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Original Article

Mechanistic approach to investigate the induction of toxicity by magnesium oxide nanoparticles on testicular, nervous and muscular tissues of albino rats

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Abstract

Nanoparticles are used extensively in various industries, such as agriculture, food packaging, medical diagnostics and electronics. However, their increasing usage raises concerns regarding potential health hazards and environmental risks. This study examined the impact of intra-peritoneal injections of magnesium oxide (MgO) nanoparticles on the brain, testis, and muscles of male albino rats. Mature male rats (n=20) after acclimatization were randomly divided into four groups (G_0, G_1, G_2, G_3). The rats in the treated groups (G_1-G_3) were given MgO NPs @ 25 mg/kg, 50 mg/kg and 75 mg/kg respectively for ten consecutive days. G_0 rats served as untreated control group. Results indicated that MgO NPs induced clinical alterations in exposed rats. The exposed organs including brain, and testis gained more weight and their stress parameters [reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS)] increased significantly in a dose dependent manner. Antioxidant enzymes including catalase (CAT), peroxidase (POD), reduced glutathione (GSH) and superoxide dismutase (SOD) reduced significantly in studied organs as compared to control ones. The treated rats have shown atrophy of neurons, microgliosis, cytoplasmic vacuolization, and congestion. Changes in the testis include inflammation, sloughing of cells, damaged spermatogonia, necrosis of spermatids, spermatogonia and arrest of spermatogenesis process. Conclusively, it is suggested that persistent application of nanomaterials at environmentally relevant concentrations may induce adverse toxicological effects in targeted and non-targeted exposed animals.

Keywords: Albino rats, MgO NPs, Oxidative stress, Antioxidant enzymes, Histopathology

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Introduction

Nanotechnology has fastened the pace of advancements in all fields of science and technology (ALRashdi et al., 2023; Elbehary et al., 2023; Mansoor et al., 2023). This has led to the extensive use of nanomaterials including several metallic oxides like MgO, ZnO, NiO and TiO_2 (Shahid et al., 2023; Altaf et al., 2024; Öziç et al., 2024). Nanomaterials (NMs) have demonstrated significant advantages and technological benefits in the fields of biomedical sciences, agriculture and environmental sector, engineering, food and cosmetics (Mansour et al., 2023). However, their increasing production and usage multiply the chances of their release into the surrounding environment, making human exposure to NMs inevitable (Anjum et al., 2023; Aslam et al., 2023). This exposure over an extended period may affect the living organisms inhabiting the ecosystem (Azam et al., 2022; Krishnaveni et al., 2023).

The MgO NPs owing to their unique physicochemical characteristics have garnered significant attention in various fields, including medicine, electronics, and environmental science. They are used extensively for their antibacterial, anticancer activities, and drug delivery capability (Nejati et al., 2022; Mwafy et al., 2023). MgO nanoparticles have been studied for their potential role in precise drug delivery for cancer therapy (Di et al., 2012; Iqbal et al., 2024). They can be filled with anti-cancer medications and directed toward specific tumor locations, thereby overcoming generalized side effects and increasing their effectiveness (Danhier et al., 2010). Magnesium oxide nanoparticles possess unique magnetic and optical properties under which the image quality can be enhanced to a greater extent (Ahmad et al., 2020).

MgO NPs are also found to regulate the inflammatory response in several conditions including arthritis, inflammatory bowel disease and other important diseases (Fahmy et al., 2022). The potential applications and beneficial aspects of these nanoparticles in predominantly all fields of science have overlooked adverse effects that these NPs can impose to public health and environment. This research examined the impact of MgO NPs varying doses on the oxidative stress indicators and antioxidant enzyme levels in the rat model. Studying these potential risks to biological systems provided crucial information that will be helpful in mitigating hazards linked with NPs usage in several industries. Moreover, safe dosage levels can also be assessed based on these experiments in addition to their toxicological effects profiling. In the case of MgO NPs, there is not much data available revealing their hazards at different dosage levels and describing their underlying mechanisms (Noori and Kareem, 2019; El-Dawy et al., 2023). Oxidative stress leads to an imbalance between the generation of reactive oxygen species (ROS) and the body's capability to neutralize these harmful by-products and is also a critical parameter in studying nanoparticle-induced toxicity. Excessive generation of ROS can lead to damaging cellular components (lipids, proteins, and DNA), and cause various pathological conditions (Boğa et al., 2024).

Through a series of biochemical markers and histopathological indicators, this study seeks to elucidate the correlation between MgO nanoparticle exposure and their neurological, muscular and testicular toxicities shown via oxidative damage. By investigating the extent of toxicity and the mechanisms driving oxidative stress, the study provides valuable insights into the potential health risks of MgO nanoparticles, paving the way for safer application and regulation in various industries.

Material and Methods

Nanoparticles, albino rats and experimental design

MgO-NPs were obtained from the Institute of Physics of the Islamia University of Bahawalpur, Pakistan. All the chemicals and different reagents used during the current experimental trial were of analytical grade. Twenty mature male Rattus norvegicus albinos weighing between 140 and 160 gm were acquired from the animal house of the Islamia University of Bahawalpur. All the rats were free from any type of illness and were in good health condition. Fifteen days before the start of the trial, the rats were shifted to laboratory for acclimatization. Standard diet and clean fresh water were provided ad-libitum to the rats daily for the entire trial period. A 12-hour light/dark cycle was followed and the humidity (65±5%) and the temperature 24±1°C were kept. The rats were handled throughout the experiment following the guidelines on the care and use of laboratory animals.

Groups and doses

Rats were randomly grouped into four groups (G_0, G_1, G_2, and G_3) and kept separately in wire cages

throughout the experiment. Group G_0 served as the control. In Group G_1, rats were injected with MgO NPs at a dosage of 25 mg/kg/day, while group G_2 received 50 mg/kg/day, and group G_3 was given 75 mg/kg/day intraperitoneally for 10 days. All the rats were observed daily for any abnormalities.

Tissue homogenates, oxidative stress and antioxidant enzymes

The collected organs/tissues (brain, muscle, and testis) were separately triturated in petri dishes and homogenized using chilled double distilled water. The homogenates were then centrifuged at 3000 rpm for 10 minutes. The sediment obtained after centrifugation was collected and stored at -20°C for further analysis. Several parameters related to oxidative stress and antioxidant status were examined, including reactive oxygen species (ROS) (Hayashi et al., 2007), reduced glutathione (GSH) (Jollow et al., 1974; Raza et al., 2022), Thiobarbituric acid reactive substances (TBARS) (Grewal et al., 2005), and various antioxidants such as catalase (CAT), superoxide dismutase (SOD) (Akram et al., 2021; Kakkar et al., 1984), and peroxidase (POD) (Misra and Fridovich, 1977). Absorbance readings for POD, SOD, and CAT were taken at wavelengths of 470 nm, 560 nm, and 240 nm, respectively, while ROS and TBARS were measured at wavelengths of 505 nm and 532 nm. A UV-Vis spectrophotometer was used for these measurements.

Histopathology analysis

10-day-old rats in all the groups were humanely euthanized and dissected. Various tissues including the brain, muscle and testis were collected at the necropsy. The absolute organ weight of each organ was recorded. The relative weight of different organs was calculated as Organ-Weight-to-Body-Weight Ratios. Each collected organ was preserved separately in 10% formaldehyde solution for histopathological lesions (Shaker et al., 2023). Approximately, 4-6 µm thick sections were taken using the Leica rotary microtome from each organ. Following conventional histological protocols, the collected sections were then coated in paraffin wax, processed and stained with hematoxylin and eosin (H&E). A light microscope was used to observe deleterious effects of nanoparticles on various organs.

Statistical analysis

Using the IBM statistical program package, the ANOVA test was used for statistical evaluation of data collected from oxidative and antioxidant enzymes (SPSS). Rats injected with NPs and those not, were compared for the standard deviations of all investigated parameters, including antioxidant and oxidative enzymes, using Tukey's test. $P \leq 0.05$ was set as a reliable threshold.

Results

Behavioral changes

MgO NP treated rats indicated behavioral and neurological changes like depression, weakness, and weight loss.

Relative weight of visceral organs

The relative weight of various organs of rats exposed to different concentrations of MgO NPs was measured. After two weeks of intraperitoneal injection with various doses of MgO NPs, a substantial increase in relative weight of the brain and testes was recorded. The values showed a considerable increase in the relative weight of the brain and testis indicating adverse effects of MgO NPs which appeared to be dose-dependent (Fig. 1).

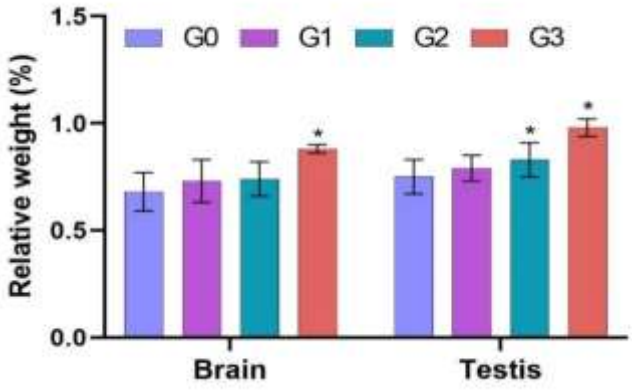

Figure-1. Photograph showing comparison of relative weight of different organs of rats exposed to varying doses of MgO NPs

Oxidative stress and anti-oxidative parameters

Brain

In brain, ROS and TBRAS indicated abnormally high values in rats treated with higher doses of MgO NPs (50 mg/kg/day and 75 mg/kg/day). The quantity of antioxidant enzymes reduced significantly in albino rats at higher doses of NPs indicating stress as compared to the control group (Figure 2).

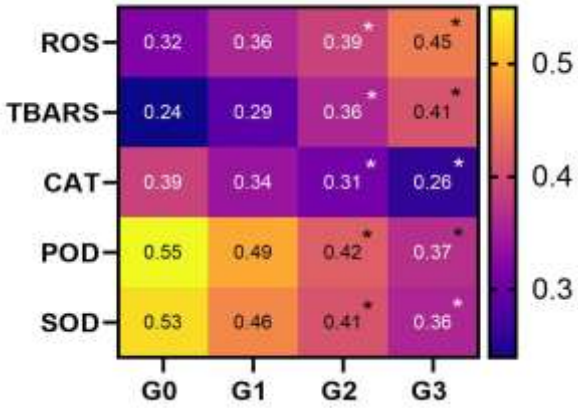

Figure-2. Photograph showing comparison of oxidative stress and antioxidant profile of brain of rats exposed to varying doses of MgO NPs

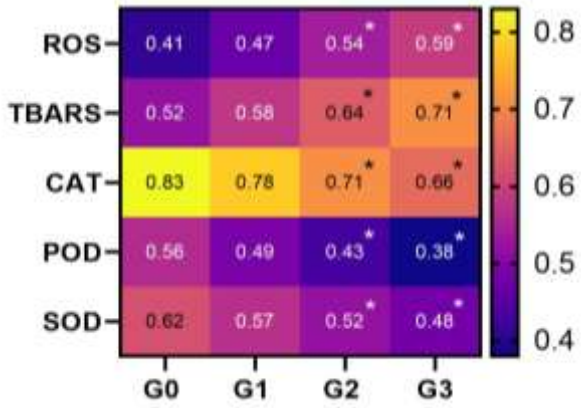

Figure-3. Photograph showing comparison of oxidative stress and antioxidant profile of testis of rats exposed to varying doses of MgO NPs

Testis

In case of testis, the oxidative stress biomarkers were significantly increased in rats reared in groups G_2 and G_3. The contents of CAT and POD non-significantly changed in treated group G_1 as compared to the control group. These enzymes, however, present significant changes in G_2 and G_3 treated groups. In testis, the contents of SOD significantly reduced in treated groups (G_1, G_2, and G_3) as compared to the control group (Figure 3).

Histopathology of different visceral organs

Testis

The MgO NP treated groups have shown adverse changes including inflammation, sloughing of cells, damaged spermatogonia, increase in cell debris in seminiferous tubules, hypospermatogenesis, necrosis of spermatids, spermatogonia and arrest of spermatogenesis process (Fig 4). The severity of various histopathological alterations in the testis of MgO NPs exposed and unexposed groups of male albino rats have been tabulated against varying doses. (Table 1).

Table-1. Histopathological alterations in the testis of MgO NPs exposed and unexposed male albino rats

Parameters	Groups/Treatments			
Histopathological lesions	**G_0 (Control)**	**G_1 (25mg/kg/day)**	**G_2 (50mg/kg/day)**	**G_3 (75mg/kg/day)**
Inflammatory processes	-	++	++	++
vacuolation of epithelia of seminiferous tubules	-	+	++	+++
Reduction in diameter of seminiferous tubules	-	++	++	+++
Partial germ cell arrest	-	+	++	+++
Decrease frequency of normal seminiferous tubules	-	+	++	+++
Degeneration and damage of spermatogonia	-	+	++	++
Hypospermatogenesis	-	+	++	+++
Sloughed cells	-	+	++	+++
Increase cell debris in lumen of seminiferous tubules	-	+	++	+++
Disorganization of spermatogonia and Sertoli cells	-	+	++	+++
Necrosis of spermatogonia and Sertoli cells	-	+	++	+++
Arrest of process of spermatogenesis	-	++	++	+++
Increased germ cell depletion in seminiferous epithelium	-	++	++	+++
Necrosis of spermatids	-	+	+++	+++

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

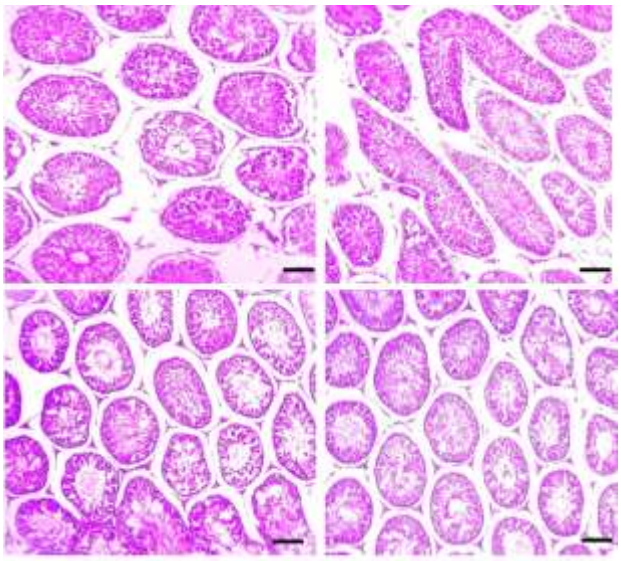

Figure-4. Photomicrograph of testes of albino rats treated with MgO NPs (75mg/kg body mass) showing various pathological ailments like arrest of spermatogenesis, degeneration of seminiferous tubules and necrosis of epithelium of seminiferous tubules. H&E Stain;400X

Brain

Microscopic observation of the brain of treated rats exhibited different ailments like necrosis of neurons, atrophy of neurons, microgliosis, cytoplasmic vacuolization, and congestion post-treatment (Fig 5). The severity of various histopathological alterations in the brain of MgO NPs exposed and unexposed male albino rats has been shown (Table 2).

Muscles

The histopathological alterations observed in the muscles of rats were atrophy and necrosis of myocytes, edema, inflammatory cell infiltrate, and intrafiber necrosis at low dosage levels. Higher dosage leads to severe to very severe changes especially inflammatory cell infiltration, and degeneration of muscle fibers (Fig 6; Table 3).

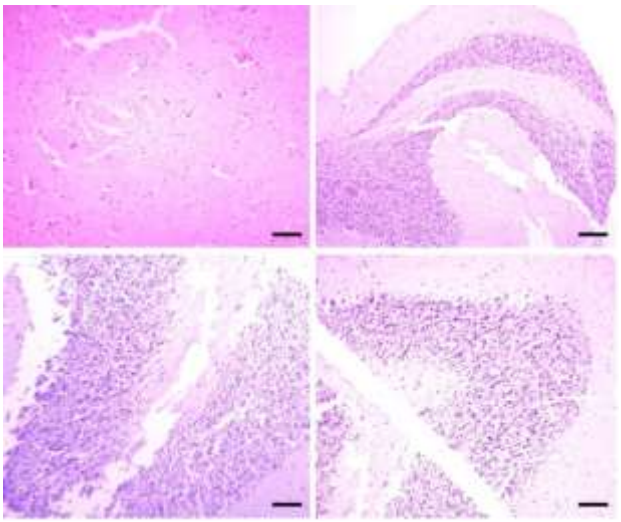

Figure-5. Photomicrograph of brain of albino rats treated with MgO NPs (75mg/kg body mass) showing various pathological ailments like eccentric neurons, microgliosis, degeneration of neurons, atrophy of neuron and necrosis of neuron. H&E Stain;400X

Table-2. Histopathological alterations in the brain of MgO NPs exposed and unexposed groups male albino rats

Parameters	Groups/Treatments			
Histopathological lesions	**G_0 (Control)**	**G_1 (25mg/kg/day)**	**G_2 (50mg/kg/day)**	**G_3 (75mg/kg/day)**
Eccentric nuclei of neuron	-	+	++	+++
Hypertrophy of cytoplasm of neurons	-	+	+++	+++
Edema	-	+	++	++
Microgliosis	-	+	++	+++
Atrophy of neuron	-	+	++	+++
Necrosis of neuron	-	+	++	++
Inflammatory reactions	-	+	++	++
Neuronal degeneration	-	+	+++	+++
Vacuolation of neuron	-	+	++	+++

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

Table-3. Severity of various histopathological alterations in muscle of MgO NPs exposed and unexposed groups male albino rats.

Parameters	Groups/Treatments			
	G_0 (Control)	G_1 (25mg/kg/day)	G_2 (50mg/kg/day)	G_3 (75mg/kg/day)
Atrophy of myocytes	-	++	+++	+++
Necrosis of myocytes	-	+	++	+++
Inflammatory cell infiltration	-	+++	++++	++++
Intrafiber necrosis	-	+	++	+++
Muscle mass loss	-	+	++	+++
Edema	-	+	++	+++
Degeneration of muscle fibers	-	++	+++	++++

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

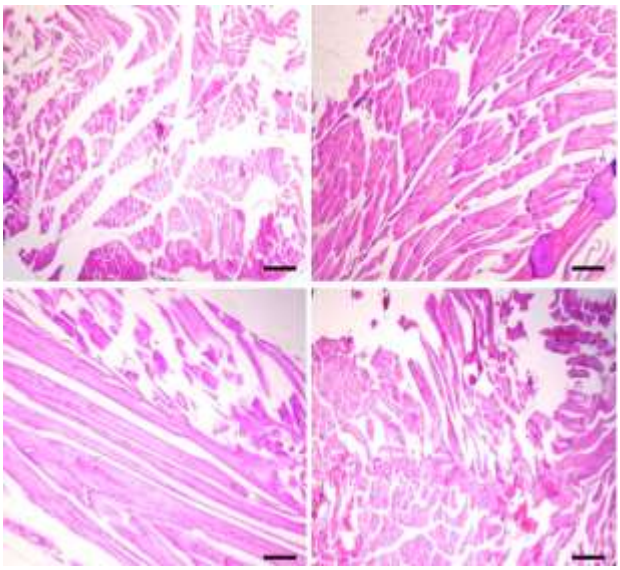

Figure-6: Photomicrograph of muscles of albino rats treated with MgO NPs (75mg/kg body mass) showing various pathological ailments like necrosis of myocytes, atrophy of myocytes, intra-fiber necrosis, degeneration of neurons, atrophy of neuron and necrosis of neuron. H&E Stain;400X

Discussion

Different reports have indicated that estimation of hemato-biochemical and histopathological (Hussain et al., 2017; Hussain et al., 2019; Hussain et al., 2020) and oxidative stress biomarkers play a pivotal role in monitoring/screening of toxic effects of various compounds. MgO NPs are important small biomaterials having diversified usage in medicinal, microbiological, environmental, agriculture, energy production and public health management (Sisubalan et al., 2024). Their continuous and long term exposure at higher concentrations is however associated with health risks (Rempel et al., 2020). There is not much data available on the underlying mechanisms of the toxicological effects of these NPs on living organisms. This study aimed at exposing laboratory rats to three different doses of MgO NPs and examining their toxicological effects. The results have shown a significant increase in reactive oxygen species and Thiobarbituric acid reactive substances (TBARS) levels in various visceral organs in a dose-dependent manner. These elevated levels can be attributable to increased ROS production and lipid peroxidation along with glutathione depletion, which leads to tissue damage in the visceral organs (Mangalampalli et al., 2018). This study demonstrated that the exposure of rats to MgO NPs can lead to the induction of oxidative stress in multiple visceral organs/tissues like the brain, testis, muscles in a dose-dependent manner. Higher ROS and TBARS content in MgO-exposed rats can lead to the generation of free radicals. It is noted that these NPs induce oxidative stress via interaction with polyunsaturated fatty acids in the cell membrane of various visceral organs including the brain, testis and muscles (Sudhabose et al., 2024). The physiological abnormalities can lead to inflammation of various organs in the MgO NP exposed rats which might be due to the reduction in total protein content in different tissues.

There are few reports available regarding the toxicological effects of MgO NPs in terms of oxidative stress and genotoxicity in the isolated cells of various visceral organs (Kazmi et al., 2023; Sudhabose et al., 2023). Exposure to different concentrations of metallic oxide nanoparticles leads to the generation of ROS depending upon dosage and

duration of exposure resulting in protein denaturation and lipid peroxidation thereby inducing genotoxicity (Feng et al., 2000). In addition these MgO NPs also induce toxicity via the liberation of Mg ions like that of ZnO ions (Hajibeygi et al., 2021). The genotoxic effects might be due to the interaction of these metallic oxide NPs with the sulfur containing proteins resulting in free radical formation and blockage of respiratory enzymes thereby leading to cell death (Feng et al., 2000). The dosage and length of exposure to NPs is often related to oxidative stress if exposure continues (Ali et al., 2024). Moreover it has been found that direct nanoparticle exposure leads to more DNA damage in metabolically active organs and tissues (Dong et al., 2022; Sibiya et al., 2022). Observations regarding genomic mutations and chromosomal abnormalities have led to the mutagenic/carcinogenic nature of these MgO NPs (El-Hamaky et al., 2023; Hamida et al., 2020). MgO NPs induce DNA damage via oxidative stress, direct binding of DNA, and inhibition of its repair mechanism (Hamida et al., 2020). ROS cause oxidation of nucleotide bases and break in the strands which can lead to conformational changes and strand crosslinks (Khan et al., 2022; Sial et al., 2023). These changes as well as suppression of DNA repair mechanism might induce genotoxicity (Li et al., 2018). Such genotoxic effects have been reported for other nanoparticles as well (Attia et al., 2018; Tulinska et al., 2022). Moreover, increased DNA damage in brain, testis and muscles and other organs are indications of systemic genotoxicity. Chronic exposure to high doses of MgO NPs have similar genotoxic effects as reported for other metallic oxide nanoparticles as well (Cavallo et al., 2023; Gurram et al., 2023).

The antioxidant enzyme including CAT, SOD, GSH and POD values reduce in the rats exposed to high dose of MgO nanoparticles. This reduction in values in exposed organs and tissues might be correlated with inflammatory response. The NPs might trigger inflammation and oxidative stress which cause damage to DNA in the tissues and organs of the affected rats (Srisuvetha et al., 2020). This oxidative injury can also trigger apoptosis and necrosis thereby leading to loss of functioning and cell death (Ali et al., 2024; Samim et al., 2023). The oxidative injury noticed in this study can be due to the generation of reactive oxygen species, oxidative signaling cascade activation and antioxidant depletion (Balkrishna et al., 2021; Venkatappa et al., 2022). ROS interact with macromolecules (proteins, lipids, DNA) and causes oxidative changes. Increased TBARS due to MgO NPs can result in compromising the integrity of cell membrane. Significant decline in GSH in high NP dosage exposed rats further elaborated the impairment of antioxidant defenses against ROS (Arslanbaş and Coşar, 2019; Mazaheri et al., 2019). Studies carried out earlier for examining the nanotoxicity of metallic oxide nanoparticles also reported decline in antioxidant enzymes in different experimental models (Aziz and Abdullah, 2023; García-Medina et al., 2022; Ghafarifarsani et al., 2023; Naguib et al., 2023). The oxidative stress induced by NPs can be more pronounced and can inhibit the antioxidant enzymes functioning as a secondary response (Sanati et al., 2022). CAT and SOD detoxifies H_2O_2 and superoxide radicals and POD clears lipid peroxides (Kumar et al., 2023). Their reduction impairs the activity to neutralize reactive oxygen species and leads to oxidative damage. The ROS dysregulation in response to decline in antioxidant enzymes have consequences leading to biomolecular oxidation and cell injury (Ghorbani et al., 2023). It has also been postulated that interaction of Mg2+ ions intracellularly could result in lowering the values of proteins and different antioxidant enzymes as seen in various organs and tissues (Dolmetsch et al., 1998). These Mg2+ ions activate transcription elements (NF-kB) that result in free radicals formation leading to changes in the homeostasis of cells which enhance Mg2+ influx during stimulation of endoplasmic reticulum and production of superoxide anions responsible for mitochondrial perturbation and cell damage (Xia et al., 2008).

Conclusion

This study demonstrates the systemic toxicity in rats exposed to MgO nanotoxicity manifested through oxidative stress, the decline in antioxidant activity, genotoxicity and clastogenic effects on various organs and tissues including the brain, testis and muscles. These NPs also exhibited elevated ROS levels, lipid peroxidation, DNA damage, and reduction in antioxidant enzymes and glutathione levels. MgO NPs therefore directly or indirectly affect the living cells suggesting their adverse health effects in vivo in rats in a time and dose dependent response.

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Conflict of Interest: None.
Source of Funding: None.

Data Availability Statement

The data/findings of the study are available upon request to the corresponding authors.

Ethical Approval

All applicable national, international, as well as institutional guidelines mentioning the care and use of animals were strictly followed.

Contribution of Authors

Afzal G: Designed the experiment and prepared the initial draft of manuscript
Ullah MI, Alhakamy NA, Rajeh N, Rehan S & Iqbal R: Reviewed literature, critiqued and edited the final draft of manuscript
Ali N & Khan A: Reviewed literature and prepared the initial draft of manuscript
Afzal M & Iqbal MS: Data collection & analysis and prepared the initial draft of manuscript
Hussain R: Concieved idea and designed the experiment

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