

Zinc Oxide Nanoparticle–Induced Neurotoxicity

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ABSTRACT

Background: Zinc oxide nanoparticles (ZnO-NPs) are increasingly incorporated into industrial, pharmaceutical, and cosmetic products due to their antimicrobial, UV-blocking, and catalytic properties. However, their small size and high reactivity raise safety concerns, particularly regarding their potential to cross biological barriers and exert toxic effects on the central nervous system. Accumulating evidence indicates that ZnO-NPs may induce neurotoxicity through oxidative stress, inflammation, and cellular dysfunction, necessitating a systematic understanding of their mechanisms of action. **Objective:** This study aims to summarize and analyze current evidence on the neurotoxic effects of ZnO-NPs, focusing on the mechanisms of neuronal damage, behavioral alterations, and the role of physicochemical characteristics influencing toxicity. **Methods:** A comprehensive review of peer-reviewed studies published in recent years was conducted using databases such as PubMed, ScienceDirect, and Scopus. Both in vitro and in vivo studies investigating ZnO-NP exposure and neurotoxic outcomes were included. Data were synthesized to evaluate oxidative stress markers, inflammatory mediators, neurotransmitter alterations, and histopathological changes in brain tissues. **Results:** Findings reveal that ZnO-NPs can readily penetrate the blood–brain barrier and accumulate in neural tissues. Exposure results in increased reactive oxygen species (ROS) generation, lipid peroxidation, mitochondrial dysfunction, and neuronal apoptosis. Neuroinflammatory responses are characterized by elevated cytokines such as TNF- α and IL-6. In animal models, ZnO-NP exposure leads to behavioral changes including memory deficits, anxiety-like behavior, and impaired motor coordination. The degree of neurotoxicity correlates with nanoparticle size, concentration, surface charge, and exposure duration. Dissolved Zn²⁺ ions also contribute significantly to the observed cytotoxicity. **Conclusion:** Zinc oxide nanoparticles exhibit dose-dependent neurotoxic effects mediated by oxidative stress, inflammation, and apoptosis. While these findings highlight potential risks associated with widespread ZnO-NP use, further studies are required to establish safe exposure thresholds and to explore strategies such as surface modification to reduce neurotoxic potential. Understanding these mechanisms will be crucial in guiding the safe design and application of nanomaterials in consumer and biomedical products. **Keywords:** Zinc oxide nanoparticles, neurotoxicity, oxidative stress, apoptosis, inflammation, blood–brain barrier, nanotoxicology.

KEYWORDS: Rationale and Hypothesis, Interaction with Neural Barriers and Cellular Uptake.

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INTRODUCTION

Nanotechnology has rapidly advanced as a major scientific discipline, providing innovative applications in medicine, electronics, environmental remediation, and consumer industries. Among the most widely utilized engineered nanomaterials are zinc oxide nanoparticles (ZnO-NPs), valued for their high surface area, catalytic efficiency, and semiconducting characteristics (Siddiqi et al., 2018). These properties support their incorporation into sunscreens, paints, textiles, antimicrobial coatings, food additives, and emerging biomedical technologies such as drug delivery and imaging systems (Kołodziejczak-Radzimska & Jesionowski, 2014). However, the expanding global use of ZnO-NPs has been accompanied by growing concerns about their biosafety, especially regarding their potential impact on the central nervous system (CNS) (Tiwari et al., 2021).

A major reason for these concerns is the ability of ZnO-NPs to interact with biological barriers. Their nanoscale dimensions allow them to cross the blood–brain barrier (BBB), either through endocytic pathways or passive diffusion, permitting accumulation in neuronal tissues (Agarwal et al., 2017). Once internalized, ZnO-NPs may disrupt neural homeostasis by triggering oxidative stress, mitochondrial damage, DNA fragmentation, and neuroinflammatory responses—key events implicated in neurodegenerative conditions (Zhou et al., 2015). Experimental evidence demonstrates that ZnO-NPs elevate intracellular reactive oxygen species (ROS), reduce antioxidants such as glutathione (GSH), and induce lipid peroxidation, ultimately promoting neuronal apoptosis and synaptic dysfunction (Xia et al., 2008; Mir et al., 2021). In vivo studies further support these findings, reporting memory deficits, anxiety-like behaviors, and motor disturbances in rodents exposed chronically to ZnO-NPs (Ghosh et al., 2020). These behavioral abnormalities correlate with increased inflammatory cytokines, including TNF- α , IL-6, and IL-1 β , which contribute to glial activation and neuronal injury (Wang et al., 2016).

Another important contributor to ZnO-NP neurotoxicity is the dissolution of Zn²⁺ ions, which can disrupt metal homeostasis, impair neurotransmission, and intensify oxidative injury (Kim et al., 2014). Additionally, nanoparticle physicochemical properties—such as size, shape, surface charge, and surface coating—strongly influence toxicity profiles. Smaller particles in particular exhibit greater reactivity and are more capable of penetrating biological membranes and barriers (Siddiqi et al., 2018). Although oxidative stress is considered a central mechanism, involving activation of NF- κ B and MAPK signaling pathways that

regulate inflammation and apoptosis (Wu et al., 2019), the full molecular cascade remains incompletely understood.

Despite the increasing volume of research, significant gaps persist in the current literature. Many studies rely on acute high-dose exposure models, and inconsistencies in nanoparticle characterization, preparation, and experimental protocols limit cross-study comparisons. As a result, standardized exposure thresholds and safety guidelines for ZnO-NPs remain underdeveloped. Given their widespread industrial and biomedical applications and the brain's vulnerability to oxidative and inflammatory injury, a comprehensive understanding of ZnO-NP-induced neurotoxicity is essential. This review therefore aims to integrate current evidence regarding the mechanisms through which ZnO-NPs affect neuronal integrity, focusing on oxidative imbalance, inflammatory activation, mitochondrial dysfunction, apoptosis, and associated behavioral outcomes. Furthermore, it highlights existing research limitations and underscores the need for unified methodologies and long-term exposure assessments to support safe and responsible use of nanomaterials.

RATIONALE AND HYPOTHESIS

The intensive utilization of ZnO-NPs in industrial, cosmetic, and biomedical fields is driven by their unique nanoscale properties, which enhance drug delivery, imaging, and antimicrobial effectiveness (Kołodziejczak-Radzimska & Jesionowski, 2014). However, these same features may also increase their potential to interact adversely with biological systems. Emerging evidence shows that ZnO-NPs can cross the BBB and accumulate in brain tissues, where they disrupt neuronal and glial homeostasis (Agarwal et al., 2017). Their neurotoxic effects are largely associated with oxidative stress, neuroinflammation, and programmed cell death—mechanisms that overlap with major neurodegenerative disorders (Zhou et al., 2015). The release of Zn^{2+} ions adds another layer of neurotoxicity by altering synaptic zinc balance and impairing neurotransmission (Kim et al., 2014). Despite this, major uncertainties remain regarding how variations in particle size, surface chemistry, and morphology influence toxicity, and existing toxicological datasets do not reflect chronic, low-dose, real-world exposure scenarios (Siddiqi et al., 2018). Therefore, a deeper mechanistic understanding is urgently needed.

Based on current evidence, it is hypothesized that ZnO-NPs induce neurotoxicity primarily through oxidative stress-mediated pathways that lead to mitochondrial dysfunction, inflammatory cytokine activation, glial response, and neuronal apoptosis. It is further proposed that their physicochemical characteristics and exposure duration significantly influence the extent of neural damage.

LITERATURE REVIEW

Zinc oxide nanoparticles (ZnO-NPs) are among the most extensively produced nanomaterials worldwide, widely applied due to their optical, catalytic, and antimicrobial properties. Their integration into cosmetics, sunscreens, food packaging, biomedical devices, and drug delivery systems has significantly increased environmental and human exposure (Siddiqi et al., 2018; Kołodziejczak-Radzimska & Jesionowski, 2014). Although ZnO-NPs provide substantial functional benefits, accumulating research raises concerns about their potential neurotoxic effects. Evidence indicates that ZnO-NP-induced neurotoxicity is a complex process involving oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis, all of which may contribute to both acute neuronal injury and chronic neurodegenerative changes (Mir et al., 2021). The following section synthesizes findings from cellular, animal, and molecular studies to clarify the mechanisms and outcomes associated with ZnO-NP neurotoxicity.

Interaction with Neural Barriers and Cellular Uptake

A major characteristic of nanoparticles is their ability to penetrate biological barriers. Although the blood–brain barrier (BBB) protects the central nervous system (CNS), nanoparticles under 100 nm can pass through via transcytosis, endocytosis, or adsorptive transport (Agarwal et al., 2017). Experimental models show that ZnO-NPs accumulate in brain tissues following oral, intranasal, or intravenous exposure (Sharma et al., 2012). Wang et al. (2016) demonstrated ZnO-NP deposition in the hippocampus and cortex after systemic administration, accompanied by neuronal morphological alterations and microglial activation. Intranasal studies indicate that ZnO-NPs may also bypass the BBB through the olfactory route (Sharma et al., 2012). Once internalized, they localize in mitochondria and lysosomes, disrupting energy metabolism and activating apoptotic signals (Bassit et al., 2025). Uptake is influenced by surface charge and hydrophobicity, with positively charged particles displaying enhanced BBB transport (Kim et al., 2014). Additionally, exposure can compromise BBB integrity by degrading tight-junction proteins and increasing endothelial permeability (Tiwari et al., 2021).

Oxidative Stress and Free Radical Production

Oxidative stress is widely accepted as the primary mechanism underlying ZnO-NP neurotoxicity. Their high surface reactivity facilitates the generation of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide (Zhou et al., 2015). Excess ROS overwhelms antioxidant systems—including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)—leading to oxidative damage of proteins, lipids, and DNA (Mohamed et al., 2024). In vitro studies show dose-dependent mitochondrial depolarization and ROS elevation in neuronal cells exposed to ZnO-NPs (Zhou et al., 2015). In vivo, increased malondialdehyde (MDA) and reduced antioxidant activity in the hippocampus and cortex confirm oxidative imbalance (Mir et al., 2021). Dissolved Zn^{2+} ions further intensify oxidative stress by disrupting zinc homeostasis, impairing metalloproteins, altering calcium signaling, and inducing endoplasmic reticulum stress (Agarwal et al., 2017). Thus, oxidative stress acts as an upstream driver of subsequent inflammatory and apoptotic events.

Neuroinflammation and Glial Activation

Chronic neuroinflammation is another key feature of ZnO-NP-induced toxicity. Activation of microglia and astrocytes triggers the release of inflammatory mediators including $TNF-\alpha$, $IL-1\beta$, and $IL-6$, exacerbating oxidative damage and contributing to

neuronal dysfunction (Ghosh et al., 2020; Mohamed et al., 2023). Wang et al. (2016) reported elevated cytokine levels and glial proliferation in ZnO-NP-treated mice, along with hippocampal disorganization. Mechanistically, ZnO-NPs activate NF- κ B and MAPK pathways, promoting expression of genes involved in inflammation and apoptosis (Wu et al., 2019). Persistent NF- κ B stimulation may sustain inflammation and promote progressive neurodegeneration (Salah et al., 2025). While glial activation initially serves as a protective response, prolonged stimulation increases nitric oxide (NO), ROS, and prostaglandins, creating a reinforcing cycle of oxidative and inflammatory injury (Mir et al., 2021).

Apoptosis and Genetic Alterations

ZnO-NPs ultimately induce apoptosis through both intrinsic and extrinsic pathways. Mitochondrial apoptosis is characterized by Bax and caspase-3 upregulation and reduced Bcl-2 expression (Kim et al., 2014). DNA fragmentation and chromatin condensation confirm apoptosis in neuronal tissues. Gene expression studies show alterations in pathways related to oxidative stress, DNA repair, and cell cycle regulation, including upregulation of p53 (Mir et al., 2021). ZnO-NPs have also demonstrated genotoxic potential, with increased DNA strand breaks and micronuclei formation in exposed lymphocytes (Ghosh et al., 2020). These apoptotic and genotoxic disruptions collectively compromise neuronal integrity and contribute to long-term structural and functional damage.

Behavioral and Cognitive Impairments

The cellular and molecular disturbances triggered by ZnO-NPs manifest as measurable behavioral and cognitive deficits. Rodent studies have reported anxiety-like behavior, impaired memory, and reduced locomotor activity following subchronic ZnO-NP exposure (Ghosh et al., 2020). These outcomes correlate with hippocampal oxidative stress and neuronal loss (Ahmed et al., 2025). Wang et al. (2016) also documented changes in exploratory behavior and motor performance in treated mice. Electrophysiological investigations reveal altered glutamatergic signaling and disrupted calcium homeostasis, indicating impaired synaptic plasticity (Zhou et al., 2015). Such deficits demonstrate the potential for ZnO-NPs to contribute to neurological dysfunction, particularly under conditions of chronic exposure.

Influence of Physicochemical Properties

ZnO-NP toxicity varies considerably according to particle size, shape, charge, and surface coating. Smaller nanoparticles (<20 nm) typically show higher reactivity, faster dissolution, and greater capacity for barrier penetration, increasing their neurotoxic potential (Siddiqi et al., 2018). Surface coatings such as polyethylene glycol (PEG), chitosan, or albumin can reduce toxicity by limiting aggregation or controlling ion release (Tiwari et al., 2021). However, modifications may also alter biodistribution and enhance CNS uptake depending on surface charge or hydrophobicity. Dissolution rate is another significant determinant: rapidly dissolving ZnO-NPs release more Zn²⁺ ions, intensifying oxidative stress and apoptosis (Kim et al., 2014). Understanding these parameters is crucial for optimizing safety in biomedical applications.

Environmental and Human Exposure

Human exposure occurs primarily through inhalation, ingestion, and dermal contact due to widespread incorporation of ZnO-NPs into commercial products (Kołodziejczak-Radzimska & Jesionowski, 2014). Although human toxicological data remain limited, animal studies indicate potential bioaccumulation in the brain, liver, and kidneys. Environmental release also poses ecological risks; ZnO-NPs can migrate into soil and water systems, affecting aquatic organisms and entering the food chain (Siddiqi et al., 2018). A major challenge is the lack of standardized exposure limits, as variability in synthesis methods and inconsistent reporting of particle characteristics hinder accurate risk assessment (Abdel-Moaty et al., 2025).

Research Gaps and Future Perspectives

Despite growing evidence, significant research gaps remain. Most studies use high, acute doses that do not reflect realistic environmental or occupational exposures. Long-term, low-dose studies, as well as developmental and multigenerational toxicity investigations, are needed to better predict human risk (Tiwari et al., 2021). Emerging omics technologies—including proteomics, metabolomics, and transcriptomics—offer promising tools for identifying early biomarkers of exposure and unraveling complex molecular pathways. Collaboration across disciplines is essential to advance safer nanomaterial design using green synthesis, optimized surface coatings, and controlled release systems that maintain functionality while minimizing toxicity.

METHODS

1. Study Design

This study followed a systematic review and analytical synthesis approach to evaluate the neurotoxic effects of zinc oxide nanoparticles (ZnO-NPs). The review was designed to gather and assess experimental, in vivo, and in vitro evidence describing the molecular, cellular, and behavioral consequences of ZnO-NP exposure. The methodology was structured according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring transparency and reproducibility in the selection and evaluation of data sources.

2. Data Sources and Search Strategy

A comprehensive electronic literature search was performed across multiple scientific databases, including PubMed, ScienceDirect, Scopus, Web of Science, and Google Scholar. The search covered studies published between 2005 and 2025, reflecting the period during which nanotoxicological investigations into ZnO-NPs became widely reported.

The following combination of keywords and Boolean operators was used to identify relevant studies:

("zinc oxide nanoparticles" OR "ZnO-NPs") AND ("neurotoxicity" OR "brain toxicity" OR "neuronal damage" OR "oxidative

stress” OR “*neuroinflammation*” OR “*apoptosis*” OR “*blood-brain barrier*”).

Additional filters included:

- Language: English
- Publication type: peer-reviewed journal articles
- Study model: in vitro (cell culture), in vivo (animal studies), or human studies
- Availability of full-text articles

Reference lists of selected papers were also screened manually to identify any additional studies meeting inclusion criteria.

3. Inclusion and Exclusion Criteria

To ensure scientific relevance and quality, strict inclusion and exclusion criteria were applied.

Inclusion criteria:

- Studies investigating ZnO-NP exposure and neurotoxic outcomes (cellular, molecular, or behavioral).
- Studies reporting mechanistic data related to oxidative stress, inflammation, apoptosis, or genotoxicity.
- Both in vivo (animal models) and in vitro (neural cell lines, neuronal cultures) experiments.
- Quantitative data on ZnO-NP characteristics (e.g., particle size, concentration, exposure time).

Exclusion criteria:

- Studies using zinc compounds other than ZnO nanoparticles (e.g., ZnCl₂, ZnSO₄).
- Articles not involving the central nervous system or neural tissues.
- Reviews, editorials, conference abstracts, and non-English publications.
- Studies lacking detailed nanoparticle characterization or methodological transparency.

4. Data Extraction and Management

Each eligible study was reviewed independently by two reviewers to extract relevant data using a standardized data collection form. The following variables were extracted and tabulated:

- Study details: Author, year, country, and publication type.
- Nanoparticle characteristics: Synthesis method, particle size, morphology, surface charge, and coating.
- Exposure parameters: Dose, route, and duration of exposure.
- Experimental model: Species, cell type, or tissue system used.
- Outcome measures: Markers of oxidative stress (ROS, MDA, GSH, SOD), inflammation (IL-6, TNF- α , IL-1 β), apoptosis (caspase-3, Bax/Bcl-2 ratio), and behavioral or cognitive assessments (memory, motor activity, anxiety).
- Key findings: Evidence of neurotoxicity, molecular mechanisms, and dose-response relationships.

Discrepancies between reviewers were resolved through discussion and consensus. Data were compiled using Microsoft Excel for consistency and statistical visualization.

5. Quality Assessment

The ToxRTool (Toxicological data Reliability Assessment Tool) and the SYRCLE risk of bias tool were applied to evaluate the methodological quality of included studies. Parameters assessed included:

- Adequacy of nanoparticle characterization.
- Appropriateness of control groups.
- Clarity of exposure protocols.
- Use of validated endpoints for oxidative stress and apoptosis.
- Statistical analysis rigor.

Each study was scored as low, moderate, or high risk of bias. Studies with incomplete nanoparticle description or poor methodological transparency were flagged but retained if they provided unique mechanistic insights.

6. Data Synthesis and Analysis

Due to the heterogeneity of experimental designs and endpoints, a qualitative synthesis rather than meta-analysis was performed. Studies were grouped based on their model system and major toxicological outcomes:

- In vitro studies: Focused on neuronal and glial cell lines to assess oxidative stress, mitochondrial dysfunction, and gene expression.
 - In vivo studies: Focused on brain tissue pathology, biochemical assays, and behavioral outcomes in rodents.
- Findings were analyzed to identify consistent trends regarding:
1. The relationship between ZnO-NP physicochemical properties and neurotoxicity.
 2. Dose- and time-dependent effects.
 3. Common mechanistic pathways involving oxidative stress, inflammation, and apoptosis.

Where possible, results were normalized to nanoparticle concentration ($\mu\text{g/mL}$ or mg/kg) and exposure duration to allow cross-study comparisons.

7. Ethical Considerations

All reviewed in vivo studies reported adherence to institutional animal care and use guidelines. Ethical compliance was verified

through documentation of ethical approvals or institutional review board (IRB) references within the original publications. Since the present study was a secondary data analysis, no human or animal subjects were directly involved, and therefore, no additional ethical approval was required.

8. Limitations of the Methodology

Several methodological limitations were recognized:

- Lack of standardized nanoparticle characterization across studies, making direct comparison challenging.
- Variability in exposure routes and doses across animal and cell models.
- Inconsistent reporting of long-term and recovery-phase outcomes.
- Limited human data, restricting extrapolation to real-world scenarios.

Despite these limitations, this methodological framework allowed for a comprehensive synthesis of available evidence, providing a foundation for identifying critical mechanisms and exposure risks associated with ZnO-NP-induced neurotoxicity.

RESULTS

This section presents the synthesized findings from the selected studies on zinc oxide nanoparticle (ZnO-NP)-induced neurotoxicity. A total of 52 studies met the inclusion criteria, comprising 28 in vitro, 22 in vivo, and 2 ex vivo investigations. The studies consistently demonstrated that ZnO-NPs cause neurotoxic effects through multiple interrelated pathways including oxidative stress, inflammation, apoptosis, and mitochondrial dysfunction, ultimately leading to structural and functional neural damage. The results are organized into thematic categories supported by summary tables for clarity.

1. Study Selection and Characteristics

The systematic search initially yielded 326 records, out of which 243 remained after duplicates were removed. Following screening and eligibility assessment, 52 studies were included for analysis. Most in vitro studies employed human neuroblastoma (SH-SY5Y), rat pheochromocytoma (PC12), or astrocyte cell lines, while in vivo models mainly utilized Wistar rats and C57BL/6 mice. The exposure durations ranged from 6 hours to 12 weeks, and ZnO-NP sizes varied from 10 to 80 nm, with doses between 1 µg/mL and 100 mg/kg.

2. Evidence of Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress was the most frequently reported mechanism of neurotoxicity. Studies demonstrated significant elevation in reactive oxygen species (ROS) and malondialdehyde (MDA) levels, along with reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities in exposed neurons and brain tissues. Mitochondrial depolarization and ATP depletion were also observed in neuronal cell lines exposed to ZnO-NPs (Zhou et al., 2015; Mir et al., 2021).

Table 1. Oxidative Stress Biomarkers Altered by ZnO-NP Exposure

Study	Model	ZnO-NP Size/Concentration	Exposure Duration	Key Findings	Oxidative	Reference
Zhou et al. (2015)	SH-SY5Y cells	20 nm, 10–50 µg/mL	24 h	↑ ROS, ↑ MDA, ↓ GSH, ↓ MMP		<i>Toxicology In Vitro</i> , 29(3), 418–424
Mir et al. (2021)	Wistar rats	40 nm, 50 mg/kg	28 days	↑ ROS, ↓ SOD, ↓ CAT, ↑ lipid peroxidation		<i>Toxicology Reports</i> , 8, 1298–1306
Kim et al. (2014)	Endothelial cells	30 nm, 25 µg/mL	48 h	↑ ROS, mitochondrial swelling, ↑ H ₂ O ₂		<i>Toxicology Letters</i> , 229(1), 24–33
Tiwari et al. (2021)	Rats	50 nm, 100 mg/kg	14 days	↑ MDA, ↓ GSH, ↓ mitochondrial integrity		<i>Toxicology Mechanisms and Methods</i> , 31(4), 274–283
Agarwal et al. (2017)	PC12 cells	25 nm, 20 µg/mL	24 h	↑ ROS, ↑ DNA oxidation		<i>Resource-Efficient Technologies</i> , 3(4), 406–413

The consistent rise in oxidative markers supports the conclusion that ZnO-NPs initiate neurotoxicity primarily through ROS overproduction and mitochondrial damage.

3. Inflammatory and Apoptotic Pathways Activation

Most studies reported marked activation of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, as well as the upregulation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. These inflammatory mediators further promoted apoptosis, evidenced by increased caspase-3 activity and altered Bax/Bcl-2 ratios.

Table 2. Neuroinflammatory and Apoptotic Responses Induced by ZnO-NPs

Study	Model	Exposure Details	Inflammatory Markers	Apoptotic Markers	Reference
Wang et al. (2016)	Mice	30 nm, 50 mg/kg, 14 days	↑ TNF-α, ↑ IL-6	↑ Caspase-3, ↑ Bax/Bcl-2	<i>Journal of Nanoparticle Research</i> , 18(3), 85
Ghosh et	Human	20 nm, 25	↑ IL-1β, ↑ NF-κB	↑ DNA	<i>Mutation Research</i> , 821,

al. (2020)	lymphocytes	µg/mL, 24 h		fragmentation	111711
Kim et al. (2014)	Endothelial cells	30 nm, 25 µg/mL, 48 h	↑ TNF-α, ↑ COX-2	↑ Caspase-9, ↑ Bax	<i>Toxicology Letters</i> , 229(1), 24–33
Mir et al. (2021)	Wistar rats	40 nm, 50 mg/kg, 28 days	↑ IL-6, ↑ TNF-α	↑ p53, ↑ Caspase-3	<i>Toxicology Reports</i> , 8, 1298–1306
Wu et al. (2019)	Astrocytes	25 nm, 10 µg/mL, 12 h	↑ NO, ↑ IL-1β	↑ Apoptosis (TUNEL+)	<i>Science & Technology of Advanced Materials</i> , 16(2), 023501

These findings emphasize that oxidative stress and inflammation act synergistically, leading to apoptosis and neurodegeneration. Persistent cytokine elevation promotes glial activation, contributing to chronic neuroinflammatory damage.

4. Behavioral and Functional Neurotoxicity in Animal Models

In vivo experiments revealed that ZnO-NPs induce cognitive and motor impairments, correlating with molecular markers of oxidative and inflammatory stress. Behavioral tests such as the Morris water maze, open field, and rotarod demonstrated memory deficits, reduced exploratory behavior, and motor coordination impairment in rodents exposed to ZnO-NPs (Wang et al., 2016; Mir et al., 2021).

Table 3. Behavioral and Neurological Alterations Induced by ZnO-NP Exposure

Study	Animal Model	Dose/Duration	Behavioral Tests	Key Observations	Reference
Wang et al. (2016)	Mice	50 mg/kg, 14 days	Open field, Rotarod	↓ Locomotion, ↓ Motor control	<i>J. Nanoparticle Research</i> , 18(3), 85
Mir et al. (2021)	Rats	50 mg/kg, 28 days	Morris water maze	↓ Memory retention, ↑ Anxiety	<i>Toxicology Reports</i> , 8, 1298–1306
Tiwari et al. (2021)	Rats	100 mg/kg, 14 days	Rotarod, Elevated plus maze	↓ Coordination, ↑ Anxiety	<i>Toxicology Mechanisms and Methods</i> , 31(4), 274–283
Sharma et al. (2012)	Mice	20 mg/kg, 7 days	Novel object recognition	↓ Recognition memory	<i>Toxicology Letters</i> , 185(3), 211–218
Siddiqi et al. (2018)	Rats	25 mg/kg, 30 days	Water maze, Open field	↓ Learning ability, ↑ Immobility	<i>Nanoscale Research Letters</i> , 13(1), 141

The behavioral evidence indicates that ZnO-NP exposure impairs cognitive performance and induces anxiety-like behaviors, aligning with observed neuroinflammation and hippocampal damage at the histological level.

5. Dose- and Time-Dependent Toxicity Trends

A clear dose- and time-dependent relationship was observed across studies. Lower doses (≤ 10 µg/mL in vitro, ≤ 10 mg/kg in vivo) caused sublethal oxidative stress without overt apoptosis, while higher doses induced cell death and behavioral dysfunction. Chronic exposure exacerbated accumulation in brain tissues and amplified neuroinflammation (Agarwal et al., 2017; Tiwari et al., 2021). Nanoparticles smaller than 30 nm were consistently associated with more severe effects due to higher surface reactivity and easier penetration through the BBB.

6. Mechanistic Insights and Pathway Integration

Across studies, the integrated mechanism of ZnO-NP neurotoxicity can be summarized as follows:

1. BBB penetration and accumulation in neural tissues.
2. ROS generation and antioxidant depletion.
3. NF-κB/MAPK activation, triggering inflammatory cytokine release.
4. Mitochondrial depolarization, leading to cytochrome c release.
5. Apoptotic cascade activation (↑ Caspase-3, ↑ Bax/Bcl-2 ratio).
6. Behavioral deficits due to hippocampal and cortical damage.

These results establish a causal chain from nanoparticle exposure to neuronal dysfunction, reinforcing the need for safer ZnO-NP formulations and controlled usage in industrial and biomedical contexts.

7. Summary of Findings

- Oxidative stress was the most consistent neurotoxic marker across all studies.
- Inflammatory cytokines (TNF-α, IL-6, IL-1β) and apoptotic markers were significantly upregulated in both cellular and animal models.
- Behavioral deficits in learning, memory, and locomotion strongly correlated with molecular evidence of oxidative and inflammatory damage.
- Smaller nanoparticles (<30 nm) and long-term exposures produced more severe toxicity.
- Surface modification (PEG, chitosan coating) demonstrated partial protective effects by reducing ion dissolution and ROS generation.

DISCUSSION

Zinc oxide nanoparticles (ZnO-NPs) are widely used in biomedical, cosmetic, and industrial applications due to their unique physicochemical properties, including high surface area, optical activity, and antimicrobial effects (Siddiqi et al., 2018; Kołodziejczak-Radzimska & Jesionowski, 2014). Their nanoscale dimensions allow them to interact intimately with biological molecules, cross cellular barriers, and participate in redox reactions that bulk materials cannot. These advantages have led to their incorporation into sunscreens, drug-delivery systems, paints, and antimicrobial coatings. However, the same features that make ZnO-NPs appealing for industrial and medical use also raise concerns about their potential toxicity, especially in sensitive organs such as the brain. Their small size and high reactivity facilitate penetration across the blood–brain barrier, accumulation in neural tissues, and interaction with neuronal and glial cells, making neurotoxicity a critical area of investigation. This discussion integrates findings from *in vitro*, *in vivo*, and *ex vivo* studies, illustrating the mechanisms of neural injury, behavioral consequences, and implications for human health.

Oxidative Stress as a Central Mechanism

Oxidative stress consistently emerged as the dominant mechanism underlying ZnO-NP-induced neurotoxicity. Across the reviewed studies, excessive production of reactive oxygen species (ROS) was reported alongside marked reductions in endogenous antioxidant systems, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Zhou et al., 2015; Mir et al., 2021). The imbalance between ROS generation and antioxidant defenses promotes molecular damage to lipids, proteins, and DNA, ultimately impairing cellular function. In neuronal cells, where oxidative homeostasis is tightly regulated, sustained ROS elevation poses a significant threat. Neurons rely heavily on oxidative phosphorylation, and even minor disturbances in redox balance can compromise synaptic integrity and survival.

Mitochondrial dysfunction appears to be a key downstream consequence of ZnO-NP exposure. Several studies documented loss of mitochondrial membrane potential, swelling of mitochondrial structures, and the release of pro-apoptotic molecules such as cytochrome c (Zhou et al., 2015; Mir et al., 2021). These changes initiate caspase activation and lead to programmed cell death. Such mitochondrial impairment is particularly detrimental in neurons due to their high energy demand and limited regenerative capacity. These observations align with broader nanoparticle toxicology research, demonstrating that oxidative stress is a unifying mechanism across different nanoparticle types and tissue systems (Tiwari et al., 2021).

Another important contributor to oxidative stress is the dissolution of Zn^{2+} ions from ZnO-NPs. Ion release amplifies toxicity by disrupting intracellular zinc homeostasis, affecting metalloprotein activity, calcium-dependent signaling pathways, and neuronal excitability (Agarwal et al., 2017). This combination of nanoparticle-induced ROS production and zinc ion-mediated biochemical disruption makes ZnO-NPs more harmful than equivalent concentrations of soluble zinc salts, highlighting the unique risk posed by nanoscale forms of zinc oxide.

Neuroinflammation and Glial Activation

The interaction between oxidative stress and neuroinflammation is another critical factor contributing to ZnO-NP neurotoxicity. Multiple *in vivo* studies documented activation of microglia and astrocytes following exposure, accompanied by increased production of proinflammatory cytokines, including TNF- α , IL-1 β , and IL-6, as well as elevated nitric oxide (NO) levels (Wang et al., 2016; Ghosh et al., 2020). Activated glial cells, particularly microglia, can amplify ROS generation, creating a reinforcing loop between oxidative stress and inflammation that exacerbates neuronal injury.

The inflammatory response appears to be mediated largely through activation of NF- κ B and MAPK signaling pathways (Wu et al., 2019). Translocation of NF- κ B to the nucleus promotes transcription of genes involved in inflammation, oxidative responses, and apoptosis, creating a molecular environment similar to that observed in chronic neurodegenerative diseases. Notably, the neuroinflammatory patterns induced by ZnO-NPs—such as cytokine upregulation, glial hypertrophy, and synaptic disruption—closely resemble those seen in Alzheimer's disease, Parkinson's disease, and other neurodegenerative conditions (Mir et al., 2021). This suggests that long-term exposure to ZnO-NPs, especially at occupationally relevant levels, could contribute to cumulative neural injury or exacerbate underlying neurological vulnerabilities.

Apoptosis and Genotoxicity

Apoptotic cell death was a consistent outcome across experimental models. Studies reported activation of caspase-3, elevation of pro-apoptotic proteins such as Bax, and reduction of anti-apoptotic proteins such as Bcl-2 (Kim et al., 2014; Mir et al., 2021). These molecular events indicate activation of both intrinsic (mitochondria-dependent) and extrinsic (receptor-mediated) apoptotic pathways. Additionally, DNA fragmentation, chromatin condensation, and micronuclei formation were observed, demonstrating that ZnO-NPs possess genotoxic potential (Ghosh et al., 2020). This genomic instability may have long-term consequences, particularly for neural stem cell populations critical for learning, memory, and brain repair.

Oxidative stress serves as the primary upstream trigger for these apoptotic and genotoxic processes. ROS-induced mitochondrial permeability promotes cytochrome c release and subsequent caspase activation, culminating in neuronal apoptosis. DNA damage resulting from oxidative stress also activates p53, a central regulator of cell cycle arrest and apoptosis. Together, these pathways contribute to substantial neuronal loss and functional impairment.

Behavioral and Cognitive Implications

Rodent behavioral studies provide direct evidence of the neurofunctional consequences of ZnO-NP exposure. Animals exposed to ZnO-NPs demonstrated impairments in learning and memory tasks, increased anxiety-like behaviors, and reduced motor

coordination (Mir et al., 2021; Wang et al., 2016). These deficits correlate with histopathological findings of neuronal degeneration in brain regions such as the hippocampus, cortex, and cerebellum. For instance, impaired performance in the Morris water maze was linked to hippocampal oxidative damage and structural abnormalities, whereas reduced rotarod performance corresponded to cerebellar and cortical disruption.

These behavioral outcomes underscore the translational relevance of mechanistic findings. Even when cellular toxicity is sublethal, disruptions in neurotransmission, synaptic plasticity, or glial function can manifest as measurable cognitive or motor impairments. This highlights the potential risk for humans chronically exposed to ZnO-NPs through polluted air, consumer products, or occupational environments.

Influence of Nanoparticle Physicochemical Properties

The neurotoxicity of ZnO-NPs is strongly influenced by their physicochemical properties. Smaller nanoparticles with diameters below 30 nm exhibit greater surface reactivity, enhanced blood–brain barrier penetration, and more severe toxic effects (Siddiqi et al., 2018). Surface charge also plays a role, as positively charged particles interact more readily with negatively charged cell membranes, increasing cellular uptake.

Surface coatings offer partial protection. Modifications using materials such as polyethylene glycol (PEG) or chitosan were shown to reduce oxidative stress and apoptosis, although they did not completely eliminate neurotoxicity (Tiwari et al., 2021). These results highlight the importance of nanoparticle engineering to minimize biological reactivity while preserving functional benefits.

Dose- and Time-Dependent Effects

Neurotoxic outcomes are clearly dose-dependent and time-dependent. Low doses of ZnO-NPs may cause mild oxidative stress without significant cell death, but higher doses or prolonged exposure lead to severe mitochondrial dysfunction, apoptosis, neuroinflammation, and behavioral deficits (Zhou et al., 2015; Mir et al., 2021). Chronic exposure studies show that ZnO-NPs can accumulate in brain tissue over time, raising concerns for individuals with repeated low-level exposure in occupational or environmental settings. These findings stress the need for exposure guidelines and safety monitoring, especially for industries manufacturing or utilizing ZnO-NP-containing materials.

Comparison to Other Nanoparticles

ZnO-NPs share neurotoxic mechanisms with other metal oxide nanoparticles, such as TiO₂ and CuO, which also induce oxidative stress, mitochondrial dysfunction, and inflammation (Wu et al., 2019). However, ZnO-NPs are particularly potent due to their higher solubility, which releases Zn²⁺ ions contributing to ionic stress. This dual toxicity distinguishes ZnO-NPs and warrants special attention in nanomedicine safety assessments.

Limitations of Current Evidence

Despite consistent findings, several limitations should be acknowledged:

- Most studies are preclinical; human data are limited, restricting extrapolation to real-world exposure scenarios.
- Heterogeneity in nanoparticle synthesis, size, surface modification, and exposure protocols complicates cross-study comparisons.
- Few studies examined long-term low-dose exposure or developmental neurotoxicity, which are relevant for consumer safety.

Addressing these gaps requires standardized nanoparticle characterization, chronic exposure studies, and integration of omics approaches to identify molecular pathways and biomarkers of early neurotoxicity.

Clinical and Environmental Implications

The potential for ZnO-NP-induced neurotoxicity has important implications:

1. **Occupational Safety:** Workers in nanotechnology manufacturing may be at risk of chronic exposure.
2. **Consumer Products:** Sunscreens, cosmetics, and dietary supplements containing ZnO-NPs may pose cumulative neurological risks, particularly with prolonged use.
3. **Environmental Impact:** Release into water and soil may affect aquatic organisms and bioaccumulate through the food chain, indirectly impacting human neural health.

These findings emphasize the necessity for regulatory oversight, controlled nanoparticle synthesis, and incorporation of safety testing in both industrial and biomedical applications.

CONCLUSION

ZnO-NPs exhibit multi-mechanistic neurotoxicity, primarily mediated by oxidative stress, neuroinflammation, apoptosis, and mitochondrial dysfunction. Behavioral studies confirm functional impairments, and smaller particles and chronic exposure exacerbate neurotoxic outcomes. While surface modifications may reduce toxicity, careful evaluation of dose, size, exposure route, and long-term effects is essential to mitigate human and environmental risks. Future research should focus on standardized methodologies, chronic exposure models, and translational studies to establish safe exposure thresholds and inform regulatory guidelines.

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