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Review Article

In vivo and *in vitro* genotoxic effects of zinc oxide nanoparticles (ZnO NPs): A comprehensive review

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ABSTRACT

Zinc oxide (ZnO) nanoparticles (NPs) are extensively utilized in various consumer products, raising concerns about their potential impacts on human health. Although cytotoxic impacts of ZnO NPs on mammalian cells have been well-documented, their genotoxicity remains a topic of ongoing research with conflicting outcomes in existing literature. This comprehensive review seeks to offer a unified overview of the current state of knowledge concerning the genotoxic impacts of ZnO NPs on both humans and animals. The review focuses on elucidating the molecular processes underlying genotoxic events caused by ZnO NPs and considers the influence of nanoparticle size and form on their interactions with biological systems. Notably, oxidative DNA damage induced by dissolved Zn^{+2} ions emerge as a primary mechanism in *in vitro* analysis, showcasing the genotoxic potential of ZnO NPs. However, the majority of studies concentrate on acute exposure scenarios, leaving a critical gap in understanding the consequences of long-term exposure, which could shed light on intracellular bioaccumulation, DNA repair processes, and cellular survival mechanisms. While evidence suggests that ZnO NPs possess genotoxic properties, further investigations, particularly in extended exposure periods, are essential for assessing the risk posed by these NPs in both animal and human DNA integrity comprehensively.

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Introduction

Nanotechnology is a far-reaching technology with tremendous applications in various aspects, including general medicine, veterinary medicine, agriculture, aquaculture, and food production. The EU has adopted a definition of nanomaterials that includes unbound particles, whether they are organic, artificial, or accidental (Aljabali et al. 2020). The scientific community should analyze the toxicity of these technologies because of the promising successes of nanotechnology research, notably in the treatment of chronic illnesses like cancer (Maher et al. 2023).

NPs have drawn a lot of attention in recent years because of their distinctive characteristics and useful properties in agricultural and related industries. NPs can be generically categorized as inorganic and organic, depending on the primary component. Fullerenes and carbon nanotubes are examples of organic NPs. Inorganic NPs include

metals (Al, Bi, Co, Cu, Au, Fe, In, Mo, Ni, Ag, Sn, Ti, W, Zn, etc.), metal oxides (Al_2O_3, CeO_2, CuO, Cu_2O, In_2O_3, La_2O_3, MgO, NiO, TiO_2, SnO_2, ZnO, ZrO_2, etc.), and quantum dots. Metal-based NPs are often utilized in biomedical applications such as cancer treatment, bio-imaging, tissue engineering, autophagy, gene delivery, drug delivery, anti-inflammatory activity, and diabetes treatment (Mousavi et al. 2022) as shown in Fig. 1. Their harmful effects on the activity, diversity, and quantity of flora and animals are closely observed (Shrestha et al. 2013). The main causes of NPs' cytotoxicity include their physicochemical qualities, contamination of poisonous elements, fibrous structure, high surface charge, and production of radical species (Fard et al. 2015).

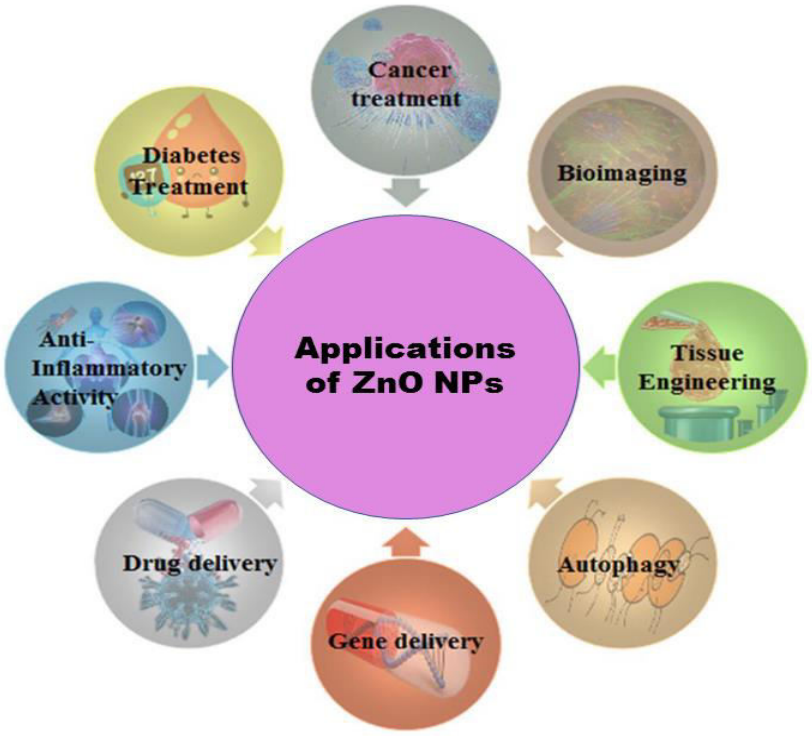

Fig. 1: Biomedical Applications of ZnO NPs

Among metal oxides, ZnO NPs have garnered the greatest interest as a potential anticancer drug and ZnO NPs powders are widely used in cosmetics (sunscreens, foot care, and ointments), pigments and coatings (ultraviolet protection, fungicide in paints), electronic devices, and catalysts. Skin exposure is predominant to final products (sunscreens but also paints), and airway exposure is predominantly workplace exposure to NP dust (factories producing NPs but also paint factories).

The ZnO NPs are more potent compounds than ZnO. In addition, it has high permeability and is easily absorbed in the gastrointestinal tract (GIT), which enhances its utility as a potential therapeutic compound (Baltić et al. 2013). However, ZnO NPs are vulnerable to oxidative reactions with different organic materials. High exposure to ZnO NPs results in loss of DNA methylation has `been found in treated human MRC5 lung fibroblast cells (Pogribna and Hammons 2021). Studies have demonstrated that ZnO NPs can induce cytotoxicity in a number of different cell types (Maher et al. 2023). In the size range of 1-100 nm, at least 50% of these particles must have one or more exterior dimensions. As the size of a material decreases, the value of its surface properties grows (Khan et al. 2019). ZnO NPs have undergone tests for *in vitro* chemical effectiveness and phytochemical screening. Limited and inconsistent indications of potential genotoxicity associated with ZnO NPs have been reported. Fig. 1 illustrates the applications and impacts of ZnO NPs in both *in vivo* and *in vitro* situations.

Despite the extensive utilization of ZnO NPs, there remains uncertainty regarding the safety of this compound for human health. This comprehensive review provides a synthesis of the existing literature regarding the *in vitro* and *in vivo* toxicity of ZnO NPs. It delves into the chemical mechanism

responsible for ZnO NPs-induced DNA damage and examines the potential risks associated with inhalation and dermal exposure, as well as their effects on diverse toxicological endpoints *in vivo*, bio-distribution patterns, and impacts on various cell lines *in vitro*.

Exposure pathways

Effect of dermal exposure

ZnO NPs have a substantially greater UV protection value and are far less opaque and whiter than traditional ZnO powder. Therefore, ZnO NPs are preferred over larger particles for usage in cosmetics. The skin is the most common route of exposure for consumers, but inhalation is more common among industrial workers who come into contact with ZnO NPs (Osmond and Mccall 2010). The global ZnO NPs market is estimated to be valued at US 1,800–2,100 mt yr^{-1}(Keller et al. 2014). Customers are most likely to be exposed to ZnO NPs through the skin because they are used in various types of cosmetics. The stratum corneum, or the outermost layer of skin, has been shown in several studies to be an effective barrier against the migration of ZnO NPs into the deeper layers of the epidermis (Roberts et al. 2017). ZnO NPs have been proven not to penetrate the healthy, undamaged skin of humans or pigs. Sebum flow frequently adequately eliminates NPs, despite their ability to be trapped in skin folds or the ostium of hair follicles. However, skin damage, like that produced by prolonged sun exposure, might reduce this protective barrier, increasing the risk of toxicological effects from ZnO NPs. Proliferating cells in the epidermis appear to be the only target of cytotoxic or genetically damaging effects. This is why it is cautioned against using ZnO NPs on flawed skin. ZnO NP absorption and interaction with the intestinal mucosa should be given equal weight. Toxicity may be increased because of a breakdown of the mucosa barrier caused by particularly severe intestinal illness. Fig. 2 illustrates the mechanistic insight into the toxicity of ZnO NPs, both *in vivo* and *in vitro* contexts.

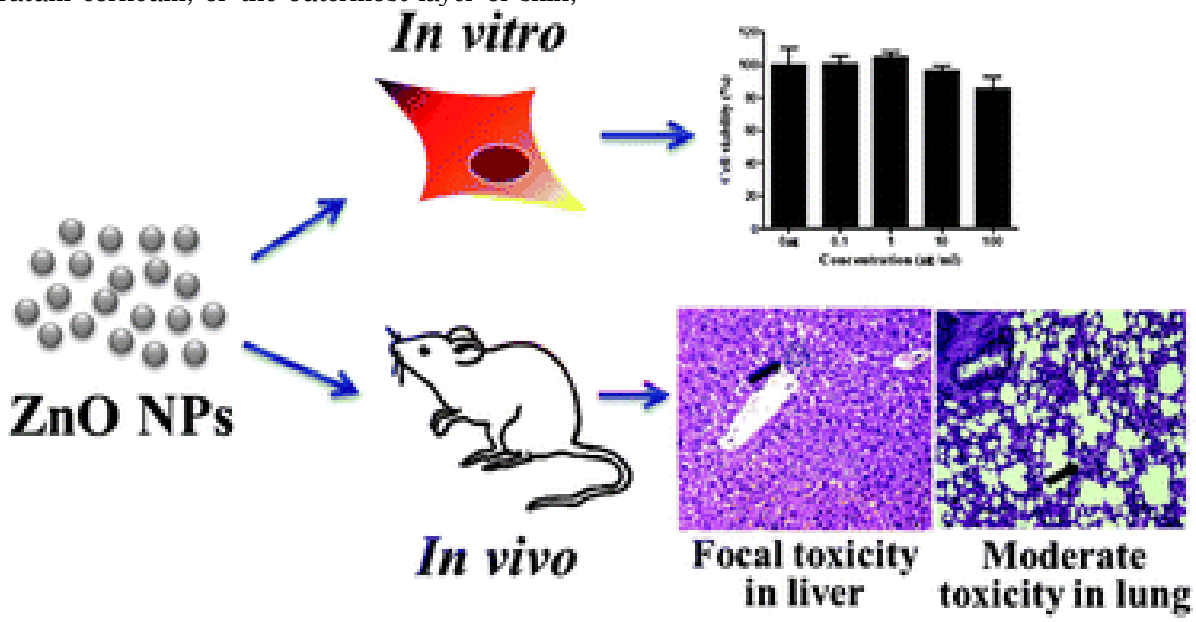

Fig. 2: Mechanistic insights into ZnO NPs toxicity *in vivo* and *in vitro*

Effect of airway exposure

Inhalation into the airways is the major mode of engagement for anyone working with chemicals, cosmetics, or paint. The bronchiolar and alveolar areas of the peripheral airway sites are accessible to nano-sized particles. If the mucociliary transport mechanism fails to remove them effectively, NPs can potentially affect alveolar cells, leading to toxic, genotoxic, or inflammatory outcomes (Osmond and Mccall 2010). Inhaling ZnO NPs poses a significant risk, and in this situation, risk evaluation is urgently needed (Vandebriel and De Jong 2012). Kao et al. (2012) reported that an elevated concentration of cytosolic Zn2+ was observed in both bronchoalveolar lavage (BAL) cells and white blood cells of rats after they inhaled 38 nm ZnO NPs. ZnO NPs are so minute that they can go through the tracheobronchial tree. Particles less than 100 nm in diameter, in particular, can enter the circulation undetected and cause serious health problems. According to Cho et al. (2012a) findings, rats subjected to a single intratracheal administration of ZnO NPs with a diameter below 10 nm demonstrated an inflammatory reaction marked by eosinophilic, fibrotic, and

granulomatous effects. This resulted in the recruitment of eosinophils and neutrophils into the bronchoalveolar lavage (BAL) fluid.

A newly formulated framework, put forth in a recent publication, suggests that with highly soluble NPs like ZnO NPs, the protein corona undergoes digestion within lysosomes because of the organelle's acidic environment and the presence of lysosomal enzymes. Following this process, the NPs swiftly dissolve, leading to the destabilization of lysosomes caused by the release of Zn^{+2} (Cho et al. 2012b). Exposure of the airways to ZnO NPs leads to a rise in intracellular Zn2+ levels. The generation of reactive oxygen species (ROS) is likely a significant contributor to the ensuing inflammatory reaction. While lung mucus can capture the majority of metal oxide NPs, it may not entirely encapsulate ZnO NPs (Vandebriel and De Jong 2012).

Genotoxicity effect of ZnO NPs

Golbamaki et al. (2015) claim that the genotoxic effects of NPs may be divided into two types: primary genotoxicity and secondary genotoxicity. Distinguishing from secondary genotoxicity, which results from the generation of ROS during particle-induced inflammation. The small size, extensive surface area, and unique physicochemical properties of NPs can render their genotoxic effects difficult to anticipate (Kwon et al. 2014). The biological properties are affected by the manufacturing procedure, the tendency to aggregate or agglomerate, and the surface coating. Particle sizes change throughout the production process. The tendency of NPs to aggregate because of their surface characteristics necessitates the use of dispersants to mitigate this issue. Coating the NP's surface represents an effective strategy for preventing aggregation. These variables play a substantial role in influencing the toxicity of NPs. Earlier studies showed that smaller NPs are more able to penetrate cell membranes, leading to more extensive DNA damage. Intracellular accumulation of NPs is most likely to occur during mitosis (Kwon et al. 2014). Particles such as silver (Ag) and ZnO NPs have the potential to dissolve in water, leading to the release of ions. Unlike Ag, Zn is a crucial component found in numerous enzymes and transcription factors within human cells. Upon ingestion, ZnO NPs can undergo degradation, resulting in the formation of Zn^{+2} ions. This process activates a range of signaling pathways and cascades, including increased calcium flow, upregulation of specific genes, and the release of inflammatory markers, among other effects (Saptarshi et al. 2015).

Solubility is an important aspect of the toxicity of NPs such as Ag, Cu, and ZnO. Similar characteristics may be found in NPs of Ag, Cu, and ZnO. (Bondarenko et al. 2013). The toxicity of ZnO NPs is heavily dependent on their solubility and affected by variables like the pH within tissues, cells, and organelles in their immediate surrounding (Liu et al. 2016). However, there is conflicting data on whether or whether NP-induced toxicity is size-dependent. Cells exposed to larger micrometer-sized particles experienced far higher DNA damage. These divergent results underscore the necessity to explore the impact of NP's size on both nanotoxicity and Nano genotoxicity. It is crucial to offer accurate descriptions of NP sizes if making comparisons to prior research. nano toxicity of ZnO NPs has been the subject of several studies and reports over the past decade. However, because of their cytotoxic effects, most of the research has focused on ZnO NPs. Most of the research examines the dose-response relationship between ZnO NPs concentration and its influence on cell viability. However, DNA damage may occur at concentrations orders of magnitude lower than those responsible for cytotoxicity. Zn ion involvement cannot be ruled out entirely. Stress is a common trigger for the lysosome-dependent breakdown known as autophagy. Activation of autophagy as a crucial controller of cellular toxicity induced by ZnO NPs (Roy et al. 2014). Earlier results showed an upregulation of autophagy marker proteins and an increase in autophagosome formation. Autophagy induction was discovered to be critically dependent on reactive oxygen species generation. Inhibition of cell death and a decrease in the production of autophagy marker proteins were observed when antioxidant enzymes were activated. Previously, shown that autophagy is critically involved in ZnO NPs-induced toxicity. Inhibiting oxidative stress and autophagy may reduce cellular damage (Hackenberg et al. 2014). Moreover, zinc oxide nanoparticles are deemed safe and biocompatible within the human body. Numerous scientific publications elucidate the potential antibacterial mechanisms associated with zinc oxide nanoparticles, which involve: (1) ROS Production: Zinc oxide nanoparticles trigger the generation of ROS, including the hydroxyl radical (OH^{+}) and peroxide (O^{-2}). This induction of oxidative stress leads to disruptions in the bacterial cell membrane, DNA damage, and eventual bacterial cell demise. (2) Zn^{+2} Ion Release: The dissolution of zinc oxide nanoparticles results in the liberation of Zn^{+2} ions. These ions interact with bacterial cells, notably targeting the cell membrane, cytoplasm, and nucleic acids. Consequently, the cellular integrity collapses, culminating in bacterial cell death; and (3) direct interactions between zinc oxide nanoparticles and bacterial cell membranes through electrostatic forces that damage the plasma membrane and cause a leakage of intracellular components (Sharmila et al. 2018). These ROS production and multifaceted mechanisms are collectively explained in Fig. 3. However, exposure to ZnO NPs causes ROS

induction and increases the expression of genes. A growing body of evidence indicates that ROs play essential roles in NP-mediated toxicity (Kong et al. 2016; Saini et al. 2016). Hence, we deduce that exposure to ZnO NPs leads to DNA oxidation prompted by ROS within cells. Notably, cells exposed to ZnO NPs exhibited escalated DNA damage, evidenced by the migration of DNA away from the nucleus and the configuration of a comet tail-like structure. The augmentation in products resulting from oxidative DNA damage and breaks in DNA strands serve as confirmation of the DNA-damaging impact of ZnO NPs.

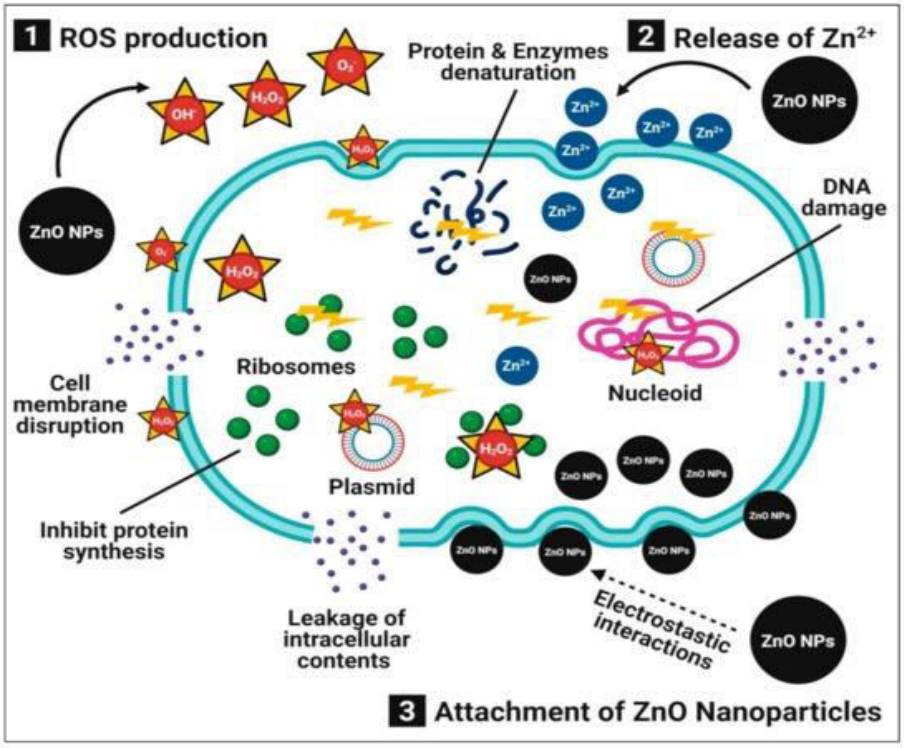

Fig. 3: ROS production and multifaceted mechanisms induced by ZnO NPs

Inducing oxidative DNA damage through the activation of autophagy pathways by ZnO NPs could potentially shift the equilibrium towards promoting cell death (Pati et al. 2016). Kononenko et al. (2017) showed that genotoxicity and cytotoxicity are concentration dependent. Damage to DNA and chromosomes was followed by a decrease in glutathione S-transferase and catalase activities.

The amount of DNA damage is influenced not only by the tested NPs but also by the exposed target cell, specifically its genetic and proteomic characteristics. Several research teams have found that ZnO NPs can initiate the p53 pathway (Ng et al. 2011; Roy et al. 2014; Sharma et al. 2012a). ZnO NPs often induce apoptosis through the p53 pathway in response to DNA damage. However, when ZnO NPs were introduced to a fibroblast cell line with p53 knockdown. Ng et al. (2011) observed both resistance to ZnO NPs-induced apoptosis and an increase in cellular mutation proliferation. Photogenotoxicity caused by ZnO NPs is critical. Wang et al. (2013) examined the extent of dose-dependent oxidative DNA damage induced by ZnO NPs in HaCat human skin keratinocytes when these cells are exposed to UV (ultraviolet) and Vis (visible) light. The authors state that photo genotoxic potential exists when ZnO NPs are exposed to UV light. The usage of ZnO NPs in sunscreen makes a critical examination of these results all the more important. Demir et al. (2014) investigated ZnO NP-induced DNA damage in human and mouse cell lines with the micronucleus test and comet assay. Additionally, they observed anchorage-independent cell proliferation following NPs treatment, which could potentially indicate a cellular transition toward a cancerous state. Previously, showed that the DNA damage caused by rod-shaped ZnO NPs was much higher than that caused by spherical NPs in peripheral blood mononuclear cells (Bhattacharya et al. 2014). The coating on ZnO NPs can also influence their genotoxic potential. In a prior investigation, it was

demonstrated that NPs coated with poly (methacrylic acid) (PMAA) induced more sustained DNA damage in exposed animals compared to control animals (Yin et al. 2010).

NPs induce genotoxicity, inflammation, and other cellular impairments by generating reactive oxygen species (ROs). DNA damage in human epidermal cell line A-431 and human liver cell line HepG2 was detected by single-cell microgel electrophoresis (comet) because of exposure to ZnO NPs. Patel et al. (2016) found that the 431-cell line produced ROS after being treated with ZnO NPs. According to research conducted by Condello et al. (2016) ZnO NPs can enter human colon cancer cells through passive absorption, endocytosis, or diffusion. The specific method of entry was found to be influenced by the aggregation state or condition of NM.

The use of the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay is a widely adopted method for assessing the production of ROS. Catalase, superoxide dismutase, malondialdehyde, decreased glutathione (GSH), and elevated glutamate levels were some of the oxidative stress markers analyzed. High cellular absorption and evidence of photogenotoxicity of ZnO NPs in *Allium cepa* (Kumari et al. 2011).

Sharma et al. (2011) conducted their research with primary human epidermal keratinocytes since they are a good cell type of target for ZnO NPs, which are commonly found in cosmetics. After 6 hours of treatment at a concentration of 8 g/mL, transmission electron microscopy showed that DNA fragmentation can occur because of the cellular uptake and internalization of ZnO NPs, as demonstrated by Sharma et al. (2011) They observed that exposure to ZnO NPs had genotoxic effects on primary human nasal mucosa. It's worth noting that volatile xenobiotics often enter the body through the nasal mucosa (Sharma et al. 2011). Evidence of the genotoxic potential of ZnO NPs was found in human nasal mucosa cells cultured in an air-liquid interface and in the sustained release of IL-816. Treatment of ZnO NPs also induced DNA damage and pro-inflammatory cytokine production in human adipose-derived mesenchymal stem cells. After being exposed to NPs, stem cells' migratory potential was severely diminished. Intriguingly, after 24 h, cells internalized significant quantities of ZnO NPs, and the particles remained bioaccumulated in the cytoplasm for more than three weeks. The dynamics of cellular uptake and efflux should be better understood in future studies. The prolonged presence of NPs within cells can pose significant concerns, as even low initial exposure doses may lead to substantial intracellular accumulations through repeated interactions over time (Hackenberg et al. 2013).

Furthermore, DNA damage further increased 24 hours after exposure, possibly because NPs were still present in the cells and ROS generation was ongoing (Hackenberg et al. 2011). Ghosh et al. (2016) researched to investigate the genotoxic effects of ZnO NPs on human peripheral blood mononuclear cells. They observed a significant reduction in mitochondrial membrane potential and minor genotoxic effects (Ghosh et al. 2016). An earlier study found that DNA damage was dramatically elevated in human cells exposed to ZnO NPs, as described in Table 1 (Branica et al. 2016). In their *in vivo* study, Alaraby et al. (2015) did not find any evidence of toxicity or an elevation in oxidative stress. They also did not observe significant alterations in the number of mutant clones or DNA damage as determined by the comet assay. However, they observed noticeable changes in the expression of the Hsp70 and p53 genes. ZnO NPs were shown to be cytotoxic and inflammatory in a human monocyte cell line by Sahu et al. without causing any detectable DNA damage (Sahu et al. 2014).

This observation was substantiated by heightened proportions of DNA exhibiting tails, along with increased length and intensity of DNA tails, notably pronounced at the 500 mg/kg dosage level. Additionally, their findings indicated a notable augmentation in the count of micronucleated cells (Ramadan et al. 2022).

In a study by Attia et al. (2018) the neurotoxic and genotoxic effects of ZnO NPs were explored in rats through oral exposure. Their research demonstrated that administration of both doses (40 and 100 mg/kg) resulted in heightened levels of inflammatory cytokines. Furthermore, the study revealed an upsurge in apoptotic markers, including caspase-3 and Fas. The outcomes of the study collectively suggest the occurrence of ZnO NP-induced neurotoxicity following repeated oral exposure. This neurotoxicity appears to be attributed to mechanisms involving oxidative stress, genotoxicity, inflammatory responses, and apoptosis.

Shahzad et al. (2019) have researched to examine the genotoxic effects of ZnO NPs on tilapia (*Oreochromis mossambicus*). Their study revealed a correlation between the severity of DNA damage and the concentration of ZnO NPs. Furthermore, they deduced that ZnO-NPs possess the capability to accumulate within soft tissues, potentially leading to respiratory difficulties due to oxidative stress. The study also highlighted the induction of antioxidant defense mechanisms by elevating glutathione (GSH), alongside instances of organ pathology and genotoxicity attributed to ZnO-NP exposure.

In vivo studies

The genotoxicity of ZnO NPs in living organisms has been the focus of only a limited number of studies. When ZnO NPs were administered to animal models, they induced toxicity that was

strongly associated with significant DNA damage in bone marrow and peripheral blood cells. Wound healing was also impaired, along with increased inflammation and inhibition of DNA repair mechanisms (Pati et al. 2016). In their study, Sharma et al. (2012a) used a mouse model to examine the outcomes of subacute oral exposure to ZnO NPs over two weeks. They observed DNA damage in liver cells because of NPs accumulation. A reduction in glutathione, glutathione-S-transferase, and glutathione peroxidase, and an increase in malondialdehyde and catalase, were seen in Lymnaealuteola freshwater snails after exposure to ZnO NP for 24 and 96 hours. Lymnaealuteola freshwater snails, following exposure to ZnO NPs for 24 and 96 hours, exhibited reduced levels of glutathione, glutathione-S-transferase, and glutathione peroxidase. Additionally, there was an increase in the level of malondialdehyde and catalase in these snails. Intestinal gland cells exposed to ZnO NPs showed genotoxic effects. Biodistribution and genotoxicity effect in mice, after oral ingestion and intraperitoneal injection (Li et al. 2012). The heart-damaging effects in rats were previously observed after exposure to ZnO NPs (Baky et al. 2013). Zhao et al. (2013) detected DNA damage in zebrafish embryos and larvae in their study.

A prior comparison of the toxic effects of ZnO NPs and Zn ions demonstrated that the ions alone were only partially accountable for the toxic effects. In contrast, when inhaled, triethoxyccaprylylsilane-coated ZnO NPs did not cause DNA damage in rat lung cells. *In vivo* study conducted by Ghosh et al. (2016) bone marrow cells exhibited a decrease in mitochondrial membrane potential. The study also observed the formation of micronuclei, the cell cycle arrested at the G0/G1, and an increase in oxidative stress.

In their study, Ng et al. (2017) discovered that exposure to ZnO NPs led to notable toxicity in melanogaster F1 progenies. The activation of ROS by ZnO NPs was linked to a significant decrease in the viability of the flies from the egg stage to adulthood. Anand et al. (2017) investigated the impact of ZnO NPs on the fruit fly Drosophila melanogaster. They found that after consuming food containing ZnO NPs at concentrations ranging from 0.1 to 10 mM, phenotypic changes such as segmented thorax and single or deformed wings were inherited by subsequent generations, as also reported in previous studies (Hu et al. 2010; Jacobsen et al. 2015) and detailed in Table 1.

***In vitro* studies**

ZnO NPs-exposed cells were genotoxic in alkaline standard comet tests (Monteiro-Riviere et al. 2011). According to Golbamaki et al. (2015) there were significant increases in the length, percentage of DNA in the tail, tail moment, and Olive tail moment after PMEF cells were treated with four different types of NPs at both the tested dosages. ZnO NPs are non-mutagenic in the Ames test and are water soluble (Nam et al. 2013).

Diethyoxydiphenylsilane/triethoxycaprylylsilane cross-polymer coated ZnO was shown to be nongenotoxic in Ames assays (Landsiedel et al. 2010). The micronucleus test (Osman et al. 2010) found that poly methyl acrylic acid-coated ZnO was much more genotoxic than uncoated ZnO when applied to WIL2-NS human lymphoblastoid cells (Yin et al. 2010). The comet test revealed consistent DNA damage in human nasal mucosa exposed to ZnO (Hackenberg et al. 2011).

Different concentrations of ZnO NPs were used to test the least inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which produced outstanding antibacterial activity in comparison to their bulkier counterparts (Mohd Yusof et al. 2021). The ZnO NPs, which are metal oxide NPs, have been employed frequently for their antibacterial qualities. Numerous bacteria have been discovered to have reduced activity when exposed to ZnO (Mohd Yusof et al. 2019).

ZnO NPs have become recognized as possible antibacterial agents with the emergence of multi-drug resistance pathogens. This is primarily because of their greater abilities to fight against a variety of diseases. Furthermore, the majority of biological processes in an animal's body depend on zinc, which is known as an essential trace element. As a result, it has been shown that using ZnO NPs considerably improves the health and output of farm animals (Mohd Yusof et al. 2019). Due to their unique physicochemical characteristics, ZnO NPs can be a potential method for creating nanovaccines and antiviral drugs, particularly against RNA viruses like the coronavirus that causes severe acute respiratory syndrome (SARS) and the human immunodeficiency virus (HIV). ZnO NPs most likely antiviral mechanisms involve preventing virus entry into cells and deactivating the virus through virostatic potential (Nasrollahzadeh et al. 2022). Table 1 shows the exposure and genotoxicity effect of ZnO NPs.

Table 1: Exposure and effects of ZnO NPs *in vivo* and *in vitro* studies

Nanomaterial's characteristics	***In vivo* and *in vitro***	**Exposure**	**Methods**	**Results**	**Reference**
Size 75–85 nm	Human peripheral blood mononuclear cells, and organs of rats	20, 40, 80 and 100 µg/ml *in vitro* and 25, 50, and 100 mg/kg bw *in vivo*	Comet assay Cytotoxicity assay	Observe ZnO-NP induced genotoxic response and ROS production leading to apoptotic cell death	(Ghosh et al. 2016)
Size 59 and 68 nm	Female wild-type C57BL/6JBonTac (C57) mice	2, 6, 18 µg/animal	Comet assay	Dose-dependent DNA damage	(Jacobsen et al. 2015)
Size 22 nm	Freshwater snail Lymnaealuteola	10, 21, 32, and 33µg/mL for 96 h	Comet assay	Exposure of ZnO NPs increased DNA damage in study Animal	(Ali et al. 2012)
Size 28 ± 5 nm	Drosophila melanogaster	0.1, 1, and 10 mM	TUNEL assay ROS detection assay	Exposure to ZnO NPs increased DNA fragmentation and resulted in phenotypic alterations that were passed on to the progeny	(Anand et al. 2017)
Size 30 nm	Mice's liver and kidney cells	300 mg/kg for 14 days	Comet assay	DNA damage was observed in liver and kidney cells	(Sharma et al. 2012b)
Size 15–25 nm	SHSY5Y human neuroblastoma cell line	20, 30, and 40 µg/mL for 3 and 6 h	Comet analysis DNA oxidative damage	ZnO NPs caused micronuclei in all cases, as well as H2AX phosphorylation and DNA damage	(Valdeiglesias et al. 2013)
Size 10–50 nm	Rat kidneys and epithelial cell line (NRK-52E)	12.5 and 50.0 mg/mL	Comet assay	DNA damage form ZnO NPs DNA was observed statistically significant	(Uzar et al. 2015)
Size 100 nm	Sea Urchin	1 ng/kg food for 3 weeks	Comet assay	Nucleus damage in immune cells and deformed larvae, provoked immune cell damage	(Manzo et al. 2017)
Size 104.17 ± 66.77 nm	Mouse embryonic fibroblast and embryonic fibroblast knockout cell lines	Sub-toxic dose (1 µg/mL) for 12 weeks, Short-term exposure (0.3125 to 40 µg/mL) for 48 h	Comet assay	Short-term ZnO NPs exposure induce ROS, and genotoxicity. No effects after long-term exposure.	(Annangi et al. 2016)
Size 200–250 nm	Cells isolated from mice	0–500 µg/mL for 24 h	Comet assay Micronucleus assay	DNA damage in peripheral blood and bone marrow cells.	(Pati et al. 2016)

Size 17 nm	Human malignant melanoma skin (A375) cell line	5, 10, and 20µg/mL for 24 and 48 h	Comet assay	A gradual nonlinear increase in cell DNA damage was observed as concentration and duration of exposure of ZnO NPs	(Alarifi et al. 2013)
Size 50 nm	Different organs of mice	2.5 g/kg for 30 min	Micronucleus Assay	ZnO NPs induces liver toxicity	(Li et al. 2012)
Size < 35 nm	Human lymphocyte	1.0, 2.5, 5.0, and 7.5 µg/mL for 2 weeks	Comet assay	ZnO NPs induced DNA damage	(Branica et al. 2016)
Size 50 - 100 nm	Embryo-larva zebrafish	1, 5, 10, 20, 50, and 100 mg/L for 144 h	Single cell gel electrophoresis	ZnO NPs induced DNA damage	(Zhao et al. 2013)
Size 75 ± 5 nm	Human lymphocyte cells	0, 125, 500, 1000 µg/mL for 3 h	Comet assay	1000 µg/mL ZnO NPs induced significant genotoxic effects	(Sarkar et al. 2014)
Size 30 ± 5 nm	Erythrocytes and organs of male rats	100, 200, 300, 400, and 500 mg/kg for 10 weeks	Comet assay Micronucleus Assay	Dosage-dependent increase in DNA fragmentation for both the comet assay and the micronuclei test	(Ramadan et al. 2022)
Size > 100nm	Erthrocytes and liver of tilapia (Oreochromis mossambicus)	0, 0.5, 1.0, and 1.5 mg/L for 14 days	Biochemical assay Comet assay	A direct correlation between the concentration of ZnO-NPs and the extent of DNA damage	(Shahzad et al. 2019)

Summary of Genotoxicity Mechanism of ZnO NPs

Although the evaluation of genotoxic effects related to ZnO NPs varies, there is compelling evidence suggesting their potential to harm human cell DNA. The comet assay, micronucleus test, chromosomal aberration assay, and H2AX method are just a few of the methodologies and endpoints that have all suggested genotoxic events. The Fpg-modified comet assay and interactions with antioxidants, like N-acetylcysteine support the relationship between oxidative stress and DNA damage. Studies have confirmed that ZnO NPs are internalized by cells. Cellular distribution, confirmed by transmission electron microscopy (TEM) and alternative techniques like side scatter flow cytometry, reveals localization within cell organelles, with lysosomal inclusion being noteworthy. Lysosomes, with their acidic conditions, cause ZnO dissolution, releasing Zn^{+2} ions. This release might begin in the cellular medium itself. The release of Zn^{+2} ions, both inside and outside the cell, is thought to be one of the main causes of DNA damage. The ions of Zn^{+2} affect DNA integrity in a dose-dependent manner even when they do not penetrate the nucleus of the cells. Lysosomes discharging Zn^{+2} into the cytoplasm act as a trigger for ROS generation, supported by the DCFH-DA assay in various research. Indicators of oxidative stress, including reduced GSH, increased malondialdehyde, superoxide dismutase, and catalase, are examined following exposure to ZnO NPs. When DNA integrity is compromised, lysosomes transform into autophagosome, which can be seen using transmission electron microscopy (TEM), or protein like LC3 II and beclin-I as markers. While few research has been done on DNA damage after the following NPs exposure, there is evidence that the presence of trapped NPs in intracellular compartments and continuous ROS-induced DNA damage may have hindered repair (Scherzad et al. 2017). Fig. 4 illustrates the induced genotoxicity of ZnO NPs.

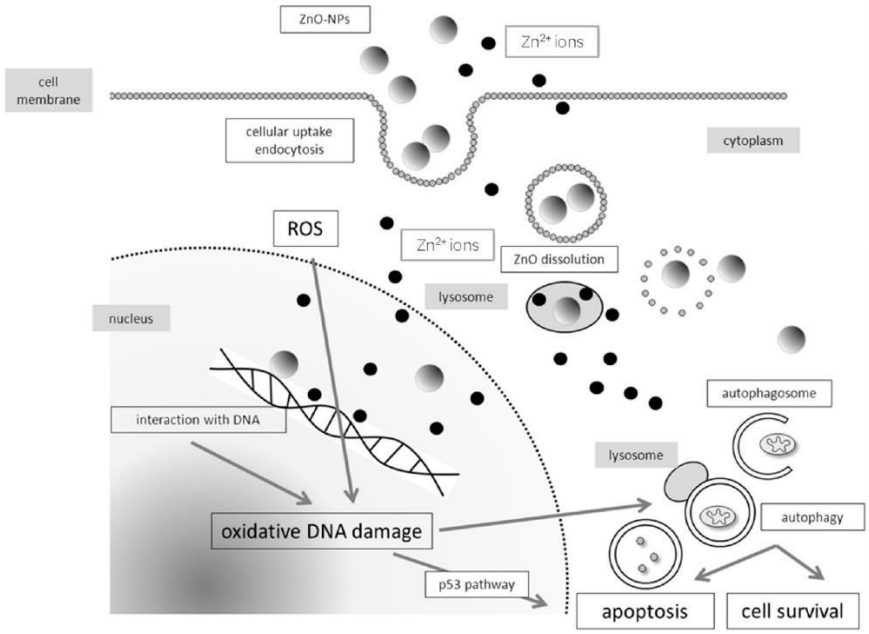

Fig. 4: A model summarizing the genotoxicity mechanisms of ZnO NPs

Conclusion

The potential genotoxicity associated with ZnO NPs remains largely unclear as of now. Because of disparities in the available data, offering recommendations or accurately assessing the risk linked to ZnO NPs utilization proves to be an immensely challenging endeavor. While research has predominantly centered on the cytotoxic implications of ZnO NPs, there is emerging recognition that these nanoparticles may fall within the category of nanomaterials capable of compromising DNA integrity. Consequently, a need for further investigations into the genotoxic effects of ZnO NPs is evident. To provide dependable genotoxicological insights, it becomes essential to characterize the physicochemical attributes of the NPs consistently and comprehensively under scrutiny. Most genotoxicological inquiries concerning ZnO NPs presently concentrate on acute exposure scenarios. The way forward entails the advancement of sophisticated diagnostic systems and the continuous exploration of the intricate biological mechanisms governing DNA damage. Although ZnO NPs exhibit effectiveness and promise, the presence of genotoxic safety concerns necessitates a thorough evaluation before advocating their responsible and secure incorporation. In essence, the trajectory of ZnO NPs as effective materials is intertwined with a comprehensive resolution of their potential genotoxicity. Striking a balance between their potential benefits and associated risks is pivotal for their ethical and secure integration into various.

applications. As further investigations unfold, collaborative endeavors across disciplines and meticulous examinations will prove indispensable in navigating the complex landscape of genotoxicity ZnO NPs, ensuring their safe and responsible application.

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Ethical statement

No Ethical permissions were required for this article.

Availability of data and material

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