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Review Article

Impacts of ZnO nanoparticles on brain and spleen in male albino rats, A comprehensive exploration of diverse exposure routes

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ABSTRACT

Nanotechnology is rapidly developing in the fields of industry, medicine, and nutrition. This study aimed to evaluate the effect of zinc oxide nanoparticles (ZnO Nps) toxicity on rats' Spleen and brain. In the brain, ZnO NPs cause alteration in histopathological and structural changes including necrosis, gliosis, and spongiform change. In spleen tissue results show that ZnO NPs exposure manages to induce alteration in various studied hematological parameters like neutrophils, platelets, and eosinophils which are found to increase significantly. However, hemoglobin content, PCV, and MCV decrease with increasing doses of ZnO NPs significantly DNA damage in rats. A flow cytometric study was performed for a better understanding of the underlying mechanism of the ZnO NPs-mediated toxicity. From the present investigation, an increase in ROS production, a decrease in MMP, and increased apoptosis were exhibited. The accumulation of nanoparticles has also shown a dose-dependent increase in the spleen and brain. Therefore, ZnO NPs may have the potential to induce a toxic effect even when exposed intermittently.

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Introduction

Food animals Nanotechnology is a term used to define areas of science and engineering in which phenomena occurring at nanoscale dimensions are used in the design, characterization, manufacture, and applications of materials, structures, devices, and systems). "Nanotechnology" is generally defined as science, engineering, and technology applied at the nanoscale, or at least one dimension, should be between 1 and 100 nm (Bayda et al. 2019). It has the power to significantly alter human existence, economic events, and societal dynamics (Moshed et al. 2017). The development, evaluation, and use of nanomaterials in the domains of material science, biology, chemistry, and physics constitute the cutting-edge field of nanotechnology. Nanomaterials stand out from their bulk counterparts due to their unique surface chemistry and chemical reactivity. Since there are more reactive sites available, an increase in the surface-to-volume ratio of nanomaterials causes an increase in reactivity or toxicity. The study of all poisonous substances that negatively impact living things and their surroundings is known as toxicology. Nanoparticles have been more exposed to the environment and people during the past several decades due to developments in the science of nanotechnology and its subsequent uses. When analyzing NP's effects on human health, their presence in the environment is crucial (Naz et al. 2020).

It is yet not understood which mechanisms allow nanoparticles to interact with biological systems in a way that causes nontoxicity (Singh et al. 2017). Materials such as NPs, nanotubes, nanocomposites, and nanostructures are considered nanomaterials if at least one dimension is less than 100 nm (Assadian et al. 2018). Nanoparticles have opened a lot of opportunities for scientific and industrial endeavors because these nanomaterials' properties are manipulated at a very small scale (Atale et al. 2017). These nano-sized materials have diverse applications in the textile industries, nan medicines, pigments and dyes, solar systems and capacitors, field energy storage, wastewater treatment, food industries, regulation of plant metabolic pathways, lithium-ion batteries, sensors, and catalytic applications (Isaac et al. 2013). Some of the distinguishing reported types of nanoparticles include photo chromatic NPs, core-shell NPs, polymer-coated magnetite NPs, metal oxide NPs FeONPs, CuONPs, MgONPs, ZnONPs, metal dioxide NPs CeO_2 NP, TiO_2 NPs, ZrO_2 NPs, and inorganic NPs, AgNPs, CoNPs, FeNPs, AuNPs, PdNPs (Mirzaei and Darroudi 2017; Dhoble and Kulkarni 2018). Each of these nanoparticles has a specific size, shape, morphology, texture, and applications (Waris et al. 2021).

Zinc (Zn) is one of the most widely used micronutrients (Boreiko 2010). Zinc oxide (ZnO) accounts for the largest industrial use of Zn compounds. Zn is used primarily in the rubber industry, as a heat conductor, white pigment, and to absorb ultraviolet (UV) light. ZnO NPs have also been used in a variety of products including semiconductors, catalysts, and paints. Further, these particles are increasingly found in consumer products, such as sunscreen, because of the strong UV absorption properties of ZnO (Choi et al. 2015) Products containing ZnO NPs may release nanoparticles or Zn ions, which may be translocated from the environment into the human blood circulation and accumulate in organs, potentially leading to toxicity (Baky et al. 2013; Sahu et al. 2014). ZnO NPs are one of the well-known metal oxide nanoparticles regarding nanomedicine and biomedical application (Rahdar et al. 2020b). This is due to their rapid and cost-effective synthesis methods and a good level of biocompatibility with living systems under consideration along with a good potential for antibacterial and antifungal activity reported for various microorganisms. Preparation methods for ZnO NPs can be mainly classified into two such as (i) physical or chemical methods and (ii) biological methods (Mirzaei and Darroudi 2017). Chemical synthesis is that the concentration of the chemicals used in the process can influence the size and shape of the particles considerably. It is known that it is po process but different concentrations and ratios of chemicals (Bandeira et al. 2020) possible to obtain particles from a few nanometers (5–10 nm) up to micrometric size using the same. There are different biological sources for the synthesis of metallic NPs including plant extracts, microorganisms and enzymes which can reduce the metal ions within the solution into NPs; in fact, it has been proposed that they can act as reducing, capping and stabilizing agents for the NPs biosynthesis as a novel method without any toxic chemicals (Shabaani et al. 2020).

In general, it is suggested that the toxic effects produced by ZnO NPs in different tissues or cell lines are mediated by increased oxidative stress and inflammation (Liang et al. 2018). ZnO possesses antihyperglycemic activity in diabetic rats after daily treatment for different periods (Ahmed et al. 2020). A variety of studies have shown that most nanoparticles have toxic effects on male germ cells (Mesallam et al. 2019). In contrast, ZnO (Engineered nanoparticles) ENPs also have the potential to reduce the viability of certain immune cells, including macrophages, and to impair macrophage activity by downregulating the genes coding for major histocompatibility complex class II molecules. It is important to understand the influence of ZnO ENPs on immune cells to evaluate the potential of these nanoparticles to affect immunological responses (Watson et al. 2015). Excessive zinc is reported to induce toxic effects. Release of metallic cations Zn^{2+} from ZnO NPs is also proved to be toxic in micro-organisms and rodents. ZnO NPs might enter via different routes to reach blood flow and induce adverse impacts on organs (Ben-Slama et al. 2015). Preliminary results indicated ZnO NPs affected organ systems may show inflammation, altered heart rate and functions, and oxidative stress (K Handral and Kelmani R 2018). ZnO NPs have shown “toxicity” in mice brain tumor cell lines when compared with similar-sized particles of Al_2O_3, TiO_2, Fe_3O_4, and CrO_3. In addition, in vitro studies, demonstrated that ZnO NPs induced the apoptosis of neural stem cells and disturbed the ion channel current in primary hippocampal neurons (Deng et al. 2009; Attia et al. 2018). However, unintended exposure to human beings during handling, storage, and disposal causes occupational hazards raising questions on health risk assessment regarding the toxicity, including cytotoxic, genotoxic, and pro-inflammatory effects (Singh et al. 2019). Most of them attribute the toxicity to particle dissolution and the ability to induce ROS. It has been reported that exposure to ZnO NPs induces cytotoxicity in neuronal progenitor cells and neurons (Sudhakaran et al. 2020).

The use of nanoparticles based on metals and their oxides is of great interest. One of the well-studied metals affecting biological objects is zinc (Zn) and its oxide (O). Zn is an active element and exhibits strong reduction properties. It can easily oxidize to form ZnO. Zn plays an important role in the human body since it is one of the most important trace

elements. Zn is found in all tissues of the human body, with the highest concentration found in myocytes (85% of the total zinc content in the body) (Król et al. 2017). ZnO NPs have been extensively used in the manufacture of ceramics, photocatalysis, and ultraviolet (UV) filters (Dimkpa et al. 2017). ZnO NPs are used as juice-clearing agents and nano-fertilizers in the food and agriculture industries, respectively. ZnO NPs can penetrate the cell membrane easily due to their much smaller size and then be distributed to various organs by systemic circulation (Hussain et al. 2020). These nanoparticles have both useful and dangerous qualities. Because ZnO nanoparticles are the commonly utilized nanomaterials in various consumer products, assessment of their toxicity is extremely important (Zhu et al. 2019; Toropov and Toropova 2021). The synthesized nanoparticles can be used for various applications like sensors, lasers, drug discovery, etc., and the synergistic effects may be measured depending on the application. In such applications where the anticancer (Jayappa et al. 2020), antibacterial (Naseer et al. 2020), antimicrobial (Supraja et al. 2018), antityrosinase (Darroudi et al. 2020), and antibiofilm (Mahamuni et al. 2019) activities of nanoparticles are utilized, shape and size play an important role. For example, when compared to rod-shaped ZnO NPs, the spherically shaped NP can release Zn^{2+} ions with much ease, and small-sized particles are more permeable to bacteria membranes (Jayachandran et al. 2021) Among these, with multifaceted benefits, ZnO NPs are exciting inorganic materials. ZnO-NPs can be used in various sectors, such as energy conservation, textiles, electronics, healthcare, catalysis, cosmetics, semiconductors, and chemical sensing (Faisal et al. 2021).

Although zinc is considered relatively non-toxic, there is growing evidence that free zinc ions can cause negative effects on cells. To assess the toxicity of a test substance *in vitro*, animal cell cultures are usually used. It is known that nerve cells are the most sensitive to exogenous influences (Mitroshina et al. 2020). It has been reported that exposure to zinc ions leads to neuronal degradation. The mechanisms of action of ZnO NPs can be reduced to the following: disruption of the cell membrane, binding to proteins and DNA, generation of reactive oxygen species (ROS), disturbance of the processes of bacterial DNA amplification, alteration (more often, down-regulation) of expression in a wide range of genes (Saha et al. 2020; Karthik et al. 2022).

Routes of exposure dependent toxicity studies

In vivo studies explored various routes of exposure to ZnO NPs (Sharma et al. 2012). The potential routes of exposure such as injection, inhalation (Ben-Slama et al. 2015) dermal exposure (Smijs and Bouwstra 2010), Oral administration, intravenous (i/v) and intraperitoneal (i/p). To evaluate the toxicity intravenous (i/v) and intraperitoneal (i/p) administration needs has to be performed (Handral and Kelmani R 2018).

Oral administration

According to (Sharma et al. 2012) oral administration of ZnO NP also produced toxicity in some animal models. ZnO NPs induced oxidative stress, DNA damage, and apoptosis in mouse liver, spleen, kidney, and brain after sub-acute oral exposure to 300 mg/kg for 14 days. Baek et al. (2012) suggested that ZnO NPs were not readily absorbed into the bloodstream via GIT after a single oral dose and were mainly excreted via feces. In the study, rats were given a single dose of 50, 300, or 2000 mg/kg and data indicated that the distribution levels were not higher than expected because of a low absorption rate. When rats were given a single oral administration of 5, 50, 300, 1000, or 2000 mg/kg, an inverse dose-dependent increase was noted in aspartate aminotransferase and alanine aminotransferase (Pasupuleti et al. 2012). Tang et al. (2021) (Teng et al. 2021) verified that oral administration of ≥ 50 mg/kg body weight of ZnO NPs (30 nm diameter) for 14 days in adult male mice caused spermatogenesis abnormalities, testicular damages, and reduced serum testosterone levels. Moreover, a significant increase in the serum levels of inflammatory cytokine (IFN-γ, IL-6, and TNF-α), the CD^{4+} and CD^{8+} cells number, and the Zn levels in the liver, spleen, and thymus of male BALB/c mice orally exposed to 50 mg ZNPs /kg b.wt for 14 consecutive days (Senapati et al. 2017).

Intravenous injection

The toxicity and kinetics of ZnO NP after intravenous (iv) injection, which is a route of distribution of NPs to target organs. For the intrinsic in vivo toxicity of ZnO NPs, it is essential to deliver NPs to target organs. This may be done by IV injection. Exposure through IV injection of ZnO NP is infrequent in the workplace or from consumer products (Choi et al. 2015). ZnO NPs (3.0 mg/kg or 30 mg/kg body weight) were not promptly absorbed from the GIT of rats after consumption and were mostly eliminated in feces. However, the ZnO NPs (30 mg/kg body weight) blood concentration peaks within 5 min and returns to normal by 48 h post-intravenous injection. Administered ZnO NPs disseminated mainly to the liver, kidneys, lung, and spleen, but not to the thymus, brain, and testes. In these rats, intravenous ZnO NPs enhanced the formation of mitotic figures in the hepatic tissues, while in the pulmonary tissues, multifocal acute damages with dark brown pigmentation were found (Fujihara and Nishimoto 2023). Rahdar et al. (2020b) reported that rats injected IV with 30 mg/kg of ZnO NPs were found to have elevated levels of mitotic figures in hepatocytes and

multifocal acute injuries with dark brown pigment were noted in lungs. Examination of organs revealed that ZnO NPs were distributed mainly to the liver, kidneys, lung, and spleen, but not to the thymus, brain, and testes. The altered distribution level was significantly reached to normal level by seventh-day post-administration along with feces excretion levels after IV injection along with biliary excretion of ZnO NPs. Fujihara et al. (2015) demonstrated that with a single intravenous exposure to neutron-activated ZnO NPs (size 10 and 70 nm, dose 120 µg/mouse) in mice, the highest concentrations of ZnO NPs were detected in the lungs and liver (< 7 h post administration) and ZnO NPs were distributed in the blood, liver, spleen, lungs, brain, and heart at 24 h post administration (Fujihara et al. 2015). We have previously performed single intravenous administration of ZnO NPs (size 58.5 nm, dose 0.2 mg/kg) in mice and the short-term tissue distribution of ZnO NPs in the lungs, liver, kidneys, and spleen was investigated up to 1 h (Yeh et al. 2012).

Intraperitoneal injection

ZnO NPs also showed toxicity to the central nervous system. Intraperitoneal (I/P) injection with ZnO NPs (100 mg/kg body weight) significantly ($p < 0.05$) improved MDA and decreased catalase and SOD activities in the brain. The study suggested that the ZnO NPs-induced prominent brain histological changes, including oedema and satellitosis, could cause behavioural changes (Rahdar et al. 2020a). Han et al. illustrated that long-term (8 weeks) IP injection of ZnO NPs (4.0 mg/kg body weight) resulted in attenuating the spatial learning and memory capacity through a change in synaptic plasticity in young Wister rats (Han et al. 2011). Li et al. (Li et al. 2012) performed a single intraperitoneal injection of ZnO NPs (size 93 nm, dose 2.5 g/kg) in mice and showed that Zn accumulated in various organs except for the brain due to the blood-brain barrier up to 72 h post-dosing. The Zn levels in the liver were the highest, followed by those in the spleen, lungs, kidneys, and heart. Amara et al. (Amara et al. 2014) performed repeated intraperitoneal injections of ZnO NPs (size 20–30 nm, dose 25 mg/kg every other day for 10 days) in rats and showed that there was no Zn accumulation in the liver, brain, and kidneys after 24 h of exposure withdrawal. Lin et al. (Lin et al. 2015) reported the accumulation of ZnO NPs (size 47.8 nm, dose 10 mg/kg) in mice in the liver, lung, kidneys, spleen, and heart 6 h after a single intraperitoneal injection.

Toxicological effects of ZnO nanoparticles on various organs of rats

ZnO NPs have been shown to exert harmful impacts on different cells, including megakaryocyte, inflammatory response, DNA damage, and apoptosis. According to recent research, the release of Zn is what causes ZnO nanoparticles to be poisonous.

Effects on body weight and organ weight

There was no significant difference in the body weight of the rat dams treated with ZnO NPs (5, 25, 50 mg kg^{-1}) during the lactating period (19 days) and their offspring. The liver and kidney weight of the treated dams or offspring did not significantly change. The spleen weight of the dams treated with ZnO NPs (50 mg kg^{-1}) increased significantly (Hussain et al. 2020). In high-dose group animals, the mean body weight was significantly decreased on day 7 whereas on day 15 showed reduced their lower dose group. The % gain in body weight was increased by 1.23% on day 7 and 8.35% on day 15, which was also statistically lower (Srivastav et al. 2016). ZnO NPs-administered rats show a significant reduction in daily weight gain. On the other hand, ZnO NPs + BV-treated rats showed a significant increment in daily weight gain compared to ZnO NP-exposed rats. Moreover, no significant difference exists in daily weight gain between ZnO NPs + BV and ZNPs/BV-treated group and the C28D and C56D control groups, respectively. On the other hand, no significant in brain weight was recorded (Eleiwa et al. 2023). The acute toxicity shows the percentage net body weight of mice treated with TiO2 NPs, and ZnO NPs for 14 d. No significant differences were observed in the percentage net body weight of mice treated with 150.00 mg/kg of TiO2 NPs or ZnO. In contrast, a significant ($p < 0.001$) reduction in the net body weight was observed at 300.00 mg/kg of ZnO NPs. But a significant reduction in the relative spleen and brain weights for the three agents (Ogunsuyi et al. 2022). Decreases in organ weight of the heart, liver, spleen, lungs, kidneys, and brain were reported at 14 days post-exposure following a single intragastric dose (size ~ 20 ± 5 nm, 100 mg/kg) in mice and repeated (28 days) intraperitoneal and intravenous exposure in rats (size 12–90 nm, dose 30.3 mg/kg for 14 and 28 days) (Ashajyothi et al.). Razooki and Rabee (2020) (Razooki and Rabee 2020) reported a slight decrease in body weight after daily intraperitoneal injection with ZnO NPs with concentrations of 1, 10, and 100 mg/kg for 14 days.

Effects on brain tissues

The ZnO NP-exposed rats had a significantly higher content of dopamine and serotonin by about 20 and 13-fold of the control rat's values. In contrast, BV treatment significantly suppressed the ZnO NPs-induced increment in brain dopamine and serotonin levels to be 10, and sixfold of the control values in the ZnO NPs + BV and ZnO NPs/BV treated group, respectively, and a significantly lower than the ZNPs-exposed rats (Eleiwa et al. 2023). The intraperitoneal administration of ZnO NPs could induce significant alterations in brain

oxidative status. The ZnO NPs could induce oxidative stress and can significantly decrease endogenous antioxidant enzymes. The current work showed a significant decrease in brain GSH levels following treatment with ZnO NPs. These results were in contrast with other studies indicating the positive effects of ZnO NPs on brain function, or with studies indicating neuroprotective potential of these nanoparticles (Rahdar et al. 2020a). ZnO NP exposure caused significant Zn accumulation in the hippocampus and induced pathological changes and a cascade of signaling effects related to cholinergic neurotransmission. The excessive Zn accumulation and consequent disturbance of cholinergic neurotransmission in the brain indicate that inhalation of ZnO NPs may be a risk factor for neurological disorders such as AD (Guo et al. 2020). Shim et al. (Shim et al. 2014; Fujihara and Nishimoto 2023) examined the toxicological effects on the brain following oral, dermal, and intravenous exposure to ZnO NPs (size 20 nm, dose 500 mg/kg) for 28 days in rats. The blood–brain barrier was not damaged and could block penetration of ZnO NPs. No accumulation in the brain was observed following intraperitoneal administration of ZnO NPs (size 93 nm, dose 2.5 g/kg) in mice (Fujihara and Nishimoto 2023).

Effects on spleen tissues

The spleen had normal histology where red and white pulp could be identified clearly and megakaryocytes were occasionally noticed. After third and fourth weeks of exposure to exceptionally high dose of ZnO NPs, megakaryocyte hyperplasia was seen in the red pulp and the number of megakaryocytes increased. In the previous study, slight enlargement of the splenic corpuscle was reported at 1 and 5 g/kg of 20 and 120 nm of ZnO NPs after two weeks of exposure (Wang et al. 2008). The histopathological examination of the spleen is highly recommended to evaluate the immune system. In the nanotreated group, thickened stroma, atrophy of white pulps with disappearance of germinal centers, expanded red pulp, and also vacuolation and apoptosis of some splenocytes were revealed. Similar observations were reported by Wang et al. (Wang et al. 2008) on splenocytes after ZnO NPs. Abd El Fadeel et al. (2022) studied that orally intake of ZnO NPs causes marked changes in spleen tissues as the thickening of capsule, thickening of trabeculae, destruction of white pulp, and red pulp expansion. Abd Elmonem et al. examined that ZnO NPs prominently restructured the radiation-induced hepatic, renal, and spleen damage. It also induced significant regularization of radiation-induced biochemical abnormalities. Coadministration of vitamin E with ZnO-NPs has potentiated its antioxidant effect (Abd Elmonem et al. 2021).

Therapeutic mechanism of ZnO nanoparticles

Our research indicates that ZnO NPs have the potential to cause oxidative stress and can dramatically lower endogenous antioxidant enzyme levels. The results of the current study demonstrated a considerable drop in brain GSH levels after ZnO NP therapy. These findings were at odds with those of previous research showing ZnO NPs to have beneficial effects on brain function or with research showing these nanoparticles to have neuroprotective potential (Guo et al. 2020). According to the previous experiments, damage to the brain tissue can led to the neuronal degeneration, satellitosis, and pre-vascular edema, and, hence, the histopathological investigation of the brain could provide valuable information regarding the brain function (Aktas et al. 2007).

Conclusion

Mechanism of cellular toxicity and death via liposomal sequestration of ZnO nanoparticles can cross the cell membrane. ZnO NPs can dissolve and release Zn+ ions due to the lower pH in lysosomes. The release ion can directly interact with the DNA because of its positive charge and lead to apoptosis. A significant increase in dopamine and norepinephrine levels in brain cerebral cortex and increased brain oxidative stress suggest neurotoxic potential of these nanoparticles. In conclusion, ZnO NPs induced obvious immunotoxicity in the brain and spleen, where oxidative\inflammatory pathway may be the potential mechanism underlying this toxicity. We recommended treading carefully when using these nanoparticles and further studies still need to predict their toxicity, especially on human health.

Author's Contribution

All the authors contributed equally.

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Consent for Participation

All the authors participated equally

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All the authors gave their consent to publish this paper.

Availability of Data

Data from this study is available with the corresponding author.

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