled water. Samples were then dehydrated for 12 hours through a graded ethanol series, followed by clearing in toluene and embedding in parafin wax. Tissue blocks were sectioned at 4 µm thickness using a microtome. The sections were rehydrated on glass slides, followed by staining with hematoxylin and eosin to obtain a clear view of the cellular parts under microscopic examination. After mounting with a suitable mounting medium, the slides were dried and and examined under light microscope to evaluate histopathological changes.

2.6 Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical significance was assessed using Student's t-test for comparison of two means. For multiple group comparisons one-way ananlysis of variance (ANOVA) was performed (GraphPad Prism 9.5.1 software, LLC, USA). Differences were considered significant for $p \leq 0.05$ level ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

The following comparisons were conducted:

a: Control (T) vs AS vs ZnO vs AS-ZnO

b: Control (T) vs AS

c: Control (T) vs ZnO

d: Control (T) vs AS–ZnO

3. Results

3.1 Variations in liver parameters

The results revealed that albumin levels did not show significant differences among treatment groups compared to the control (Fig. 1A). In contrast, a significant decrease in total bilirubin levels were observed in the AS and ZnO treated groups compared to the control (Fig. 1B). Direct bilirubin levels followed an opposite trend, with a significant elevation in the ZnO treated group (Fig. 1C). Total cholesterol levels were significantly higher in the ZnO and AS-ZnO treated groups. While AS treated group showed significant decrease compared to the control (Fig. 1D). Regarding triglycerides, a significant decrease was observed in all groups, with a particularly notable decrease in the AS-ZnO treated group (Fig. 1E).

3.2 Variations in kidney parameters

Renal functions were affected by the administration of *A.sativum* and zinc oxide. A non significant increase in urea levels was observed in the AS and ZnO treated group compared with the control, while a significant increase was observed in rabbits of AS-ZnO group (Fig. 2A). Creatinine levels were significantly higher in the AS and ZnO treated group than the control group, whereas a non significant reduction was observed in the AS-ZnO group (Fig. 2B). Uric acid levels showed non

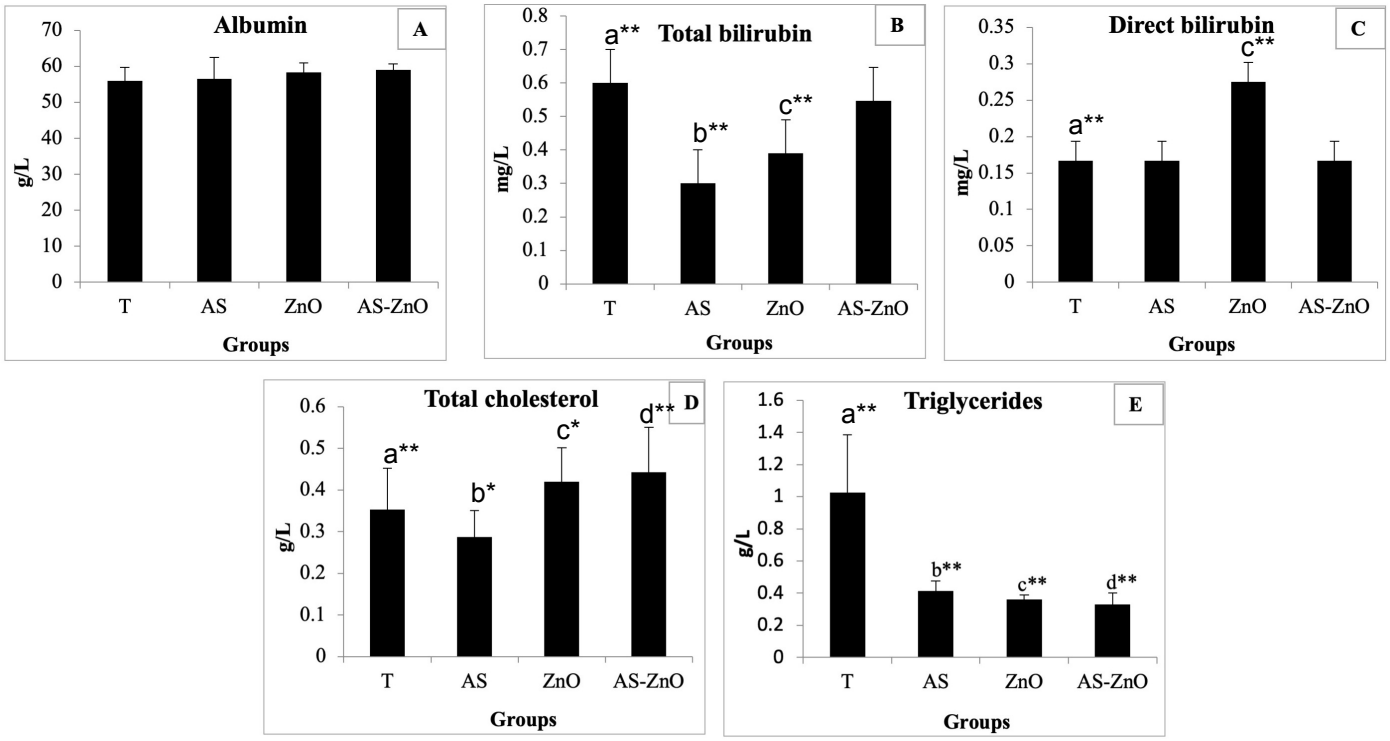

Fig. 1. *Variations in liver parameters (Mean ± SEM) in the control and treated groups after eight weeks of treatment*
Fig. 1A: Albumin levels (g/L), Fig. 1B: Total bilirubin levels (mg/L), Fig. 1C: Direct bilirubin levels (mg/L), Fig. 1D: Total cholesterol levels (g/L), Fig. 1E: Triglyceride levels (g/L)
T: Control, AS: Allium sativum, ZnO: Zinc oxide, AS-ZnO: Allium sativum-Zinc oxide

significant changes in the AS and ZnO groups, however, a significant increase was observed in the AS-ZnO group compared with the control (Fig. 2C).

3.3 Enzyme variations

The treatment with ZnO and *A.sativum* resulted in significant changes in plasma enzyme activity. A significant decrease in plasma ALP activity was observed in the ZnO and AS-ZnO groups and a non significant elevation was observed in the AS treated group compared with the control (Fig. 3A). Lactate dehydrogenase activity increased sgnificantly in the ZnO treated group. However, AS treated group revealed a significant decrease compared with the control, whereas AS-ZnO group was statistically similar to control group (Fig. 3B).

3.4 Variations in relative weight of organs

The administration of *A. sativum* and/or ZnO resulted in changes in relative organ weights. The relative weight of the liver showed a significant decrease in AS, ZnO, and AS-ZnO treated groups compared with the control (Fig. 4A). However, the relative weight of the kidney revealed significant incease in AS and ZnO treated groups. Non significant change was observed in AS-ZnO treated group compared with the control (Fig. 4B).

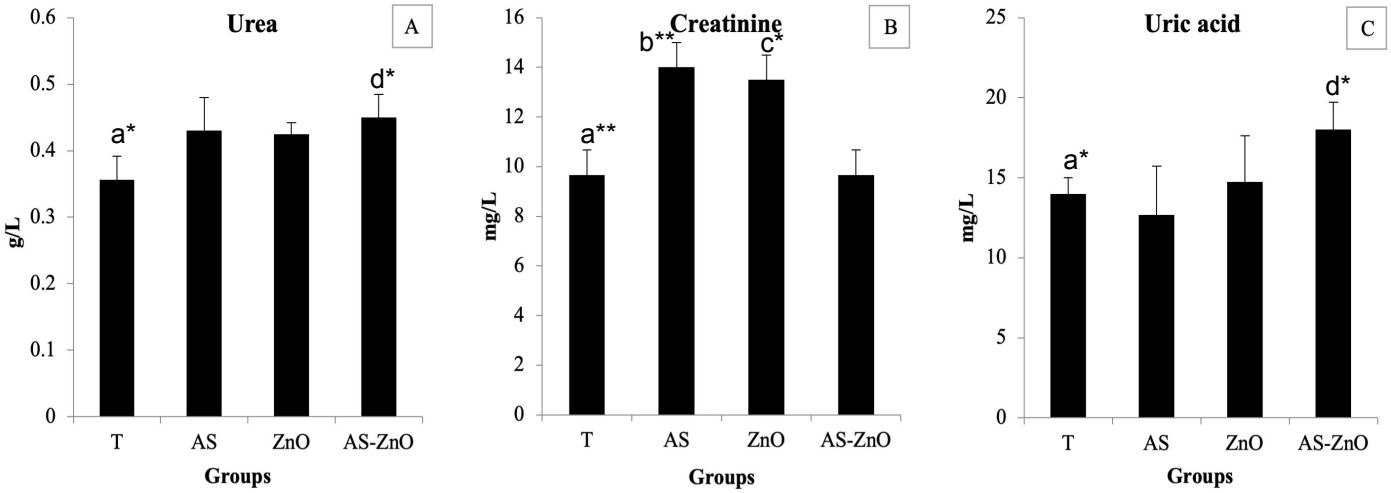

Fig. 2. *Variations in kidney parameters (Mean ± SEM) in the control and treated groups after eight weeks of treatment*
Fig. 2A: Urea levels (g/L), Fig. 2B: Creatinine levels (mg/L), Fig. 2C: Uric acid levels (mg/L)
T: Control. AS: Allium sativum. ZnO: Zinc oxide. AS-ZnO: Allium sativum-Zinc oxide

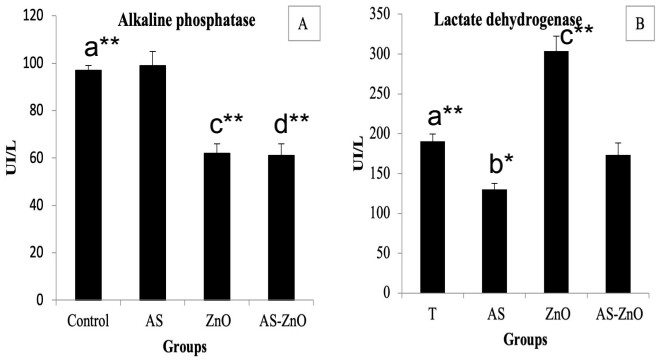

Fig. 3. *Variations in the activity of enzymes (Mean ± SEM) in the control and treated groups after eight weeks of treatment*
Fig. 3A: Alkaline phosphatase (UI/L), 3B: Lactate dehydrogenase (UI/L).
T: Control, AS: Allium sativum, ZnO: Zinc oxide, AS-ZnO: Allium sativum-Zinc oxide

3.5 Histological observations

3.5.1 Liver histopathology

Histological sections taken from the liver of rabbits treated with *A.sativum* (Fig. 5C, 5D) revealed nearly normal tissue structure compared with the control group (Fig. 5A, 5B), with intact hepatocytes and slight hypertrophy of cells but without any signs of necrosis or inflammation. However, the ZnO group (Fig. 5E, 5F) showed congestion of the centrilobular veins, cytoplasmic vacuolization, slight hypertrophy of the hepatocytes, pycnotic nuclei, and marked vacuolization accompanied by loss of membrane integrity, indicating significant cellular damage. The AS-ZnO group (Fig. 5G, 5H) showed a tissue structure closer to the control, with a marked reduction in the damage observed in the ZnO group. The hepatocytes showed a notable improvement, and better preservation of tissue architecture, with a reduction in vacuolization.

3.5.1 Kidney histopathology

Microscopic examination of kidney sections of rabbits in AS group (Fig. 6C, 6D) revealed a normal renal parenchyma with well defined glomeruli and tubules, and moderate tubular epithelial hypertrophy without any pathogonomic sign. Histological sections of kidneys from ZnO rabbit group (Fig. 6E, 6F) showed vacuolization of proximal tubular epithelial cells, moderate tubular dilatation, intra-glomerular hemorrhages, and mild inflammatory infiltration. The AS-ZnO group (Fig. 6G, 6H) revealed reduced tissue damage compared to ZnO group rabbits, with minimal vacuolization and relatively intact glomeruli.

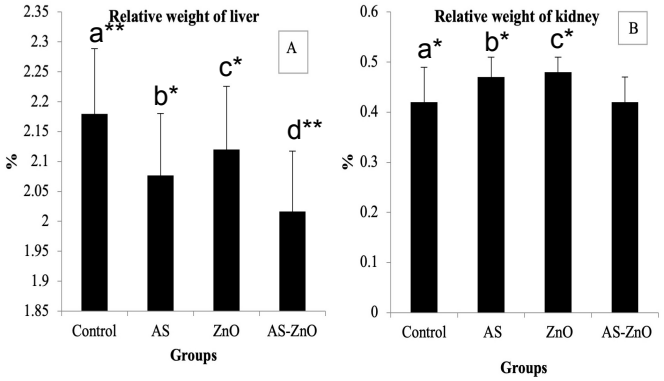

Fig. 4. *Variations in relative weight of organs (Mean ± SEM) in the control and treated groups after eight weeks of treatment*
Fig. 4A: Relative weight of liver (%), 4B: Relative weight of kidney(%)
T: Control, AS: Allium sativum, ZnO: Zinc oxide, AS-ZnO: Allium sativum-Zinc oxide

4. Discussion

The present study demonstrated alterations in hepatic and renal biomarkers following ZnO and/or *A. sativum* administration, reflecting both toxic and protective effects. Albumin levels reflect the hepatic protein synthetic function and the absence of any significant changes in its levels indicated that the hepatic protein synthesis was not disturbed, even after 60 days of exposure. Clinical studie have shown that zinc supplementation does not affect plasma albumin levels, particularly in hemodialysis patients, where overall albumin levels remain stable despite improved zinc status (Mastoor-Tehrani et al. 2024). The total bilirubin, a biomarker of hepatobiliary function, showed a significant reduction in all treated groups compared to the control. *A. sativum* is well documented for its hepative protective role by stimulating antioxidant enzymes (SOD, CAT, GPx), thereby enhancing neutralization of free radicals produced by oxidative stress and hence improves liver function (Banerjee et al. 2001). This improved hepatic function may facilitate bilirubin clearance, leading to a decrease in its levels. Garlic also has a detoxification effect by promoting liver regeneration, which could contribute to improved biliary excretion of total bilirubin and consequent lower serum bilirubin levels (Hamdy et al. 2024). ZnO may exhibit antioxidant effects, at low concentrations, by reducing hepatic oxidative stress. A study has shown that ZnO can improve hepatic antioxidant activity and regulate bilirubin levels due to its nanostructured properties (Bashandy et al. 2021). Conversely, the consumption of *A. sativum* showed non significant effect on direct bilirubin levels, suggesting no intereference garlic in bilirubin metabolism. However, ZnO caused a significant increase in direct bilirubin, likely due to hepatocellular injury and bile duct dysfunction caused by oxidative stress due to high ZnO doses (Pei et al. 2022; Al-Ragi et al. 2024). High levels of cholesterol in the ZnO and AS-ZnO groups indicate disruption in lipid metabolism. *A. sativum* is known for its lipid-lowering effects (Banerjee and Maulik 2002; Singh et al. 2009; Farid et al. 2022) and this effect was observed in present study study as well. On the other hand, the increased cholesterol levels observed in ZnO group indicates oxidative stress induced lipid dysregulation. Furthermore, the AS-ZnO group exhibited even more higher cholesterol levels, possibly due to synergistic metabolic interactions between ZnO and AS. Triglyceride decreased significantly in all treated groups, which indicates enhanced lipid catabolism. Garlic, particularly its sulfur compound – allicin, is widely known for its triglyceride lowering properties (Banerjee and Maulik 2002). ZnO promotes lipophagy through Zn^{2+} dependent activation of MTF-1/PPARα and AMPK pathways, leading to reduced lipid accumulation in the liver (Wei et al. 2018). Furthermore, Zinc also influences the β-catenin signaling pathway, enhancing lipolysis and inhibiting lipogenesis through HDAC3-mediated deacetylation (Xu et al. 2023). The combination of *A. sativum* and ZnO in this study reveals synergistic effect, which amplifies these lipid-lowering mechanisms.

Renal biomarkers (creatinine, urea, uric acid) are the products of protein metabolism. The results of present study revealed a significant increase in creatinine and non significant increase in urea levels in the ZnO treated group, reflecting zinc-induced nephrotoxicity (Chouit 2017) with a reduction in the glomerular filtration rate and the subsequent development of renal failure (Karale and Kamath 2017).

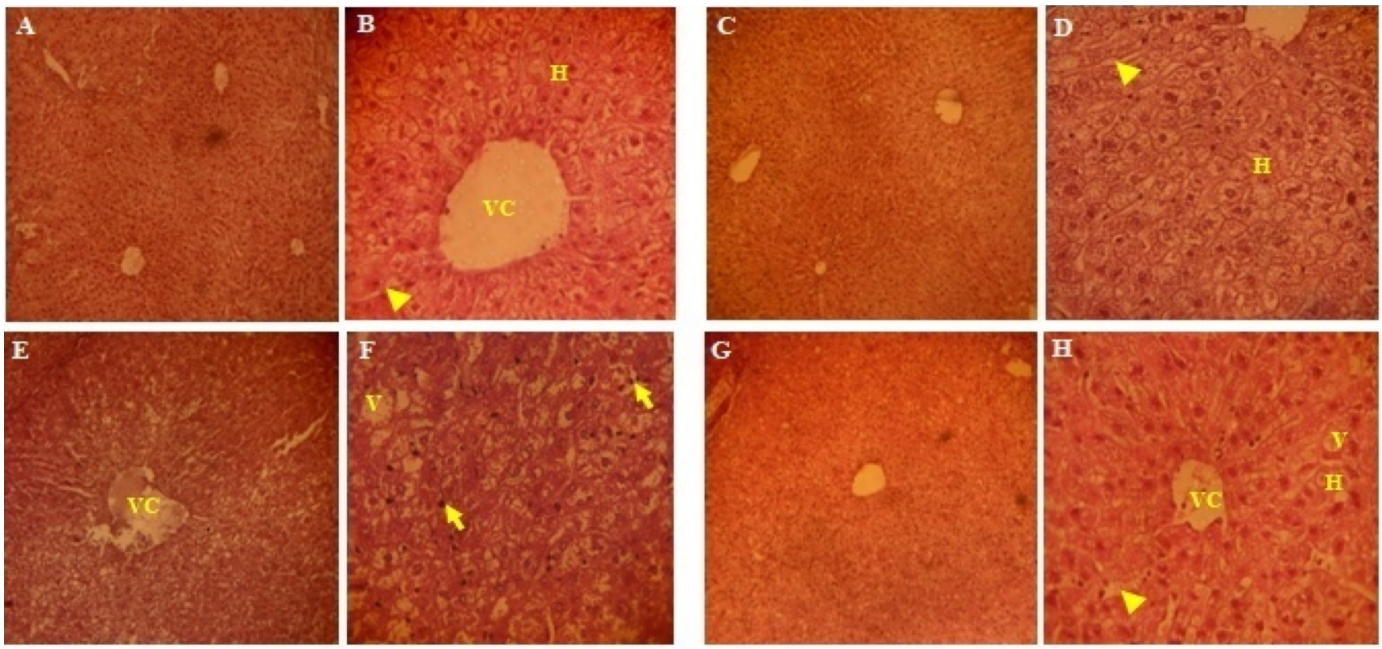

Fig. 5. *Haematoxylin and Eosin stained histological sections of the rabbit liver in control and treated groups after eight weeks of treatment*
Fig. 5A, 5B: Control, Fig. 5C, 5D : Allium sativum group, Fig. 5E, 5F: Zinc-oxide group, Fig. 5G, 5H : Allium sativum-Zinc oxide group
The magnification is 100 x and zoom 1.6x for Fig. 5A, C, E, and G ; and 400x, zoom 1.6x for Fig. 5B, D, F, and H
H: Hepatocytes, VC: Veine centrolobulaire, Arrow: Noyau pycnotique, Arrowheads: Sinusoides

According to Stohs and Bagchi (1995) the significant increase in renal functional markers after exposure to heavy metals can be attributed to cellular damage resulting from excessive free radical production. Garlic administration in the rabbits decreased the uric acid levels in the present study, corroborating earlier reports of its nephroprotective effects (Verma et al. 2021).

Alkaline phosphatase (ALP) is an indicator of liver and bile activity. The significant decrease in ALP activity observed in this study can be attributed to the fact that zinc promotes oxidative stress and excessive production of free radicals at high doses and/or over long periods of administration, which in turn decreases alkaline phosphatase activity. According to the study of Dudeja and Brasitus (1993), ROS inhibit ALP activity at the brush border membrane of the rat intestine without affecting other enzymes, and cause oxidative inactivation of brain alkaline phosphatase responsible for hydrolysis of phosphocholine (Sok 1999). The level of alkaline phosphatase (ALP) increased non significantly in the group receiving *A. sativum* alone. This may reflect the stimulation of metabolic or regenerative processes induced by the active compounds in garlic, particularly allicin and organic sulphur

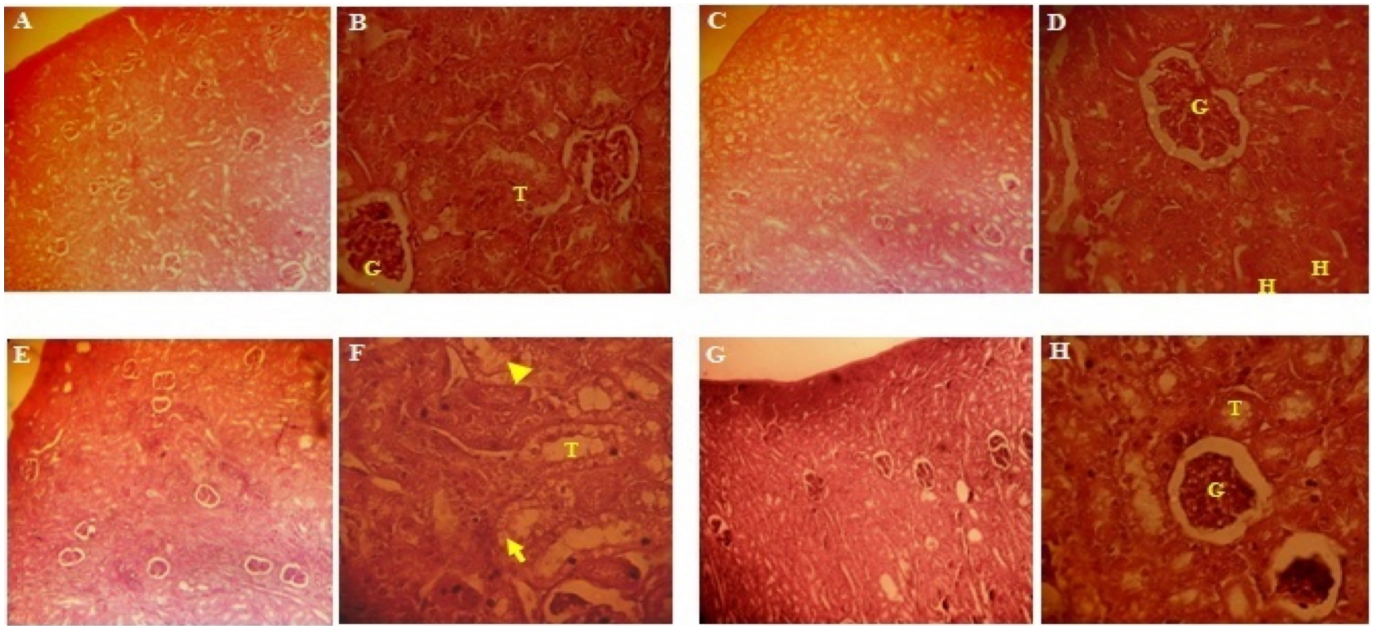

Fig. 6. *Haematoxylin and Eosin stained histological sections of the rabbit kidney in control and treated groups after eight weeks of treatment*
Fig. 6A, 6B: Control, Fig. 6C, 6D : Allium sativum group, Fig. 6E, 6F: Zinc-oxide group, Fig. 6G, 6H : Allium sativum-Zinc oxide group
The magnification is 100 x and zoom 1.6x for Fig. 5A, C, E, and G ; and 400x, zoom 1.6x for Fig. 5B, D, F, and H
G : Glomerule, T: Tubule renal, Arrow: Vacuolisation, Arrowheads: Infultration inflammatoire, H: Hypertrophie

compounds. Lactate dehydrogenase (LDH) is an enzyme that represents an important biomarker of cell damage and tissue necrosis, particularly in the liver and kidneys. The group treated with *A. sativum* alone showed a moderate decrease, reflecting reduced cell lysis due to improved cell protection against oxidative stress by increasing the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Chidoka and Amadikwa 2014). The significant increase of LDH in the ZnO-treated rabbits indicates hepatocellular damage, while its decrease in AS and AS-ZnO groups reveal the antioxidant role of garlic in preventing cellular damage (Pei et al. 2022).

Changes in organ weight are sensitive markers of harmful compounds or heavy metal toxicity (Suriyavadhana and Tpakutharivu 2011). A significant decrease in the relative weight of the liver in all treated groups was observed, with more pronounced reduction in the AS-ZnO group. This change may reflect metabolic modulation. A significant decrease in liver weight due to garlic treatment could indicate metabolic adaptation, or cellular remodelling. Garlic is also well known for having a lipid lowering effect on the liver, and could therefore eliminate lipid deposits, reducing its mass. However, The relative weight of kidney increased significantly in both the AS and ZnO treated groups compared with the control. This finding suggests that both substances may induce physiological or metabolic changes in renal tissue. However, the underlying mechanisms are likely different. A study on rats fed an excess zinc diet reported that zinc accumulated in kidney tissue, resulting in increased kidney weight (Khorsandi et al. 2018). High doses of zinc can cause renal dysfunction, inflammation and tubular damage, associated with the excessive production of superoxide anion radicals (OO^{-}), which can induce renal hypertrophy (Yanagisawa et al. 2014). In addition, the inclusion of garlic in the rat diet along with regular feed, led to enhanced performance and increased kidney weight (Ejiogu 2024).

Histological findings strongly support these biochemical trends. Garlic maintained hepatic and renal integrity in this study due to its key role in reducing oxidative stress and inflammation, which is consistent with prior studies (Ahmed 2018; Shang et al. 2019; Lee et al. 2019; Dorrigiv et al. 2020; Sheir et al. 2025). The observation of ZnO-induced toxicity, characterized by hepatic accumulation of Zn, cytoplasmic vacuolisation, membrane degeneration, necrosis and slight hepatocyte hypertrophy, has been reported earlier as well (Oyebadejo et al. 2014; Al-Ragi et al. 2024). The group treated with a combination of AS-ZnO had a structure closer to that of the control group revealing a notable reduction in the abnormalities induced by ZnO alone. Similarly, the renal toxicity induced by ZnO and characterized by glomerular dilation and tubular vacoularization and necrosis (Hosseini et al. 2018; Attal and Bouchema 2021; Liu et al. 2024) was reversed by garlic co-administration, preserving structural integrity, likely due to its potent antioxidant and anti-inflammatory properties (Tandon et al. 2008; Tugbobo et al. 2016).

5. Conclusions

In conclusion, this study demonstrated that *Allium sativum* exhibits hepatoprotective and nephroprotective properties against zinc-induced toxicity. ZnO exposure resulted in significant biochemical and histological alterations in the liver and kidneys, while garlic administration mitigated these effects by enhancing antioxidant defense and preserving tissue architecture. Combined treatment maintained most biochemical parameters within normal ranges and reduced organ damage, emphasizing the therapeutic potential of *A. sativum* in protecting against heavy metal-induced oxidative stress.

Declarations

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Conflict of interest: All authors declare no conflicts of interest

Acknowledgements: None

Ethics approval: The study was approved by the Laboratory of Animal Ecobiology and Physiology and the Faculty of Exact Sciences and Natural and Life Sciences (Approval code: AEA/LEBPA-E0612800/001/2024).

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