DOI: https://doi.org/10.4314/njbmb.v39i3.2 NJBMB (2024) 39(3), 129-135

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals/

e-ISSN: 2659-0042

Research Article

Ameliorative Potentials of N-Acetylcysteine and Vitamin C on Zinc-oxide Nanoparticles Induced Hepato-renal Toxicity in Male Wistar Rats

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OPEN ACCESS

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ARTICLE HISTORY
Received: 05/07/2024
Reviewed: 21/08/2024
Revised: 16/09/2024
Accepted: 23/09/2024
Published: 30/09/2024

CITATION
Oghenetega, O.B., Ibrahim, F.O., Oghenetega, G.E., Animashaun, R.O., Okwute, P.G. (2024). Ameliorative potentials of N-acetylcysteine and vitamin C on hepato-renal toxicity of zinc-oxide nanoparticles in male wistar rats. *Nigerian Journal of Biochemistry and Molecular Biology*. 39(3), 129-135
https://doi.org/10.4314/njbmb.v39i3.2

ABSTRACT

Zinc-oxide nanoparticles (ZnO-NPs) are prevalent in various companies and consumer products, raising concerns about their potential toxicity. Vitamin C and N-acetyl-cysteine (NAC) are known for their antioxidant properties, which may protect against cytotoxicity. However, limited information exists on their effects on ZnO-NPs-induced toxicity. This study investigates the ameliorative effects of N-acetylcysteine and vitamin C on hepato-renal toxicity of Zinc-oxide Nanoparticles in Male Wistar Rats. Twenty-five male Wistar rats (100-120g) were grouped namely; Control, ZnO-NPs, ZnO-NPs + NAC, ZnO-NPs + Vit. C, and ZnO-NPs + NAC + Vit. C. ZnO-NPs were administered orally at 200 mg/kg, with treatment groups receiving an additional 100 mg/kg of Vitamin C and NAC. After 28 days, blood samples were collected for analysis, including liver enzymes (AST, ALP, ALT), liver malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), urea, creatinine, and serum zinc levels. ZnO-NPs significantly increased liver creatinine, urea, AST, ALT, MDA, and serum zinc levels compared to the control group ($P<0.05$). NAC and Vitamin C, alone or combined, significantly reduced liver SOD levels ($P<0.05$). Co-treatment significantly decreased ALT, urea, and creatinine while significantly increasing liver SOD ($P<0.05$). NAC and Vitamin C co-treatment alleviated the toxic effects of ZnO-NPs-induced hepato–renal damage in male albino Wistar rats.

Keywords: *Antioxidant, Bromate, Cytotoxicity, Cytokines, Isorhamnetin, Quercetin*

INTRODUCTION

Zinc-oxide nanoparticles are a type of engineered nano-material that exhibit distinctive physical and chemical characteristics because they are minute, generally spanning from 1 to 100 nanometers in diameter. These nanoparticles are commonly used in many consumer products, such as sunscreens, makeup, textiles, and electronic devices because they can absorb UV radiation, provide antimicrobial properties, and function as catalysts (Rajasekar *et al.,* 2024).

The kidney and liver play crucial roles in maintaining overall health and homeostasis within the body. They are responsible for important physiological processes, including metabolism, detoxification, and waste elimination (Brzóska *et al.*, 2003; Kumar *et al.*, 2021). Despite their widespread application for societal advancement, concerns have arisen regarding the likely health risks of increased usage. Nanoparticle contact has been observed to induce cytotoxicity in various organs, including the kidney and liver.

These changes may include alterations in enzymatic activity, oxidative stress, inflammation, and cell damage. ZnO-NPs have been shown to induce reactive oxygen species (ROS) generation, causing oxidative stress and invariably cellular destruction in the kidney and liver (Hussain *et al.*, 2016; Wang *et al.*, 2017; Moatamed *et al.*, 2019; Khan *et al.*, 2020; Pei *et al.*, 2023). In the kidney, exposure to ZnO-NPs has been (Hussain *et al.*, 2016; Wang *et al.*, 2017; Moatamed *et al.*, 2019; Khan *et al.*, 2020; Pei *et al.*, 2023), linked to nephrotoxicity, which can manifest as impaired renal function, glomerular damage, tubular injury, and inflammation. In the liver, ZnO-NP exposure has been associated with hepatotoxicity, characterized by hepatic inflammation, oxidative stress, lipid peroxidation, and hepatocellular damage. Vitamin C and N-acetylcysteine in contrast, are naturally existing compounds present in numerous fruits, vegetables, and herbs, renowned for their anti-inflammatory properties and ability to counteract the toxicity caused by diverse agents, such as heavy metals, pesticides, and nanoparticles (Ay *et al.*, 2021). Given the potentially harmful effects of ZnO-NPs on kidney and liver health, exploring potential protective interventions is crucial (Hashim *et al.*, 2022; Ali *et al.*, 2024). Limited findings have focused on evaluating the protective effects of NAC and Vitamin C against ZnO-NP-induced kidney and liver toxicity. Hence this study aims to find out the ameliorative effect of N-acetylcysteine and vitamin C on Zinc-oxide Nanoparticle induced hepato-renal toxicity in Male Wistar Rats

MATERIALS AND METHODS

Animal source

For this study, 25 healthy male albino Wistar rats (100-120g) were obtained from and housed in the Animal House of the Department of Physiology, Babcock University, and were provided typical rat chow and unlimited access to drinkable water. The animals were kept in conditions where the average room temperature was 23˚C, relative humidity was 55%, and they experienced a 12-hour light-dark cycle. The animals were put arbitrarily into 5 groups (n=5 per group) and were acclimatized for 2 weeks.

Experimental design

The male albino Wistar rats were grouped into 5 (n = 5) as indicated in Table 1 below. The treatments were administered orally via an oral cannula for twenty-eight days after acclimatization.

Table 1. The Experimental Grouping

Group	Treatment
1	Distilled Water (1ml)
2	Zinc-oxide Nanoparticles (100mg/kg)
3	Zinc-oxide Nanoparticles (100mg/kg) + N-acetyl cysteine (100mg/kg)
4	Zinc-oxide Nanoparticles (100ml/kg) + Vitamin C (100mg/kg)
5	Zinc-oxide Nanoparticles (100mg/kg) + N-acetyl cysteine (100mg/kg) + Vitamin C (100mg/kg)

Drug administration

Zinc-oxide nanoparticles (CAS No: 1314-13-2) were bought from Sigma Aldrich, St. Louis, USA. Vitamin C was obtained from Emzor Pharmaceutical Industries Ltd, Lagos, Nigeria and N-acetylcysteine was obtained from Molychem, Mumbai, India. Zinc-oxide Nanoparticle was administered at 100mg/kg (Oghenetega *et al.*, 2024); N-acetyl cysteine at 100mg/kg (Kunle-Alabi *et al.*, 2017) and Vitamin C at 100mg/kg (Poli *et al.*, 2022).

Dispersion protocol for Zinc-oxide nanoparticle

ZnO NPs were made by dissolving 2mg of nanoparticles in 1 millilitre of water. The mixture was subjected to three rounds of sonication in a sonication bath for three minutes, with a 30-second rest in between, using an Elma S3000 sonicator bath (Elma Schmidbauer, Germany). The mixture was mixed using a vortex mixer for 5 minutes to achieve homogeneity.

Sample collection

After 4 weeks of treatment blood was collected from each animal under ether anesthesia through the retro-orbital sinus and centrifuged at 3000rpm for 15 mins. The sera collected were used for liver function test and kidney function test using the colorimetric method and the animal was euthanized via cervical dislocation. The kidney and liver were extracted, separated from adhering tissues and weighed using an electronic scale. The liver tissue was used for oxidative stress assay.

Determination of liver function enzymes and oxidative stress markers

The Colorimetric method was used in the determination of serum AST, ALP and ALT) levels using kits (Fortress Diagnostics Limited, United Kingdom). Malondialdehyde (MDA) assay followed Wallin *et al.'s* (1993) method, where Malondialdehyde, a lipid peroxidation byproduct, reacts with thiobarbituric acid to produce a red compound measurable at 535 nm. Superoxide Dismutase (SOD) activity was determined using Misra and Fridovich's (1971) method, which relies on the conversion of adrenaline to adrenochrome by superoxide anions, with absorbance measured at 420 nm and Catalase (CAT) assay was performed according to Aebi's (1974) method. The sample reacted with 30 mM H_2O_2 in a 50 mM phosphate buffer (pH 7.4), and the decrease in H_2O_2 absorbance at 520 nm, after reaction termination, indicated CAT activity. All colorimetric assays, including the MDA, SOD, and CAT assays, were conducted using the Spectrumlab 23A UV-Visible spectrophotometer.

Determination of serum Zinc levels, Urea and Creatinine

The colorimetric method was used to determine serum zinc, urea and creatinine levels using kits (Fortress Diagnostics Limited, United Kingdom). The procedure for estimating urea, creatinine and zinc levels was carried out according to the kits' manual. These were conducted using the Spectrumlab 23A UV-Visible spectrophotometer.

RESULTS

The result illustrated in Table 2 shows a significant reduction in weight gain in the group treated with ZnO-NPs only in comparison to the control group. Weight gain increased significantly in the group treated with NAC and Vitamin C, respectively, in comparison to the group treated with ZnO NPs only. Noticeably, the NAC and Vitamin C co-administration treated group presented no significant difference in weight gain in comparison to the group for control. No significant change was seen in the relative weights of the liver and kidneys between the treated groups in comparison to the control group.

Table 2. Impact of ZnO-NPs, N-Acetyl Cysteine, and Vitamin C on Body Weight Gain and Organ Weight.

Experimental Groups	Weight Gain(g)	Rel. Liver Weight (g)	Rel. Kidney Weight (g)
Control	86.6 ± 1.806	0.4872 ± 0.02370	0.6664 ± 0.008412
ZnONP treated	71.4 ± 1.327**	0.4775 ± 0.02156	0.6218 ± 0.006931
ZnONP + NAC	83.4 ± 1.691#	0.5560 ± 0.006528	0.6764 ± 0.04478
ZnONPF + VIT-C	85.6 ± 3.043##	0.4573 ± 0.01743	0.6926 ± 0.004836
ZnONP + NAC + VIT-C	77.2 ± 3.441	0.4474 ± 0.01547	0.6871 ± 0.008187

Data are presented as Mean ± S.E.M (n = 5) **p <0.01 when compared with Control; #p < 0.05 when compared to the Nanoparticles treatment group; ##p < 0.01 when compared to the Nanoparticles treatment group.

The result shown in Figure 1 reveals a significant rise in the serum zinc level in the group treated with ZnO-NPs in comparison to the control (p<0.001). However, the group treated with NAC and Vitamin C co-administration had a significant reduction in serum zinc level in comparison to the Zinc-oxide nanoparticles treatment group. In Figure 2, a significant reduction in liver SOD levels in the group treated with ZnO NPs in comparison to control. However, Liver SOD was significantly increased in NAC and Vitamin C single and co-administration treatment groups when compared to the zinc oxide nanoparticles treatment group. With respect to Figure 3, there was no obvious difference in the group treated with ZnO NPs in comparison to the control. Liver CAT increased markedly in the Vitamin C treatment group as against the control group.

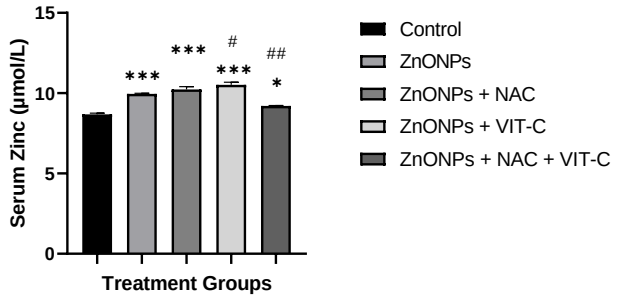

Figure 1. Impact of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Serum Zinc Levels.

Data are expressed as Mean ± S.E.M (n = 5). *p <0.05 when compared with Control***p <0.001 in comparison with Control; #p < 0.05 when compared to Nanoparticles treatment group; ###p < 0.001 in comparison to Nanoparticles treatment group.

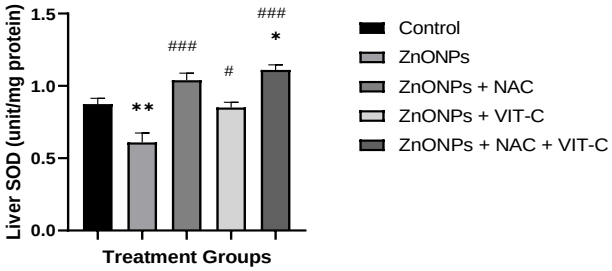

Figure 2. Impact of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Liver SOD Levels.

Data are expressed as Mean ± S.E.M (n = 5). *p <0.05 in comparison to control; **p <0.01 in comparison to control; #p < 0.05 when compared to Nanoparticles treatment group; ###p < 0.001 when compared to Nanoparticles treatment group.

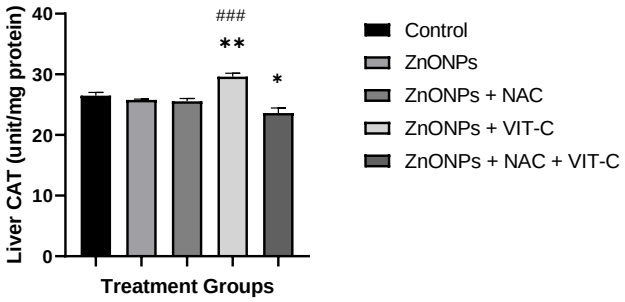

Figure 3. Impact of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Liver Catalase Levels.

Data are expressed as Mean ± S.E.M (n = 5). *p <0.05 in comparison to Control; *p <0.01 when compared with Control; ###p < 0.001 when compared to Nanoparticles treatment group.

Subsequently, we investigated the Effect of Zinc Oxide Nanoparticles, N-acetyl cysteine, and Vitamin C on Liver Malondialdehyde Levels. Zinc oxide nanoparticles were shown to increase liver MDA levels in comparison to the control as presented in Figure 4. However, Liver MDA was markedly reduced in the NAC treatment group in comparison to the zinc oxide nanoparticles treatment group. Noticeably, the Vitamin C and NAC + Vitamin C treatment groups displayed no significant discrepancy in comparison to the control. Moreover, Figure 5 indicates a significant elevation in serum AST in the group treated with zinc oxide nanoparticles. Noticeably, the NAC and Vitamin C co-administration treatment group also presented a significant increase in serum AST when compared to the control. However, the NAC and Vitamin C single administration treatment groups presented a significant reduction in serum AST in comparison to the zinc oxide nanoparticles treatment group. Although there was no displayed no significant differences in serum ALP between the treatment groups (as illustrated in Figure 6), The result shown in Figure 7, indicates a significant elevation in alanine transaminase levels in the group treated with ZnO NPs in comparison to the control. Notably this increase in ALT levels was found to be ameliorated in all the treatment groups of NAC and vitamin C alone ad combine when compared to the zinc oxide nanoparticle-only treatment group.

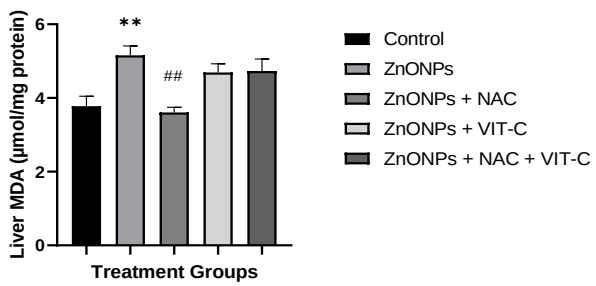

Figure 4. Effect of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Liver Malondialdehyde Levels.

Data are expressed as Mean ± S.E.M (n = 5). **p <0.01 when compared with Control; ##p < 0.01 when compared to Nanoparticles treatment group.

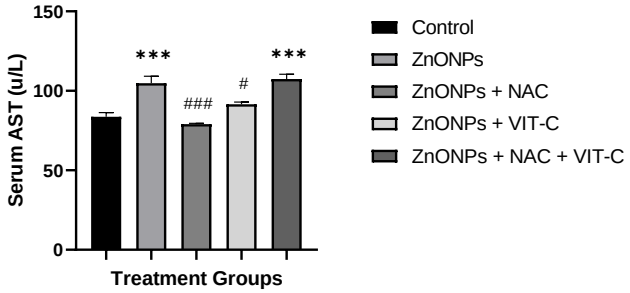

Figure 5. Effect of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Serum Aspartate Aminotransferase Levels.

Data are expressed as Mean ± S.E.M (n = 5). ***p <0.001 when compared with Control; #p < 0.05 in comparison to Nanoparticles treatment group; ###p < 0.001 in comparison to Nanoparticles treatment group.

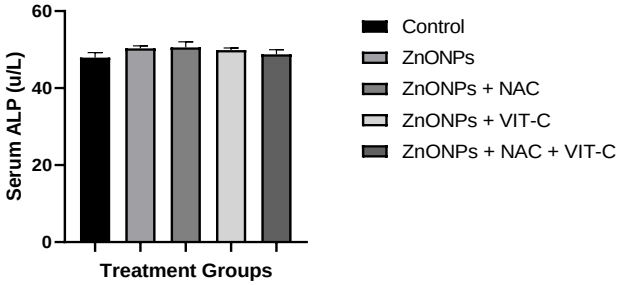

Figure 6. Effect of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Serum Alkaline Phosphatase Levels.

Data are expressed as Mean ± S.E.M (n = 5)

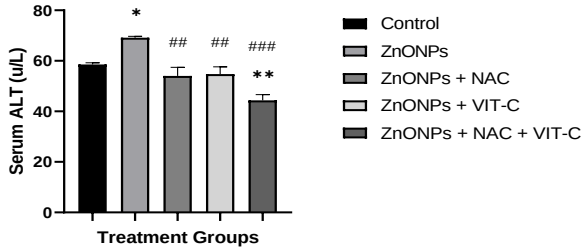

Figure 7. Effect of ZnO-NPs, N-acetyl cysteine, and Vitamin C on Serum Alanine Aminotransferase Levels.

Data are expressed as Mean ± S.E.M (n = 5). *p <0.05 in comparison with Control; **p <0.01 in comparison with Control; ##p < 0.01 in comparison to Nanoparticles treatment group; ##p < 0.01 when compared to Nanoparticles treatment group.

While investigating the impact of ZnO NPs, N-acetyl cysteine, and Vitamin C on Serum Urea Concentration, a significant elevation in urea concentration in the zinc oxide nanoparticles administration and NAC treatment groups in comparison to the control. However, urea concentration was seen to be significantly decreased in the Vitamin C and NAC + Vitamin C treatment groups in comparison to the Zinc oxide nanoparticles group (Figure 8). Similarly, a significant elevation in serum creatinine levels in the ZnO-NPs, NAC and Vitamin C treatment groups in comparison to the control (as illustrated in Figure 9). However, the NAC and vitamin C co-administration treatment group displayed a significant decrease in serum creatinine levels as against the zinc-oxide nanoparticles treatment group.

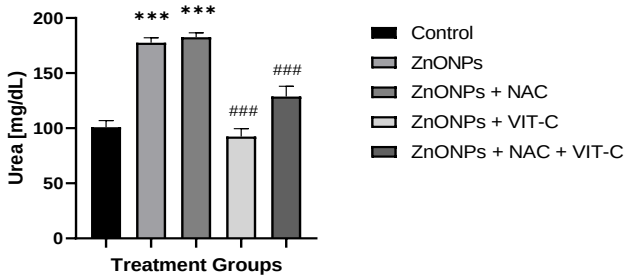

Figure 8. Impact of Nanoparticles, N-acetyl cysteine, and Vitamin C on Serum Urea Concentration.

Data are expressed as Mean ± S.E.M (n = 5). ***p <0.001 in comparison to Control; ###p < 0.001 when compared to Nanoparticles treatment group.

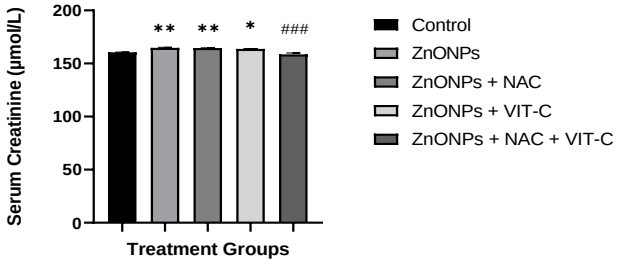

Figure 9. Effect of Nanoparticles, N-acetyl cysteine, and Vitamin C on Serum Creatinine.

Data are expressed as Mean ± S.E.M (n = 5). *p <0.05 in comparison to Control;**p <0.01 in comparison to Control; ###p < 0.001 when compared to Nanoparticles treatment group.

DISCUSSION

This research seeks to investigate the impacts of vitamin C and N-acetylcysteine against zinc oxide nanoparticle-induced hepato-renal damage. This was carried out by assessing the impacts of ZnO-NPs treatment and the single and combined administration of NAC and vitamin C, on several parameters. These parameters include liver function and oxidative stress markers, kidney function markers, relative weights of liver and kidney and serum zinc levels. To determine the effect of ZnO-NPs and the treatment compounds on liver function and oxidative stress, their impact on the level of the enzymes Malondialdehyde, Catalase, Superoxide dismutase, Alkaline phosphatase (ALP), Aspartase aminotransferase (AST), and Alanine transaminase (ALT) were ascertained. Our analysis of

the results revealed notable findings regarding the possible beneficial consequence of NAC and Vitamin C treatment against Zn-ONP-induced liver damage.

Oxidative stress stems from an imbalance between pro-oxidant and antioxidant substances within a biological system, as highlighted in research by Pizzino *et al.* (2017). This imbalance can trigger lipid peroxidation, leading to the formation aldehydes and other related compounds such as Malondialdehyde (MDA), as described in the study by Moldogazieva *et al.* (2019). Malondialdehyde is an important oxidative stress marker that occurs when free radicals attack lipids in cell membranes to cause their degradation. This typically occurs because of accumulation of free radicals possibly due to invasion or exposure to toxins. MDA levels have been widely utilized as a reliable indicator for assessing oxidative stress, as demonstrated in studies by Altun *et al.* (2018) and Ghonimi *et al.* (2021). In this study, a significantly elevated liver MDA levels was seen in the ZnO-NPs treatment group in comparison to the control group. This is in tandem with existing literature emphasizing the possibility of ZnO-NP exposure resulting in free radical accumulation in cells (Lavicoli *et al.*, 2016). These results also revealed elevated levels of Zinc in the blood, indicating significant ZnONP exposure. This increase in MDA due to ZnONP exposure in this study was found to be ameliorated by all the treatment groups, with the singular administration of NAC showing the most significant reduction in MDA in comparison to the ZnONP treatment group. SOD and CAT, vital antioxidant enzymes, respond to oxidative stress by neutralizing harmful free radicals. SOD converts superoxide anions into H_2O_2 and water, while CAT further breaks down H_2O_2 into H_2O and O_2 (Roy *et al.*, 2023). In this study, ZnONPs treatment showed no toxicity via the catalase marker, while the group treated with Vitamin C demonstrated a significant elevation in catalase concentrations. This finding is not uncommon as vitamin C is a known potent anti-oxidant compound (Dennis and Witting, 2017). Several studies have also illustrated the increased anti-oxidative properties of vitamin C on oxidative stress markers including catalase (Didier *et al.*, 2023). The reason for the inactivity of zinc oxide nanoparticles via catalase marker is not fully understood, as it could have occurred due to several factors such as cellular response variability, duration and dosage of exposure, or other complex biological effects. These results further indicate a significant reduction in superoxide dismutase due to zinc oxide nanoparticle administration. Similar to malondialdehyde, this decrease in SOD was ameliorated by all the treatment groups. The major mechanism of action of the treatment compounds vitamin C and NAC is the increased formation of physiological anti-oxidants, and the foraging of free radicals like ROS (Traber and Stevens, 2011; Tenório *et al.*, 2021).

Liver function tests (LFTs) are a series of blood tests that assess the levels of various enzymes and compounds to determine the relative health and functioning of the liver. This study carried out these tests by measuring the levels of the enzymes Alkaline phosphatase (ALP), Aspartase aminotransferase (AST), and Alanine transaminase (ALT) in the blood. These enzymes function to break down and convert proteins to energy for hepatocytes, as well as to metabolize amino acids. They are typically present in the liver but can be found in very low amounts in the bloodstream. However, damage to the liver can cause the enzymes to be released into the bloodstream, therefore it is an important marker for liver damage. Studies have correlated increased liver damage to nanoparticle exposure by evaluating elevated levels of AST, ALP and ALT levels in the blood (Samim *et al.*, 2022; Lala *et al.*, 2022). Zinc oxide nanoparticles toxicity has also been shown to result in increased liver damage (Mansouri *et al.*, 2015; Hua-Qiao *et al.*, 2016; Wang *et al.*, 2017; Moatamed *et al.*, 2019; Pei *et al.*, 2023). This may be due to oxidative stress, as nanoparticles have been shown in existing literature as well as in our results to cause oxidative stress in liver cells (Sharma *et al.*, 2012). This oxidative stress can further lead to inflammation and cell apoptosis through the intrinsic signaling pathway due to DNA damage or cell membrane disruption caused by lipid peroxidation (Redza-Dutordoir and Averill-Bates, 2016). These results indicate the development of liver toxicity due to zinc oxide nanoparticle administration, as significantly elevated levels of AST and ALT in the blood were observed. This implies that oxidative stress was also induced in hepatocytes causing increased levels of AST and ALT release into the blood compared to control. The treatment groups delivered mixed results regarding the single or co-treatments of NAC and vitamin C. Single treatment of NAC and vitamin C was found to ameliorate the elevated levels of both AST and ALT in comparison to the zinc oxide nanoparticle treatment group. However, concomitant administration of NAC and vitamin C seemed to be more potent in ameliorating elevated levels of ALT as it was significantly lower even when compared to the control.

Zinc oxide nanoparticles were seen to significantly elevate urea and creatinine concentrations in the nanoparticles administered group when in comparison to the control. The increased urea and creatinine in the body have significant physiological and pathological implications. Urea, a waste product resulting in protein metabolism, is primarily eliminated through the kidneys in the form of urine. Elevated levels of urea in the bloodstream, known as uremia, can indicate impaired renal function (Gounden *et al.*, 2022). One of the potential mechanisms through which ZnO-NPs may influence urea levels is by inducing oxidative stress and inflammation in renal cells. Oxidative stress can destroy renal structures, including glomeruli and tubules, compromising their filtration and reabsorption functions (Gyurászová *et al.*, 2020). Consequently, impaired renal function can result in decreased urea clearance and subsequent elevation of urea levels in the blood (Gounden *et al.*, 2022). Creatinine is also a waste product, generated from the breakdown of creatinine phosphate in muscle tissue, and regulated by the kidneys (Kashani *et al.*, 2020). The increased urea and creatinine concentrations in the blood due to ZnO-NP exposure are indicative of kidney damage, or impaired kidney function (Gounden *et al.*, 2022). While single administration of vitamin C and NAC were unable to ameliorate the increase in serum creatinine, their co-administration was able to mitigate the harmful consequences of zinc oxide nanoparticles on

creatinine. This indicates a potential complementary anti-oxidant mechanism between NAC and vitamin C that synergistically protects against oxidative stress, highlighted by the amelioration of the marked rise in serum creatinine due to zinc oxide nanoparticle administration. Serum urea concentrations were also significantly increased due to zinc oxide nanoparticle administration. This increase was unable to be ameliorated by a single administration of NAC but was mitigated in groups treated with vitamin C, and NAC + vitamin C combined. This implies that vitamin C treatment alone or in combination with NAC in this study shows ameliorative properties in ZnO-NPs-induced renal toxicity. The ameliorative anti-oxidant properties of NAC and vitamin C against kidney damage or impairment demonstrated in this study are in line with current existing literature that highlights this protective effect (Dennis and Witting, 2017).

CONCLUSION

Zinc oxide nanoparticles were shown to result in alterations in the physiology of the liver and kidney and induce liver oxidative stress. However, Vitamin C and N-acetylcysteine were able to ameliorate these adverse effects.

AUTHORS' CONTRIBUTIONS

Conceptualization, OBO and EGO.; methodology, OBO, PGO and FOI.; validation, OBO, FOI and EGO; formal analysis, OBO, EGO and FOI; investigation, ROO, FOI.; resources, OBO and FOI; data curation, OBO and FOI; writing—original draft preparation, EGO; writing—review and editing, PGO, ROO and OBO; supervision, OBO; project administration, FOI; funding acquisition, FOI. All authors have read and agreed to the published version of the manuscript. Ethical approval was obtained from Babcock University Health Research Ethics Committee (BUHREC) before the start of the study, with registration number 320/24.

FUNDING STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

Not applicable

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