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AMELIORATIVE ROLE OF VITAMIN C AGAINST ZINC-OXIDE NANOPARTICLE-INDUCED OXIDATIVE STRESS AND KIDNEY DYSFUNCTION IN MALE WISTAR RATS

[1]Edene, O. A and [2]*Asenuga, E. R.

[1]Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Nigeria.
[2]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin.
***Corresponding Author: E-mail:** ebunoluwa.olowu@uniben.edu Phone: +2348052720219

ABSTRACT

The protective effect of vitamin C (Vit C) against ZnO NP-induced kidney toxicity in male Wistar rats was investigated in this study. Thirty rats Obtained from the Animal house of the Department of Anatomy, University of Benin, were divided into five groups: Group A: Control (distilled water), Group B: ZnO only (70 mg/kg), and three co-treatment groups receiving ZnO (70 mg/kg) plus Vit C at 100, 200, and 500 mg/kg for 7 days (Group C, D and E, respectively). Exposure to ZnO NPs led to significantly ($p<0.05$) increased total protein, malondialdehyde (MDA), and sodium levels, modified urea and bicarbonate concentrations, altered superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, and led to severe tubular necrosis in group B. Co-administration with vitamin C at 100 and 200 mg/kg resulted in the restoration of these biochemical parameters, reduced oxidative stress, and ameliorated histopathological lesions in a dose-dependent manner. However, there was 100% mortality in the group that received 500 mg/kg vitamin C, suggesting a paradoxical pro-oxidant effect at supraphysiological doses. These findings proved that vitamin C at optimal doses (100–200 mg/kg) effectively alleviates ZnO NP-induced nephrotoxicity through antioxidant mechanisms, but high doses may likely exacerbate toxicity. Establishing therapeutic windows is essential for antioxidant augmentation in nanotoxicology.

KEYWORDS: *Nephrotoxicity, oxidative stress, ZnO-nanoparticles, vitamin C*

1.0.INTRODUCTION

The significant positive impact of nanoparticles has been observed in various sectors such as health, electronics, energy generation, and environmental science due to their variety of features that make them extremely adaptable and useful in a wide range of applications (Martínez *et al*., 2020). The manipulation and control of matter on an extremely small scale is called nanotechnology. A nanoparticle is a small particle that has a dimension between 1 and 100 nanometres. The nanoparticle materials and systems exhibit distinctive features because of their size (Bundschuh *et al.,* 2018). These little particles display outstanding physicochemical characteristics compared to bulk zinc oxide (Czyżowska and Barbasz, 2022). These characteristics give them the possibility of entering the ecosystem via several pathways, like wastewater discharge, product usage, and industrial effluents; as such, having a thorough understanding of their toxicity and behaviour is extremely important (Singh, 2019). As a result of the distinctive features and diverse applications of ZnO NPs, it has garnered important attention in research and development. They are known for their excellent antibacterial, photocatalytic, UV-filtering, and semi-conducting capabilities, making them promising for applications in medicine, electronics, cosmetics, energy, and environmental remediation (Czyżowska and

Barbasz, 2022). However, despite their advantageous characteristics, the unique properties of ZnO NPs also raise concerns with regard to the likely environmental and human health impacts (Arumugam *et al.,* 2025). Evidence indicates that Zinc oxide nanoparticles (ZnO NPs) can enter the human body through multiple pathways, including dermal absorption, ingestion, and inhalation. (Chen *et al.,* 2022). The ZnO NPs circulate through the blood and accumulate in organs once they enter the body, potentially interfering with normal activities (Khorsandi *et al.,* 2018). Studies have shown that exposure to excessive amounts of ZnO NPs or exposure for an extended period can damage the kidneys, manifesting as inflammation, oxidative stress, changes in renal function, and physical damage (Rana, 2021; Abouzeinab *et al*., 2023; Hassan *et al*., 2024). The toxicological profile of ZnO nanoparticles is influenced by multiple physicochemical and exposure-related parameters, including particle size, surface functionalization, administered concentration, and the specific route of entry into the body. Therefore, it is important to control the use of ZnO NPs to prevent or minimise any unwanted consequences (Du *et al.,* 2018).

Researchers are investigating the use of antioxidants such as vitamin C as a means of preventing or minimising the prospective renal impairment caused by ZnO NPs (Amal *et al*., 2021; Noorin *et al*., 2024; Oghenetega *et al*., 2024). Ascorbic acid or vitamin C is a water-soluble vitamin that is essential for several physiological functions within the body (Pehlivan, 2017). It is abundant in fruits and vegetables, and plays a critical role in protecting the body against oxidative damage by neutralizing free radicals (Kaźmierczak-Barańska *et al*., 2020; Doseděl *et al*., 2021; Alberts *et al*., 2025). Because it cannot be stored endogenously, continuous intake through diet or supplementation is required. Its antioxidant properties not only reduce oxidative stress but also modulate inflammatory responses and supplementation. It has been shown to attenuate kidney injury associated with ZnO nanoparticle exposure (Noorin *et al*., 2024). While initial findings suggest that vitamin C may mitigate nanoparticle-induced nephrotoxicity, further work is needed to establish optimal dosage, timing, and long-term outcomes (Noorin *et al*., 2024).

The male Wistar rat is a laboratory animal that is widely used in biomedical research. It was developed by the Wistar Institute in Philadelphia, USA. The weight of these rats is usually around 250-350 grams, and they have a life expectancy of about 2-3 years. The Wistar rats are preferred for research because it is easy to handle, docile in nature, have a high fertility rate, are able to adapt to several experimental conditions, and because it shares many similarities with humans biologically and physiologically. Wistar rats' metabolisms, cardiovascular systems, drug responses, endocrine functions, and reproductive systems are similar with humans. They are therefore useful for studying diseases and processes, toxicological studies, drug testing, and behavioural research (Patel *et al.,* 2024).

2.0 MATERIALS AND METHODS

2.1 Animal protocol

Thirty healthy adult male Wistar rats weighing between 130 and 200 g were used for this study. The rats were obtained from the Department of Anatomy Animal house, University of Benin, Benin City, Nigeria. Acclimatisation was for 2

weeks, in which the rats were exposed to 12 hours of darkness and 12 hours of light. The rats were well fed with commercial food (pellets) and water ad libitum throughout the study. The nanoparticles used in this research were purchased from Sigma Aldrich, while other chemicals used for this study are all of analytical grades and purchased from reputable companies.

2.2 Experimental Design

The thirty (30) rats were divided into groups of five, based on their weights, with six rats in each group (Yu *et al*., 2014). The groups consisted of one control group and four experimental groups. After the two weeks of acclimatisation, administration of nanoparticles (ZnO) and antioxidant (vitamin C) began. The control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, group B was administered 70 mg/kg of ZnO only, group C 70 mg/kg of ZnO and 100 mg/kg vitamin C, group D 70 mg/kg ZnO and 200 mg/kg vitamin C and group E, 70 mg/kg ZnO and 500 mg/kg vitamin C. The vitamin C was administered orally through the use of oral gavage, while the ZnO was given through intraperitoneal administration. Administration was for 7 days, and feed and water were withdrawn 24 hours before the termination of the experiment.

The rats were rendered unconscious by exposing them to chloroform, sacrificed, dissected, and the kidney tissues extracted for analysis. Blood was obtained from the heart using heparinised tubes. Plasma was then separated by centrifugation at 4000 rpm for 15 minutes, and kept in a refrigerator at 4°C. Representative samples from each organ were fixed in formalin and processed for histopathological analysis.

The kidney tissues collected were rinsed in normal saline solution, dried with filter paper, and weighed before being homogenised in four volumes of the homogenising buffer (0.1 M phosphate buffer, pH 7.4). Following that, the homogenates were centrifuged at 10,000 g for 10 minutes in a cold centrifuge, and the supernatant was stored at -4 °C for biochemical analysis.

Total protein concentration was determined using kits acquired from Randox Laboratory Limited in the United Kingdom, carefully following the biuret reaction method (Gornal *et al.,* 1949). Catalase (CAT) activity was estimated by the method described by Cohen *et al.* (1970). SOD activity was ascertained using Misra and Fridovich's (1972) methodology. Estimation of glutathione peroxidase (Gpx) was calculated according to Nyman (1959). The thiobarbituric acid assay was used to quantify malonaldehyde (Buege and Aust, 1978). Urea concentration was determined according to the method of Weatherburn (1967). The plasma creatinine concentration was determined using the Randox Diagnostic Kit, as described by Bartels and Bohmer (1972). The electrolytes sodium, potassium, chloride, and bicarbonate were analysed using an ion-selective electrode (ISE). Histopathology was carried out by following the procedure of Drury and Wallington (1980). GraphPad Prism version 10.0 software was used for the statistical and graphical analysis. The endpoint and chronological approaches reported data as mean ± SEM (standard error of mean), with a 95% confidence range defined at $p<0.05$. One-way ANOVA and Tukey comparison post-HOC test was used to compare the treatment and control groups.

3.0 RESULTS AND DISCUSSION

This study investigated the ameliorative effect of different concentrations of vitamin C on ZnO-NP-induced toxicity in the kidney of male Wistar rats for 7 days. We experienced 100% mortality with rats in Group E, which received 500 mg/kg of vitamin C alongside ZnO NPs. This unexpected finding implies a potential dose-toxicity relationship at supraphysiological levels of vitamin C. Generally, vitamin C is regarded as being safe, though high doses can exert pro-oxidant effects under some conditions, especially in the presence of transition metals such as zinc (Doseděl *et al*., 2021). Notably, the death of all rats in Group E (500 mg/kg vitamin C + ZnO) suggests that there might be a paradoxical pro-oxidant effect at supraphysiological doses. Studies have shown that high-dose vitamin C may speed up the metal-catalysed hydroxyl radical formation via Fenton chemistry, aggravating nanoparticle toxicity (Nowak *et al*., 2021). These findings affirm the importance of establishing optimal therapeutic windows for antioxidant supplementation in nanotoxicology studies

The term "total protein" describes all of the protein in the blood, including globulins and albumin. Proteins are necessary for several biological processes, such as preserving fluid balance, transferring materials throughout the blood, and bolstering the immune system. In this study, there was a significant ($p<0.05$) increase in total protein concentration observed in group B when compared to the control (Figure 1). This indicates that ZnO nanoparticles induced an elevation in the protein concentration (Yan *et al*., 2012). The accumulation of nanoparticles in the renal tissues may have led to the significant elevation in total protein concentration attributed to an acute phase response or cellular damage. Studies have shown that ZnO nanoparticles can generate reactive oxygen species (ROS), which can lead to the alteration of membrane permeability and result in leakage of intracellular proteins into the bloodstream (Yan *et al*., 2012; Mirzaei *et al*., 2025). Co-administration of vitamin C in groups C and D led to a significant reduction in total protein concentration when compared to group B, and also when group D was compared to group A (Figure 1), suggesting dose-dependent protection against nanoparticle toxicity. This is similar to the studies of Essia (2025) and Abd-elrahman *et al*. (2024). The ability of vitamin C to scavenge free radicals directly and also regenerate other antioxidants may likely be responsible for its protective effect, which stabilises cellular membranes. It has been confirmed recently that vitamin C is capable of significantly reducing ZnO-NP-induced proteinuria and preserving kidney integrity (Noorin *et al*., 2024).

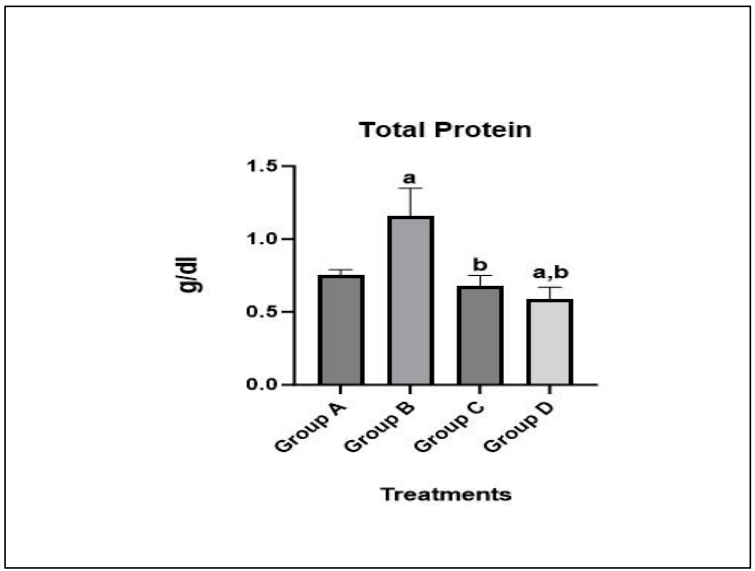

Figure 1: Total protein Concentration in the plasma of male Wistar rats exposed to varied levels of vitamin C co-administered with ZnO nanoparticles. Values are expressed as mean ± SEM, (n=5) for male Wistar rats, a and b indicate significant difference ($p<0.05$) when compared to group A and group B, respectively.
The control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, Group B, was administered 70 mg/kg of ZnO only, Group C was given 70 mg/kg of ZnO and 100 mg/kg of vitamin C, Group D was given 70 mg/kg ZnO and 200 mg/kg of vitamin C.

Superoxide dismutase (SOD) catalyses the transformation of superoxide radicals (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). SOD assists in preventing oxidative damage to cells and tissues by scavenging superoxide radicals (Anwar *et al*., 2025). The antioxidant enzyme catalase (CAT) is responsible for breaking down hydrogen peroxide (H_2O_2) into oxygen and water. In doing so, catalase contributes to the neutralisation of H_2O_2, a reactive oxygen species that may harm cells through oxidative stress, while using reduced glutathione (GSH) as a cofactor (Anwar *et al*., 2024). The antioxidant enzyme glutathione peroxidase (GPx) catalyses the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides (Jomova *et al*., 2024). The significant reduction in the activity of SOD and catalase in the kidneys of rats in group B, when compared with the control, showed that nanoparticles can induce oxidative stress (Figures 2 and 3). The chief mechanism of nanoparticle-induced nephrotoxicity is this oxidative burst, which results in lipid peroxidation and DNA damage in kidney cells (Rana, 202l; Shafiq *et al*., 2025). The reduction in the activities of SOD and catalase indicates an intense crisis of the complete antioxidant enzyme cascade, leading to the generation of superoxide radicals faster than they can be dismutated, and the kidney becomes susceptible to hydrogen peroxide accumulation. GPx is a very important antioxidant enzyme involved in breaking down hydrogen peroxide and lipid peroxide using glutathione (GSH); its significant ($p<0.05$) elevation in group B (Figure 4) indicates that the ability to repair oxidative membrane damage was heightened, and this can be an adaptive response towards oxidative stress elicited by ZnO nanoparticles. This disruption of the entire antioxidant defence system is a hallmark of nanotoxicity, resulting in ROS production exceeding the capacity of the cellular antioxidant machinery (Manful *et al*., 2025; Qin *et al*., 2026).

The co-administration of vitamin C in groups C and D dose-dependently restored SOD activity (Figure 2), with activities in those groups exceeding control levels, pointing toward antioxidant rescue (Oghenetega *et al*., 2024). Studies have shown that 200 mg/kg of vitamin C can effectively upregulate the gene expression of SOD in kidney tissues challenged with ZnO NPs (Noorin *et al*., 2024). The ability of vitamin C to restore SOD activity is important because it helps convert superoxide radicals into hydrogen peroxide, which is then neutralised by catalase and GPx. There was a dose-dependent restoration of catalase activity toward control levels in groups C and D co-administered with vitamin C, with group D (200 mg/kg vitamin C) showing marked

improvement (Figure 3). Recently, it has been discovered that aside from vitamin C's ability to restore enzyme activity, the expression of pro-inflammatory cytokines such as TNF-α and NF-κB can be activated by nanoparticle-induced oxidative stress, and this activation can also be reduced by vitamin C administration (Noorin *et al*., 2024; Alberts *et al*., 2025). There was also a restoration of GPx activity in groups C and D co-treated with vitamin C in a dose-dependent manner, with group D (200 mg/kg vitamin C) showing nearly complete normalisation (Figure 4), indicating that vitamin C has a protective role against nanoparticle-mediated antioxidant enzyme depletion, which is similar to the findings of Abd-elrahman *et al*., 2024. Findings from this study are similar to those of Noorin *et al*. (2024), who confirmed that co-administration of vitamins C and E significantly prevents the downregulation of GPx gene expression induced by ZnO NPs in renal tissues.

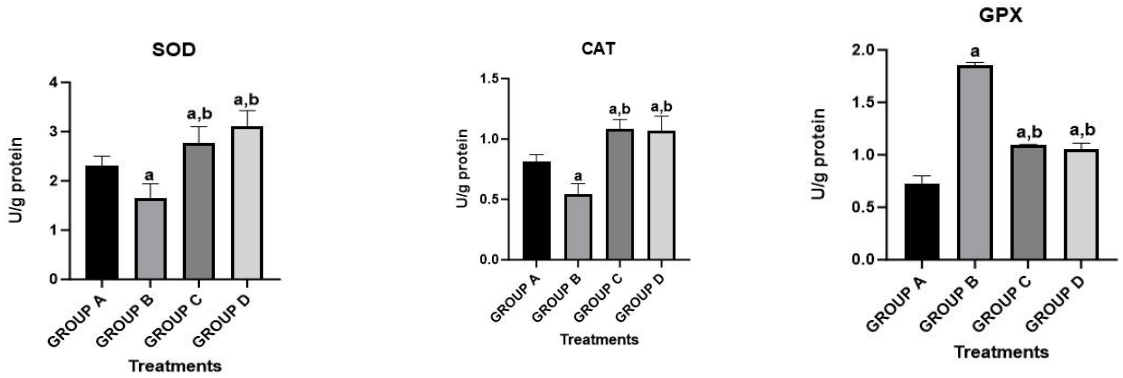

Figure 2, 3 & 4: The activity of Superoxide dismutase, Catalase, and Glutathione Peroxidase in the kidney of male Wistar rats exposed to varied levels of vitamin C co-administered with ZnO nanoparticles. Values are expressed as mean ± SEM, (n=5) for male Wistar rats, a and b indicate significant difference ($p<0.05$) when compared to group A and group B, respectively. The control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, Group B was administered 70 mg/kg of ZnO only, Group C was given 70 mg/kg of ZnO and 100 mg/kg of vitamin C, Group D was given 70 mg/kg ZnO and 200 mg/kg of vitamin C.

Malondialdehyde (MDA) is a lipid peroxidation marker produced when ROS degrade lipids in cell membranes, resulting in the creation of MDA. Lipid peroxidation can alter the integrity and function of cell membranes, thereby leading to a variety of illnesses and ageing processes (Cordiano *et al*., 2023). The concentration of malondialdehyde (MDA) levels in group B was significantly increased compared to the control group (Figure 5), indicating increased lipid peroxidation due to ZnO nanoparticle-induced oxidative stress (Belal and Gad, 2023). MDA is a stable end product of polyunsaturated fatty acid peroxidation in cell membranes, and an increase in its concentration directly relates to the extent of cellular injury induced by free radicals. The generation of hydroxyl radicals is facilitated by the fact that ZnO NPs have a high surface-area-to-volume ratio (Yan *et al*., 2012; Gultekin *et al*., 2025). Co-administration of vitamin C in groups C and D led to a significant ($p<0.05$) dose-dependent reduction of MDA levels, with group D (200 mg/kg

vitamin C) being the most effective, affirming the protective antioxidant ability of vitamin C against membrane lipid damage which is in accordance with the Oghenetega *et al*., 2024) Vitamin C is a water-soluble vitamin, which allows it to work synergistically with vitamin E to break the chain of lipid peroxidation in the aqueous phase of the cell membrane (Ogunleye *et al*., 2026).

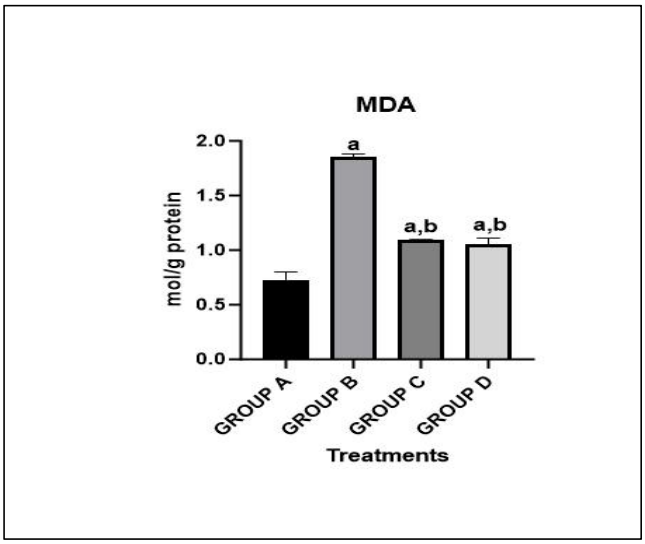

Figure 5: The concentration of MDA in the kidney of male Wistar rats exposed to varied levels of vitamin C co-administered with ZnO nanoparticles. Values are expressed as mean ± SEM, (n=5) for male Wistar rats, a and b indicate significant difference ($p<0.05$) when compared to group A and group B, respectively.
The control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, Group B, was administered 70 mg/kg of ZnO only, Group C was given 70 mg/kg of ZnO and 100 mg/kg of vitamin C, Group D was given 70 mg/kg of ZnO and 200 mg/kg of vitamin C.

Sodium is a crucial electrolyte involved in fluid balance, nerve function, and muscle contraction. Abnormal sodium levels can affect blood pressure, hydration status, and nerve function (Kavyadeepu and Mohnish, 2025). There was a significant ($p<0.05$) increase in the concentration of Na^+ in the blood of rats in group B compared to the control group (Table 1), showing ZnO nanoparticle-induced hypernatremia, which might be due to renal tubular dysfunction similar to the study of Liu *et al*. 2024. It can be suggested that ZnO NPs have a direct cytotoxic impact on tubular epithelial cells because the observed hypernatremia arises precisely from impairment of the renal tubules responsible for sodium reabsorption. This electrolyte disturbance is frequently accompanied by compromised concentrating capacity of the kidney and is a leading indicator of tubular injury, even though glomerular markers may remain normal (Goyal *et al*., 2023). Co-administration with vitamin C in groups C and D led to restoration of Na^+ levels toward normal (Table 1). The restoration of sodium balance by vitamin C emphasises its ability to preserve the structural and functional integrity of renal tubules damaged by ZnO nanoparticles (Ge *et al*., 2026).

Heart muscle, among other neuron and muscle functions, depends on potassium (K^+) (Oberman *et al*., 2026). There were no

significant changes in K^+ concentration across all groups, suggesting that ZnO nanoparticles and vitamin C did not noticeably disrupt potassium homeostasis despite other electrolyte disturbances (Table 1). This clinically signifies that the Na^+/K^+ ATPase pump activities in the basolateral membrane of the tubules remain operational. While sodium homeostasis is often altered in the early phase of toxic nephropathy, potassium levels are tightly controlled through aldosterone-dependent pathways, and changes may only be observed in severe, late-stage renal failure. The fact that hyperkalemia was absent in this study clearly indicates that 70 mg/kg of ZnO NP did not lead to renal failure or extensive cellular lysis. Another electrolyte that is essential for preserving the body's acid-base balance and appropriate fluid balance is chloride. Chloride levels that are abnormal may be a sign of kidney failure, dehydration, or certain metabolic diseases (Doseděl *et al*., 2021). The concentration of chloride ions was stable across all groups (Table 1), showing that neither ZnO nanoparticle exposure nor vitamin C co-treatment impacted chloride ion balance significantly in male Wistar rats (Sagar and Lohiya, 2024). The maintenance of chloride homeostasis relates to sodium changes passively to maintain electroneutrality; however, the stability of chloride relative to sodium indicates a possible mild anion gap metabolic acidosis rather than simple hyperchloremic acidosis. This is a consistent pattern with renal damage where loss of bicarbonate (rather than chloride retention) is the primary physiological response. The findings from this study imply that renal management of monovalent anions remains resilient even under oxidative stress induced by nanoparticles (Wang *et al*., 2023; Gonsalez *et al*., 2019). Bicarbonate is an important component of the body's buffering system, helping to maintain proper pH balance in the blood. It is regulated by the kidneys and lungs (Shrimanker and Bhattarai, 2023). In this study, the concentration of bicarbonate in blood was significantly ($p<0.05$) decreased in group B (Table 1), which is a clear indication of metabolic acidosis from renal injury induced by ZnO nanoparticles (Korus *et al*., 2025). This is a core performance indicator of renal tubular acidosis, emanating from failure of the proximal tubules to reabsorb filtered bicarbonate or failure of the distal tubules to excrete hydrogen ions. ZnO NPs can accumulate in the mitochondria of proximal tubular cells, resulting in disruption of ATP production and the functional activities of carbonic anhydrase, an enzyme essential for bicarbonate reabsorption (Yan *et al*., 2012; Anand *et al*., 2023). There was a partial restoration of bicarbonate levels in groups C and D following co-administration with vitamin C. This partial rebound implies that while vitamin C efficiently minimises oxidative stress, high-dose antioxidant therapy might not completely compensate for critical structural damage to bicarbonate transport mechanisms.

Groups and their treatments	Na^+ in Blood (mmol/L)	K^+ in Blood (mmol/L)	Cl^- in Blood (mmol/L)	Bicarbonate in Blood (mmol/L)
Group A	138 ± 1.53	4.05 ± 0.07	101.3± 1.5	24.5 ± 2.12
Group B	142 ± 0.58[a]	3.95 ± 0.07	101 ± 1.53	21 ± 1.41[a]
Group C	138 ± 1.53[b]	4 ± 0.14	101.5 ± 1.29	24 ± 1.41[b]

Group D	140 ± 1.41[b]	4.1 ± 0.07	102.3 ± 2.08	23 ± 2.12[a,b]

Table 1: The concentration of **sodium (Na^+), potassium (K^+), chloride (Cl^-), and Bicarbonate (HCO_3^-)** in the blood of male Wistar rats exposed to varied levels of Vitamin C co-administered with ZnO nanoparticles.

Values are expressed as mean ± SD, (n=5) for male Wistar rats, a and b indicate significant difference ($p<0.05$) when compared to group A and group B, respectively. The control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, Group B was administered 70 mg/kg of ZnO only, group C was given 70 mg/kg of ZnO and 100 mg/kg of vitamin C, Group D was given 70 mg/kg ZnO and 200 mg/kg of vitamin C.

Urea is a waste product produced during the breakdown of proteins in the liver. The kidneys filter it out of the blood and expel it as urine. The level of urea in the blood can indicate how well the kidneys operate (Ernstmeyer and Christman, 2024). There was a significant ($p<0.05$) decrease in the concentration of urea in blood in group B (Table 2), which is unusual for nephrotoxicity and may indicate alterations in protein metabolism or urea cycle function following exposure to ZnO nanoparticles. A decrease in blood urea nitrogen (BUN) is an uncommon finding because most nephrotoxins result in azotemia. This may imply that ZnO NPs could be causing a catabolic state or interfering with hepatic urea production rather than simply decreasing glomerular filtration. This hypothesis is corroborated by research demonstrating that ZnO NPs can accumulate in the liver and disrupt nitrogen metabolism pathways, resulting in reduced synthesis of urea despite normal kidney function (Adeyomoye *et al*., 2022). Vitamin C co-administration in groups C and D resulted in restoration of urea concentration to near control values, reflecting effective amelioration (Mohammed *et al*., 2023). These findings point to the conclusion that the main effect of the nanoparticles was extra-renal metabolic disruption, which vitamin C effectively alleviated by decreasing renal oxidative stress.

During muscle metabolism, creatine phosphate is broken down to form creatinine, a waste product. Like urea, it is removed from the body by the kidneys and filtered out in urine. Creatinine levels in the blood are used to gauge renal function (Ávila *et al*., 2025). No significant differences in creatinine were observed across all groups (Table 2), indicating that despite other renal parameters being altered, glomerular filtration rate was not disrupted at this ZnO nanoparticle dose. The absence of creatinine elevation in comparison with reduced urea levels justifies further research into the involvement of specific nephron segments. Creatinine is considered a key marker of glomerular filtration more than urea because its production is fairly constant and unaffected by protein metabolism. The normal creatinine levels in group B strongly imply that the dose of ZnO nanoparticles used in this study causes specific tubular and metabolic dysfunction without inducing substantial glomerular damage or acute kidney injury. This pattern of "tubulopathy in the absence of glomerulopathy" is a feature of certain heavy metal and nanoparticle toxicities where the proximal convoluted tubule is the main target (Koyama *et al*., 2024).

Table 2: The concentration of **Urea and Creatinine** in the blood of male Wistar rats exposed to varied levels of ZnO nanoparticles.

Groups	Urea in Blood (mg/dl)	Creatinine in Blood (mg/dL)
Group A	46.7 ± 3.51	0.67 ± 0.06
Group B	34.5 ± 3.54[a]	0.63 ± 0.04
Group C	46.5 ± 6.36[b]	0.67 ± 0.06
Group D	46 ± 1.41[b]	0.69 ± 0.01

Values are expressed as mean ± SD, (n=5) for male Wistar rats, a and b indicate significant difference ($p<0.05$) when compared to group A and group B, respectively.

The control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, Group B was administered 70 mg/kg of ZnO only, Group C was given 70 mg/kg of ZnO and 100 mg/kg of vitamin C, Group D was given 70 mg/kg ZnO and 200 mg/kg of vitamin C.

Histopathological examination showed extreme renal tubular injury in rats in Group B, characterised by patchy tubular epithelial coagulation necrosis with intraluminal casts (Figure 6). These findings are in accordance with the confirmed nephrotoxic potential of ZnO NPs, which induce proximal tubular necrosis through oxidative stress and mitochondrial disruption as reported by Noorin *et al*. (2024). It was observed that groups that were co-administered with vitamin C at 100 mg/kg (Group C) and 200 mg/kg (Group D) showed no observable lesions. This implies that there was dose-dependent nephroprotection. This protective effect of vitamin C can be attributed to its ability to scavenge reactive oxygen species and maintain cellular membrane integrity (Ziamajidi *et al*., 2023).

Notably, the death of all rats in Group E (500 mg/kg vitamin C + ZnO) suggests that there might be a paradoxical pro-oxidant effect at supraphysiological doses. Studies have shown that high-dose vitamin C may speed up the metal-catalysed hydroxyl radical formation via Fenton chemistry, aggravating nanoparticle toxicity (Nowak *et al*., 2021). These findings affirm the importance of establishing optimal therapeutic windows for antioxidant supplementation in nanotoxicology studies.

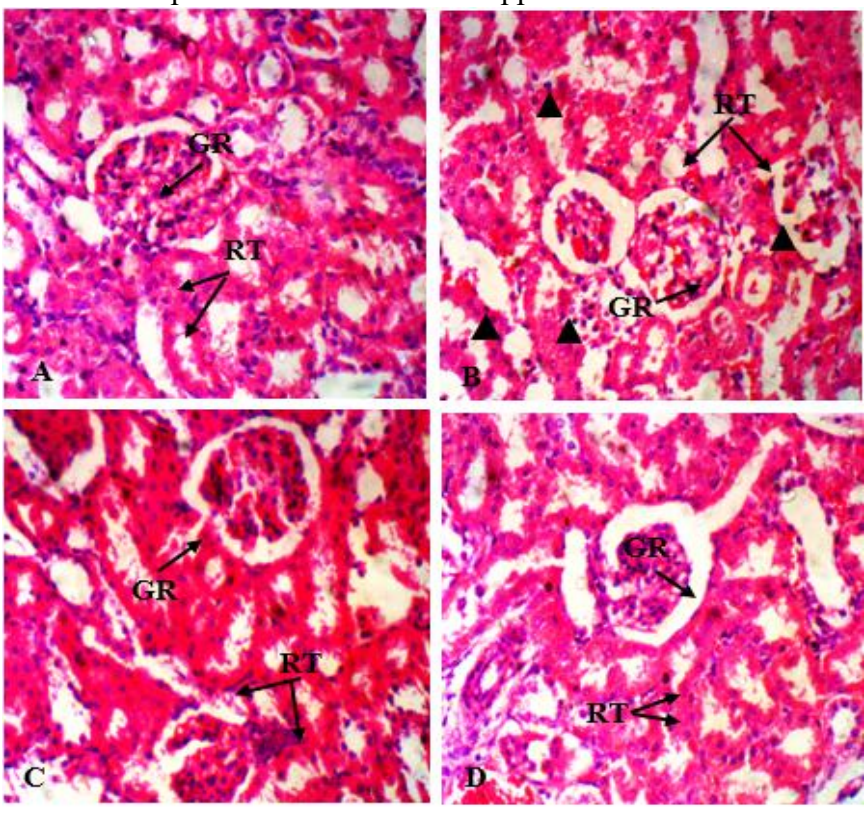

Figure 6: Photomicrographs of kidney tissue of male Wistar rats exposed to ZnO NPs and Vitamin C. Black arrow in Groups A, C, and D points to normal glomerulus and renal tubules, where no observable lesion was seen, while the black arrow in Group B points to patchy tubular epithelial coagulation necrosis with intraluminal casts. Control group (Group A) was administered 1 mL of distilled water only, while for the experimental groups, Group B was administered 70 mg/kg of ZnO only, Group C was given 70 mg/kg of ZnO and 100 mg/kg vitamin C, Group D was given 70 mg/kg ZnO and 200 mg/kg vitamin C. Plates were stained with H & E stains, Mag: X400

4.0 Conflict of Interest

All authors declare that there is no competing financial interest or personal relationship with other people or organisations that could inappropriately influence the reported work in this manuscript.

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