



RESEARCH ARTICLE

Investigation of Toxic Effects of Vanadium, Magnesium and Nickel Nanocomposite on Erythrocytes and Reproductive Organ of Male Albino Rats

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ABSTRACT

Nanoparticles and nanocomposites having potential applications in different areas are widely used in industrial and biomedical fields. Cellular inflammatory response due to exposure to nanoparticles can be monitored by measuring of inflammatory biomarkers/oxidative stress in different tissues of exposed animals. Oxidative stress parameters, contents of antioxidant enzymes and histopathological changes in erythrocytes and testicular tissues were assessed to determine the cell membrane integrity in male albino rats following 5, 10 and 15mg/kg body weight intraperitoneal administration of nanocomposites (vanadium, magnesium and nickel) daily for a period of 15 days. A dose and time dependent increase in contents of lipid peroxidation and reactive oxygen species (ROS) as well as a significant decrease in reduced glutathione (GSH) and the contents of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were measured using biochemical analysis. Statistical analysis showed significant ($P < 0.05$) variation in contents of different biomarkers of erythrocytes and testes in treated and untreated rats. Histopathological observations showed prominent testicular damage in term of degeneration of seminiferous tubules, detachment of germinal epithelium, edema and Leydig cell degeneration in rats treated with high dose depicting impaired process of spermatogenesis and reproductive toxicity. These results indicate that prolonged exposure to vanadium, magnesium and nickel nanocomposites cause significant oxidative stress and histopathological ailments. It is suggested that measuring of oxidative stress and status of antioxidant enzymes are reliable tools to determine the induction of inflammatory response in cells.

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INTRODUCTION

Nanotechnology has transformed scientific research through development of materials with enhanced properties such as increased chemical stability, surface area, and mechanical strength (Ahire *et al.*, 2022; Aslam *et al.*, 2023; Elbehary *et al.*, 2023). Among various nanomaterials, vanadium, magnesium and nickel-based nanocomposites have gained more attention among different nanomaterials due to their industrial as well as

biomedical applications such as catalysis, biosensing and drug delivery. In spite of numerous advantages of application of nanoparticles, They have been studied for immunotoxicity and cell toxicity and pose serious long term biological concerns (Elbehary *et al.*, 2023; Udourioh *et al.*, 2023). Their small size coupled with high reactivity make metal nanoparticles able to cross a cellular membrane and induce oxidative stress, inflammatory reactions and alter the integrity of membrane of cells causing damage to the cells (Maqsood *et al.*, 2023;

Mamidi *et al.*, 2024). Nanoparticles induced toxicity is especially detrimental to erythrocytes which play vital role in oxygen transport (Hermosillo-Abundis *et al.*, 2024; Iqbal *et al.*, 2024). Vanadium nanoparticles can generate reactive oxygen species (ROS) and induce lipid peroxidation of erythrocytes and erythrocytic membrane integrity and lead to hemolysis (Wang *et al.*, 2017; Younas *et al.*, 2024). Frequent application of metal based nanoparticles can be harmful as it leads to mitochondrial dysfunctions and oxidative damage in erythrocytes (Nwafili *et al.*, 2023; Shahid *et al.*, 2023). An oxidative stress mechanism, hemolytic activity and membrane destabilization induced by nickel nanoparticles have been studied (Gamasae *et al.*, 2020; El-Hamaky *et al.*, 2023).

Metal nanocomposites damage the reproductive system (Ugwu *et al.*, 2022). Oxidative stress induced injury to the Leydig cells has been associated with impaired spermatogenesis and a decrease in testosterone in the male animals exposed to vanadium (Ghosh and Banik, 2022). In excess, magnesium exposure has also been shown to induce oxidative injury in testicular tissues and impair fertility (Moshkelani *et al.*, 2020). Nickel is a genotoxic and carcinogenic agent that causes testicular atrophy, decreased sperm quality as well as DNA damage (Rizvi *et al.*, 2020). Oxidative stress is of vital importance to determine the cellular toxicity due to nanomaterials (Hamza *et al.*, 2023; Younas *et al.*, 2024). Oxidative stress, an imbalance between the antioxidant defense system and reactive oxygen species (ROS) results in damage to lipids, proteins and DNA (El-Hamaky *et al.*, 2023; Maqsood *et al.*, 2023). Vanadium nanoparticles are among the most effective in producing hydroxyl radicals and superoxide anions to damage erythrocytes and reproductive cells (Nnama *et al.*, 2022). Magnesium and nickel nanocomposites also cause overproduction of ROS in both erythrocytes and reproductive organs (Kong *et al.*, 2019; Tarasov *et al.*, 2019).

Nanoparticle-induced toxicity also involves inflammation (Summer *et al.*, 2024; Hussain *et al.*, 2025). Activated inflammatory cytokines in reproductive organs have been demonstrated to cause chronic inflammation and tissue damage in reproductive organs (Elbehary *et al.*, 2023). Excess magnesium may exacerbate inflammatory injury and oxidative stress (Zhao *et al.*, 2017). Erythrocyte membrane damage, seminiferous tubule degeneration and Leydig cell dysfunction have been observed with nanoparticles (De Palma *et al.*, 2022; Kumar *et al.*, 2024). Metal nanocomposites cause mitochondrial swelling and oxidative damage in both reproductive organs and erythrocytes (Lei *et al.*, 2023). Testicular damage associated with severe loss in integrity of seminiferous tubular epithelium and increased apoptosis of germ cells is also linked with nickel nanoparticles (Singh *et al.*, 2024). As the applications of nanomaterials increases, it is necessary to study the combined toxic effects of vanadium, magnesium and nickel nanocomposites. The individual toxicity profiles of these metals are well documented but the additive or synergistic effects of these metals are recorded. Hence, this study investigated the toxic effects of vanadium, magnesium and nickel nanocomposites in order to guarantee the safe use of nanomaterials in biomedical and industrial applications.

MATERIALS AND METHODS

Animal Selection and Maintenance: Approximately three months old 26 male albino rats having 140-150 g body mass were procured from CIDS (Cholistan Institute for Desert Study, Bahawalpur). All the rats were healthy and free from any obvious ailments. A 12hours light/dark cycle, $25\pm 1^\circ\text{C}$ temperature and humidity ($65\pm 5\%$) were maintained for all animals during the trial. Commercial poultry feed containing about 23% proteins and fresh tap water were available to each rat without any restriction. All study procedures were in accordance with the criteria outlined in the 'National Institutes of Health Guide for the Care and Use of Laboratory Animals' (NIH Publication no. 85-23, 1985). The chemicals used for determining the oxidative stress and antioxidant enzymes in erythrocytes and testes and for histopathology were of analytical grades and were procured from Sigma Aldrich (USA) and Merck (Germany).

Experimental Design: After 2-week acclimatization period, six rats were randomly allocated ($n=6$) to four groups. The treatment groups (T1, T2 and T3) were administered nanocomposite (vanadium, magnesium and nickel) at doses of 5, 10 and 15mg/kg body mass respectively, while the control group (T0) was given 10% normal saline intraperitoneally. The study concentrations were selected on the basis of previous reports on nickel (Iftikhar *et al.*, 2023), vanadium oxide (Park *et al.*, 2016) and magnesium oxide nanoparticles (Mazaheri *et al.*, 2019).

Sample Collection: Approximately, 2.5ml of blood sample from the jugular vein of each albino rat was obtained in tubes without EDTA on days 7 and 15 of experiment. 10% hemolysate of RBC was prepared from both the nanocomposite treated and untreated albino rats to determine oxidative and anti-oxidant enzymes. On days 7 and 15 of the study period, the rats (3) were euthanized from each group and the testicular tissues were carefully harvested for the histopathological examination.

Tissue preparation: In nanocomposite treated and untreated albino rats, testes were collected to determine the oxidative profile and antioxidant enzymes. About 2 ml of ice cold normal saline solution was placed in Petri dish separately for each sample (one for each tissue), homogenized and individually chopped. The homogenates were subsequently separately centrifuged at 3000rpm for 10 minutes, and the samples were stored at 4°C for further process (Wang *et al.*, 2022).

Biochemical Analysis: The contents of oxidative stress parameters like ROS (Hayashi *et al.*, 2007), thiobarbituric acid reactive substance (TBARS) (Jollow *et al.*, 1974) and reduced glutathione (GSH) (Iqbal *et al.*, 1996) were measured using UV spectrophotometer at 505, 532, 412nm, respectively. Various antioxidant indices like catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) (Akram *et al.*, 2021) were estimated in the erythrocytes and testis of the nanocomposite treated and untreated rats at wavelengths of 470, 560, 240nm, respectively (Raza *et al.*, 2022).

Histopathological Examination: Testes were separated from the rat of each group at days 7 and 15th of the trial. The organs were weighed, cleaned, and stored in a 10% neutral buffered formaldehyde solution. Using a rotary microtome, sections about 4-5 μ m thick sections were obtained. Different sections obtained from each rat were thereafter stained with hemotoxylin and eosin (H&E). To observe any histopathological alterations, microscopic observations of different sections was made using a light microscope (Nikon Eclipse 80i, Nikon Co., Tokyo, Japan) (Rani *et al.*, 2023; Ullah *et al.*, 2023).

Statistical Analysis: Data thus collected is presented as mean \pm SE. Statistical analysis of the collected data in each study group was performed utilizing IBM SPSS Statistics (version-20) and data in each group had a normal distribution. One-way analysis of variance (ANOVA) was used in statistical analysis. Finally, a post hoc Tukey's test at $P < 0.05$ significance threshold was used to compare mean values (mean \pm SE) of oxidative and antioxidant parameters in erythrocytes and testes of control and rats exposed to the NPs.

RESULTS

Oxidative stress and antioxidant enzymes: Results showed increased contents of oxidative stress parameters and decreased antioxidant enzyme in erythrocytes of nanocomposite treated albino rats as indicated in Fig. 1. Lipid peroxidation was increased remarkably in treated rats and the maximum increase was observed in the rats exposed to highest concentration of the nanocomposites as measured by thiobarbituric acid reactive substances (TBARS). Significant depletion of antioxidant defense was indicated by reduced glutathione (GSH), a key intracellular antioxidant enzyme in rats of treated groups. Initially, the reduction in contents of SOD was minimal, but with prolonged exposure it decreased significantly indicating that the ROS detox mechanism was impaired. A similar trend was found for CAT, with an extended exposure showing a significant decrease in its quantity to break down hydrogen peroxide. POD contents were relatively stable at day 7th but dropped dramatically at 15th of trial suggesting impaired peroxidative defense under chronic exposure as depicted in Fig. 2.

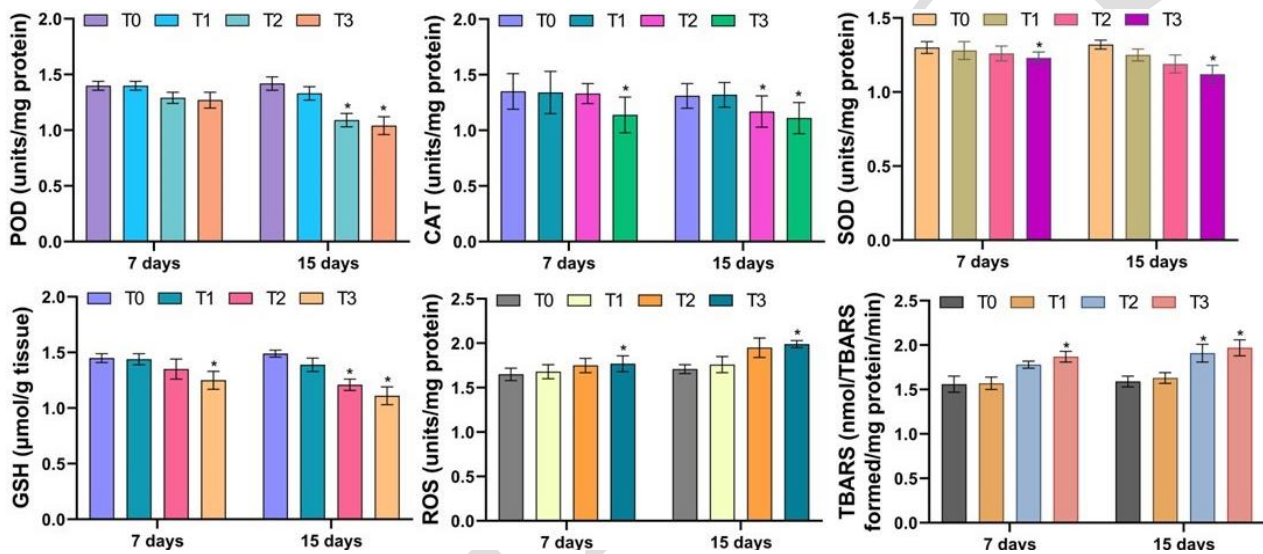


Fig. 1: Oxidative stress parameters and antioxidant enzymes in erythrocytes of albino rats treated with different concentrations of nanocomposites.

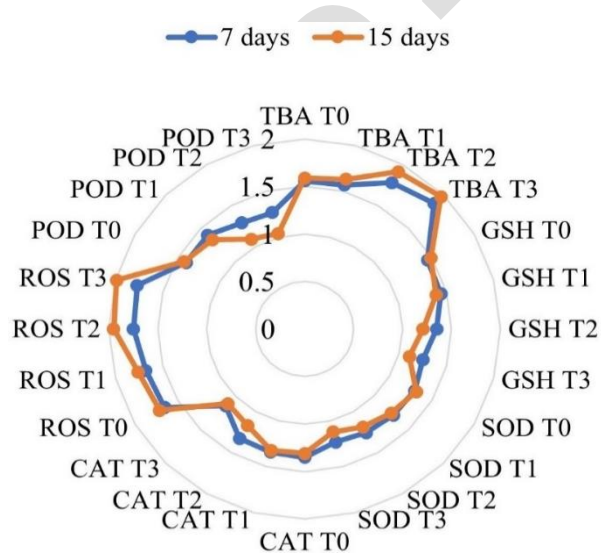


Fig. 2: Comparison of different oxidative stress and antioxidant enzymes status in erythrocytes of albino rats exposed to different doses of nanocomposite.

Treatment to albino rats induced increased contents of ROS which were more prominent in groups receiving higher concentrations at day 15th of trial. The results demonstrated the adverse effects of exposure to nanocomposite in oxidative stress biomarkers and antioxidant enzymes in the testes of albino rats (Fig. 3). TBARS (an index of lipid peroxidation) increased gradually in rats of treated groups but significantly at day 15 suggesting tissue damage. Measurements of reduced GSH, an important nonenzymatic antioxidant, showed a progressive decrease in the rats of treated groups, which reflects diminished antioxidant defense by the nanoparticle composites against oxidative stress. The rats with high exposure groups demonstrated markedly reduced contents of SOD, indicative of impaired enzymatic protection. CAT contents followed a similar trend, with reduced capacity for hydrogen peroxide decomposition. The contents of peroxidase (POD) significantly reduced in the high-dose groups at day 15. ROS level in treated groups were notably elevated especially in the highest dose receiving rats (Fig. 4).

Histopathology: Histopathological examination of testicular tissues in albino rats following administration of nanocomposite showed an accumulation of admixture of necrotic cells at high doses treated rats. Testicular architecture of the control group (T0) was normal with organized seminiferous tubules, intact basement membrane, and normal germinal epithelium. Rats treated with low doses showed mild histopathological changes including slightly disorganized germinal epithelium and minimal edema on days 7 and 15th of trial.

Moderate histopathological alterations such as germinal epithelium detachment, seminiferous tubules degeneration

and necrotic spermatids were observed in rats receiving high dose at day 7th of research trial. There were extensive damage/microscopic alterations observed on day 15 (Fig. 5) Histopathological damage in the high exposure (T3) rats continues in term of disorganized seminiferous tubules, extensive germinal epithelium degeneration, vacuolization, and edema. In this group, there was reduction in spermatogenic cells and presence of apoptotic cells indicative of significant impairment of spermatogenesis. The severity of structural/ histopathological alterations in rats exposed to vanadium, magnesium and nickel nanocomposites is indicated in Table 1.

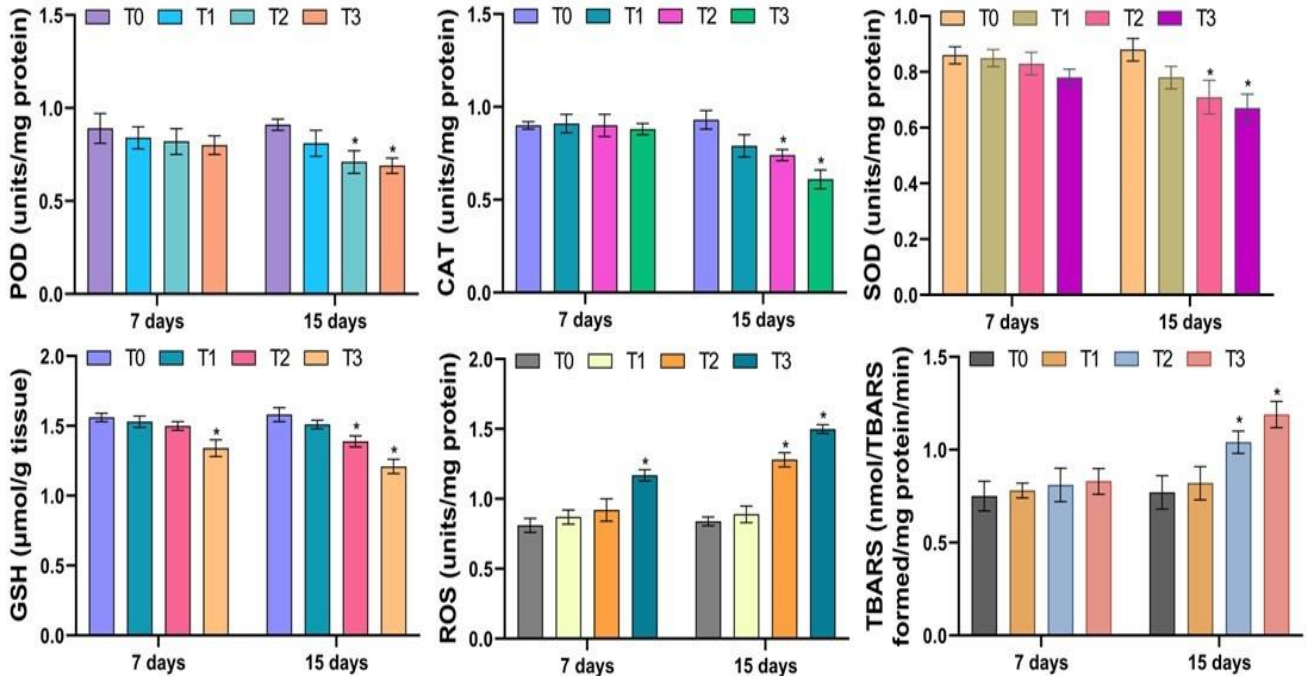


Fig. 3: Oxidative stress parameters and antioxidant enzymes in testis of albino rats treated with nanocomposites.

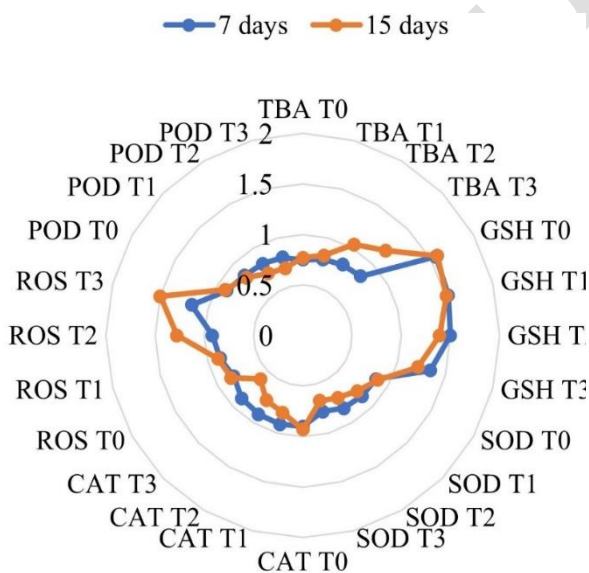


Fig. 4: Comparison of different oxidative stress and antioxidant enzymes status in erythrocytes of albino rats exposed to different doses of nanocomposite.

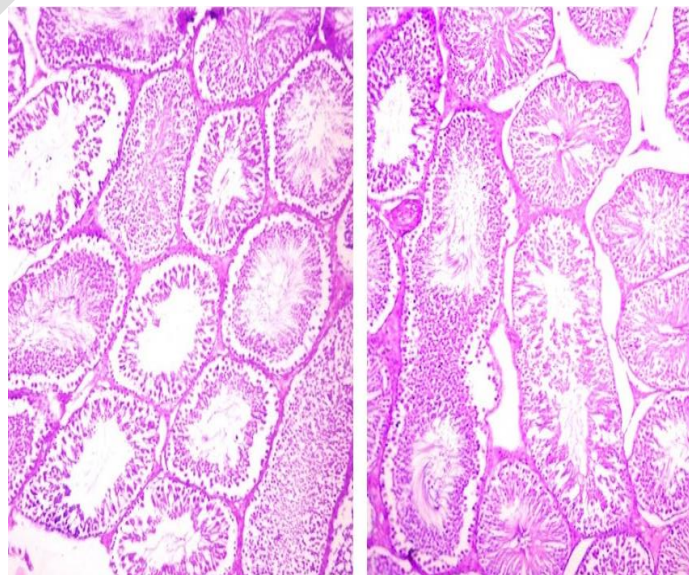


Fig. 5: Microscopic sections of testes of male albino rats showing necrosis of spermatids, detachment of germinal epithelium, arrest of process of spermatogenesis and admixture of necrotic cells in the lumen of seminiferous tubules at high dose of nanocomposite. H & E stain; 400X.

Table 1: Intensity of different microscopic alterations in testes male albino rats administered different doses of nanocomposites in rats

Parameters	Groups/Treatments			
	T0	T1	T2	T3
Histopathological lesions	-	+	+++	+++
Necrosis and disorganization of germinal epithelium	-	+	+++	+++
Pyknotic nuclei of spermatids	-	++	++	+++
Inflammatory reaction	-	+	++	+++
Sloughing of germinal cells	-	++	++	+++
Arrest of process of spermatogenesis	-	++	++	+++
Degeneration and damaged spermatogonia	-	++	++	++
Degeneration and reduction in diameter of seminiferous tubules	-	++	++	+++
Admixture of necrotic cells and debris in lumen of seminiferous tubules	-	++	+++	++++
Increase cellular debris in lumen of seminiferous tubules	-	++	++	+++
Hypo-spermatogenesis	-	+	++	+++
Decreased percentile rate of seminiferous tubules with normal cells	-	+	++	+++
Detachment of germ cell in seminiferous tubules	-	++	+++	+++
Necrosis of spermatogonia/spermatids	-	+	++	+++
Inflammatory material	-	++	++	+++

Normal (-), Mild (+), Moderate (++), Severe (+++), Very severe (++++)

DISCUSSION

The introduction of nanotechnology has made a significant change to various scientific fields ranging from medicine, industry and to environmental applications with unprecedented improvement (Suhag *et al.*, 2023). Nevertheless, the toxicological impacts of nanocomposites is crucially affected (Abbasi *et al.*, 2023). This study is concerned with the toxic effects of nanocomposites of vanadium, magnesium and nickel nanoparticles on the principal biological systems including reproductive organs and erythrocytes of male albino rats to elucidate the underlying mechanisms and offers a broader context for the safe use of nanoparticles.

Oxidative damage, a hallmark of the treated groups, was evidenced by progressive increase in lipid peroxidation (Hussain *et al.*, 2018; Ghaffar *et al.*, 2021). Over release of ROS as biomarker of oxidative stress reacted with membrane lipids leading to lipid peroxidation and compromised membrane integrity and cellular dysfunction (Kanwal *et al.*, 2024; Shafqat *et al.*, 2023). This is in line with previous studies, showing that exposure to different magnetic nanoparticles (MNPs) has led to the similar results. It is estimated that erythrocytes treated with vanadium nanoparticles showed increased lipid peroxidation (Nnama *et al.*, 2022). Similar toxic effects in magnesium and nickel nanoparticle exposed cells were also observed (Manohar *et al.*, 2022). The results of this study are also in agreement with previous findings and reinforce the pro-oxidant nature of nanocomposites (Maddheshiya and Nara, 2022). In addition, loss of membrane stability and increased hemolysis observed in erythrocytes agree with the earlier reports that nanocomposites directly disrupt bilayers by initiating lipid degradation (Lin *et al.*, 2024; Peng *et al.*, 2024).

In this study, the marked depletion of intracellular GSH levels substantiated the notion that endogenous antioxidant systems are overwhelmed by nanocomposites. As a key and important intracellular antioxidant, GSH detoxifies ROS and maintains redox homeostasis (Raj *et al.*, 2021). The depletion of GSH contents were observed in our study have also been recorded in mammals due to

metallic nanoparticles (Qiao *et al.*, 2021; Mahfouz *et al.*, 2023). The depletion could be due to excessive generation of ROS leading to rapid reduction of GSH for during detoxification processes. The GSH decline is particularly distressing because tissues become more susceptible to other injurious stimuli (Xiong *et al.*, 2021). Other studies have shown such depletion to associate with cellular dysfunction due to exposure to nanoparticles including diminished enzymatic contents and mitochondrial damage (Liu and Tang, 2020; Kumar *et al.*, 2024).

Significant reductions in contents of antioxidants including SOD, CAT, and POD were correlated with GSH depletion in rats of treated groups. Notably, SOD was decreased and particularly in high-dose treated rats. Metal nanoparticles interfere with the cellular functions and active sites of SOD and induce disorders in their integral responses (Zhao *et al.*, 2021). SOD plays a central role in decreasing the oxidative stress, its suppression probably increases superoxide radical accumulation leading to initiation of a cascade of oxidative damage (Wadan *et al.*, 2024). CAT breaks hydrogen peroxide into water and oxygen, was also impaired (Dutta *et al.*, 2024). In erythrocytes of rats treated with nickel nanoparticles, also indicated lower values for CAT contents and was attributed to nanoparticle induced structural perturbations in the enzyme (Zhao *et al.*, 2014). POD contents were found to be stable in the early stages of exposure but declined significantly with the dose and time of exposure indicating a loss of peroxidative defenses. Similar patterns in detrimental effects of prolonged nanoparticle exposure on enzymatic antioxidants have also been studied (Vlasova *et al.*, 2016; Jomova *et al.*, 2024).

ROS are highly reactive molecules damaging lipids, proteins and DNA (Juan *et al.*, 2021). The persistent ROS elevation indicates that nanocomposites disrupt mitochondrial functions (Wang *et al.*, 2024). As reported in prior studies, that the higher the concentrations of the particle, the more severe is the oxidative damage (Gravandi *et al.*, 2024; Umar *et al.*, 2024).

A significant damage in testicular tissue along with oxidative stress beyond erythrocytes was recorded in treated rats. The increase in the level of lipid peroxidation in testicular tissues was similar to that recorded in erythrocytes, and the highest dose rats showed the most pronounced increase as studied previously (Ullah *et al.*, 2023) in reproductive organs following nanoparticle exposure. Here, the increased oxidative stress parameters suggest that, if exposed for long durations the testicular membranes become disrupted in their structural integrity and are more susceptible to oxidative damage (Hussain *et al.*, 2019; Hussain *et al.*, 2020; Liu *et al.*, 2021).

The decrease in GSH level in testicular tissues probably further increases oxidative damage and in turn impairs the functions of reproductive tissues. Studies on zinc oxide and copper oxide nanoparticles reported similar reduction in GSH contents suggesting that there is a common mechanism by which nanoparticles induce oxidative stress in reproductive organs (Ajdayr *et al.*, 2021). Testicular tissues of treated rats showed significant reduction in enzymatic antioxidant contents (SOD, CAT) parallel with erythrocytes. The increased superoxide radical accumulation is likely to have significantly enhanced oxidative stress in testicular tissues. Similarly,

CAT contents reduction was pronounced at high doses and was also associated with diminished capacity to detoxify hydrogen peroxide. These findings are consistent with prior studies that found a link between reduced CAT and nanoparticle-induced reproductive toxicity (Assar *et al.*, 2022; Behairy *et al.*, 2024). No report could be found regarding the testicular changes in rats induced by nickel, magnesium and vanadium nanocomposites. However, significantly reduced body mass, poor sperm production, necrosis of seminiferous tubules and basement membranes due to intraperitoneal (45mg/kg BW) administration of nickel nanoparticles (Iftikhar *et al.*, 2023), while and toxic effects of vanadium oxide nanoparticles on heart and blood of mice (Park *et al.*, 2016) at higher doses (2 and 6mg/kg BW) have been observed. Furthermore, increased induction of oxidative stress and lower values of total antioxidant potential in rats due to magnesium oxide nanoparticles alone at higher has also been recorded (Mazaheri *et al.*, 2019). At histopathological level, albino rats administered low doses of nanocomposites exhibited mild to moderate histopathological changes including disorganization of the germinal epithelium and edema. Similarly, mild to moderate histopathological alterations due to low concentrations of nanoparticles in the testes of a rats have also been observed (Naumenko *et al.*, 2024). Severe histopathological alterations in testes of albino rats administered high doses of nanocomposites including detachment of germinal epithelium, necrosis of spermatids, arrest of spermatogenesis, admixture of necrotic spermatids and degeneration of seminiferous tubules were observed. These histopathological ailments could be due to damage to plasma membrane caused by elevated level of ROS and lipid peroxidation product (Azouz *et al.*, 2023; Ullah *et al.*, 2023). There was extensive vacuolization along with degeneration of seminiferous tubules and reduction of spermatogenic cells in albino rats in this study might also be related to impaired functions of the testis and disruption of integrity of germinal epithelium. The degeneration of Leydig cells in rats may be due to disruption of testosterone synthesis responsible to maintain healthy male reproductive system. Previously, Leydig cell dysfunctions due to nanoparticle toxicity in term of poor steroidogenic activity and hormonal imbalance has been recorded (Saoudi *et al.*, 2020; Zheng *et al.*, 2023). The testicular ailments observed in this study could also be linked to endocrine disrupting potential of nanocomposites (Bara and Kaul, 2018; Zheng *et al.*, 2023).

Conclusions: The results of this study indicated strong evidence of induction of oxidative stress and depletion of antioxidant enzymes in albino rats administered nanocomposites. This study highlighted the need for long term toxicity assessments and safety guidelines for the use of nanocomposite in biomedical fields. The determination of erythrocytic indices are important and useful tools for screening of oxidative stress induced by environmental hazards and prolonged exposure to nanocomposites.

Author contribution: The experiment was designed by YM. ZA executed the trial. GM involved in data analysis. All the other authors actively participated in preparation and finalization of the manuscript.

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