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Exploring Oxidative Stress Mechanisms of Nanoparticles Using Zebrafish (*Danio rerio*): Toxicological and Pharmaceutical Insights

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Abstract: Nanoparticles (NPs) have revolutionized biomedical and pharmaceutical applications due to their unique physicochemical properties. However, their widespread use has raised concerns regarding their potential toxicity, particularly through oxidative stress mechanisms. Oxidative stress, primarily driven by reactive oxygen species (ROS) overproduction, plays a central role in NP-induced toxicity, leading to cellular dysfunction, inflammation, apoptosis, and genotoxicity. Zebrafish (*Danio rerio*) has emerged as a powerful *in vivo* model for nanotoxicology, offering advantages such as genetic similarity to humans, rapid development, and optical transparency, allowing real-time monitoring of oxidative damage. This review synthesizes current findings on NP-induced oxidative stress in zebrafish, highlighting key toxicity mechanisms and case studies involving metallic (gold, silver, copper), metal oxide (zinc oxide, titanium dioxide, iron oxide), polymeric, and lipid-based NPs. The influence of NP physicochemical properties, such as size, surface charge, and functionalization on oxidative stress responses is explored. Additionally, experimental approaches used to assess ROS generation, antioxidant enzyme activity, and oxidative damage biomarkers in zebrafish models are examined. In addition to toxicity concerns, pharmaceutical applications of antioxidant-modified NPs are evaluated, particularly their potential in drug delivery, neuroprotection, and disease therapeutics. Challenges in zebrafish-based nanotoxicology, the need for standardized methodologies, and future directions for optimizing NP design to minimize oxidative stress-related risks are also discussed. By integrating insights from toxicity mechanisms, case studies, and pharmaceutical strategies, this review supports the development of safer and more effective nanoparticle-based therapies while addressing the challenges of oxidative stress-related toxicity.

Keywords: nanoparticles; oxidative stress; zebrafish; pharmaceutical applications

1. Introduction

Nanoparticles (NPs) are increasingly utilized across multiple industries, including pharmaceuticals, biomedical imaging, drug delivery, cosmetics, diagnostics, and environmental applications [1]. Due to their small size and high surface area-to-volume ratio, NPs exhibit unique physicochemical properties that enhance their reactivity and bioavailability, making them ideal for various biomedical and technological applications [2]. In the pharmaceutical field, NPs serve as efficient drug carriers, improving drug solubility, targeted delivery, and controlled release, reducing side effects and enhancing therapeutic efficacy [3]. Metallic NPs, such as gold (Au) and silver (Ag), are extensively applied in biomedical imaging and diagnostics due to their optical and electronic properties [4]. Additionally, lipid-based and polymeric NPs are commonly employed in drug

delivery systems, offering enhanced biocompatibility and reduced toxicity [5]. Beyond biomedical applications, NPs are widely used in cosmetics and skincare products, where they enhance product stability and skin penetration [6]. Titanium dioxide (TiO_2) and zinc oxide (ZnO) NPs, for example, are key ingredients in sunscreens, providing superior UV protection [7]. However, concerns regarding the potential toxicity of NPs, especially their ability to generate oxidative stress, have increased [8]. Environmental exposure to NPs through industrial waste, air pollution, and contaminated water sources has raised significant health concerns, necessitating further research into their long-term effects on biological systems [9].

One of the most critical toxicological effects associated with NPs is oxidative stress. This occurs when NPs induce an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms within biological systems [10]. ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), play a crucial role in cellular signaling but become harmful when generated excessively [11]. Overproduction of ROS leads to oxidative damage to lipids, proteins, and nucleic acids, contributing to cellular dysfunction and various pathological conditions [12].

Numerous studies have demonstrated that exposure to metal and metal oxide NPs, such as AgNPs, CuO NPs, and ZnO NPs, results in increased ROS production, leading to oxidative stress-induced apoptosis and genotoxicity [13]. The oxidative stress triggered by NPs can activate multiple pathways, including mitochondrial dysfunction, DNA damage, and inflammatory responses [14]. Prolonged oxidative stress has been linked to chronic inflammation, which plays a crucial role in the pathogenesis of various diseases, including cancer, neurodegeneration, and cardiovascular disorders [15]. Understanding oxidative stress as a central mechanism of NP toxicity is essential for developing safer nanomaterials with reduced adverse effects.

Zebrafish (*Danio rerio*) has emerged as a widely used vertebrate model in nanotoxicology due to its genetic similarity to humans (~70%), rapid development, and ease of maintenance in laboratory settings [16]. One of the key advantages of zebrafish is the optical transparency of embryos, allowing real-time visualization of NP interactions and oxidative stress responses in developing tissues [17]. This feature makes zebrafish an excellent *in vivo* model for assessing NP-induced oxidative damage, mitochondrial dysfunction, and inflammatory responses [15].

Studies have shown that zebrafish embryos exposed to AgNPs, TiO_2 NPs, and CdSe quantum dots exhibit significant ROS generation, leading to apoptosis and developmental abnormalities [18]. In particular, AgNPs have been reported to induce oxidative stress-mediated neurotoxicity and cardiovascular dysfunction in zebrafish models [12]. The use of zebrafish in NP toxicity assessments provides valuable insights into NP biodistribution, bioaccumulation, and their effects on different organ systems. Furthermore, zebrafish models are increasingly employed in high-throughput screening assays to evaluate pharmaceutical formulations and antioxidant-modified NPs designed to mitigate oxidative stress (Figure 1) [19].
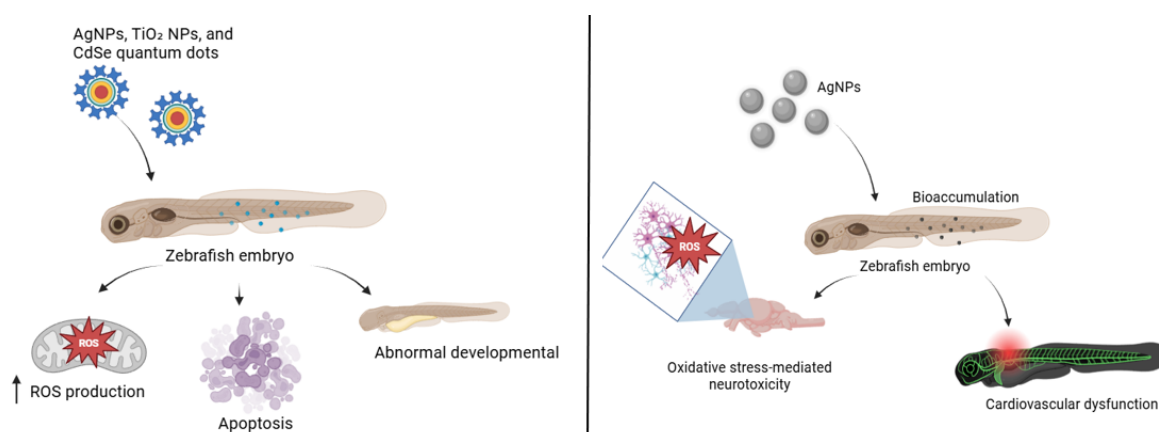

Figure 1. Nanoparticles and Oxidative Stress: Zebrafish as a Model for Toxicity and Pharmaceutical Applications.

This review aims to provide a comprehensive analysis of NP-induced oxidative stress in zebrafish, focusing on its toxicological implications and pharmaceutical applications. The study highlights key mechanisms of oxidative stress, evaluates case studies involving different NP types, and explores the role of zebrafish as a model for assessing NP safety and therapeutic potential. Additionally, the review addresses challenges in nanotoxicology and the need for standardized methodologies to improve NP risk assessment and pharmaceutical development.

2. Physicochemical Properties of Nanoparticles Influencing Oxidative Stress

2.1. NP Size and Surface Area

The size of nanoparticles plays a crucial role in their toxicity potential, as smaller NPs exhibit higher surface-area-to-volume ratios, increasing their reactivity and potential to induce oxidative stress [20]. Studies have demonstrated that reduced NP size correlates with increased ROS generation and toxicity in aquatic organisms, including zebrafish. For instance, silver nanoparticles (AgNPs) smaller than 10 nm have been shown to trigger higher oxidative stress levels compared to larger particles due to their enhanced cellular penetration and bioavailability [21,22]. Similarly, titanium dioxide (TiO_2) NPs of <20 nm have been reported to induce significant ROS production and mitochondrial dysfunction in zebrafish embryos [23,24]. Table 1 presents a synthesis of studies on the size of various NPs and their induced oxidative stress.

Table 1. Impact of NP Size on Oxidative Stress and Toxicity.

Nanoparticle Type	Small Size (≤20 nm)	Medium Size (20–50 nm)	Large Size (>50 nm)	Reference
Ag NPs	Mitochondrial Dysfunction	Moderate ROS	Minimal ROS	[11,19,22,25]
Au NPs	Pro-inflammatory Effects	Minimal Effects	Low ROS	[19,26,27]
Cu NPs	DNA Damage	Inflammation	Low ROS	[28]
Fe NPs	Cellular Damage	Mitochondrial Dysfunction	Low ROS	[29,30]
ZnO NPs	Apoptosis	Moderate ROS	Low ROS	[31–34]
TiO_2 NPs	Neurotoxicity, Behavioral Disruptions	Moderate ROS	Minimal ROS	[23,24]
CuO NPs	Genotoxicity	Cellular Stress	Minimal ROS	[35–37]
Fe_2O_3 NPs	DNA Fragmentation	Cellular Inflammation	Low ROS	[36,38–41]
CdSe QDs	DNA Damage, Apoptosis	Mitochondrial Dysfunction	Low ROS	[42,43]
ZnS QDs	Phototoxicity, Lipid Peroxidation	DNA Damage	Limited Cytotoxicity	[42,43]

Silver Nanoparticles (Ag NPs): Zebrafish embryos exposed to AgNPs of varying sizes demonstrated that smaller AgNPs (10 nm) triggered more severe ROS production and developmental toxicity compared to larger particles (50 nm) [21]. The study indicated that smaller AgNPs disrupted the antioxidant defense system, downregulating antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [44].

Gold Nanoparticles (Au NPs): Investigations on AuNPs found that particles smaller than 20 nm induced more oxidative stress than larger ones due to their ability to accumulate in tissues and penetrate cellular compartments [45]. Additionally, smaller AuNPs enhanced pro-inflammatory cytokine expression, further exacerbating ROS generation [46,47].

Copper Nanoparticles (Cu NPs) have gained attention in nanotoxicology due to their unique physicochemical properties and potential biomedical applications [36]. Their small size (≤20 nm) leads to increased oxidative stress, causing significant DNA damage and mitochondrial dysfunction in biological systems [48]. Studies have demonstrated that Cu NPs induce oxidative stress through ROS overproduction, which can trigger inflammation and apoptosis in zebrafish tissues [35,49].

Iron Nanoparticles (Fe NPs) are widely used in environmental remediation and biomedical applications. Their unique magnetic properties make them valuable for drug delivery, hyperthermia

treatment, and MRI contrast agents [39]. Studies show that Fe NPs generate ROS through Fenton reactions, causing cellular damage and mitochondrial dysfunction in zebrafish models [50]. Exposure to Fe NPs in zebrafish embryos has been associated with oxidative stress-induced apoptosis, DNA damage, and developmental abnormalities [51].

Zinc Oxide Nanoparticles: Different sizes were evaluated for oxidative stress induction in zebrafish larvae, revealing that smaller ZnO NPs led to significantly higher ROS production and mitochondrial damage [31,33]. The study suggested that the smaller NPs had increased bioavailability, leading to prolonged oxidative stress and apoptosis [34,52].

Titanium Dioxide Nanoparticles (TiO$_2$ NPs): A recent study demonstrated that TiO$_2$ NPs induce oxidative stress and neurotoxic effects in zebrafish embryos, disrupting behavioral responses and mitochondrial function [24]. These findings suggest that TiO$_2$ NPs contribute to ROS accumulation and affect neural pathways, highlighting their potential long-term toxicity in aquatic organisms and human health implications [23].

Copper Oxide Nanoparticles (CuO NPs) are widely utilized in industrial and biomedical applications due to their antimicrobial properties and catalytic activity [36]. Studies have shown that CuO NPs induce substantial ROS production, causing lipid peroxidation, mitochondrial dysfunction, and DNA fragmentation in zebrafish models. Exposure to CuO NPs in zebrafish embryos has been linked to apoptotic cell death and developmental toxicity due to their ability to disrupt redox homeostasis [28].

Iron(III) Oxide Nanoparticles (Fe$_2$O$_3$ NPs) have superparamagnetic properties. Studies have indicated that Fe$_2$O$_3$ NPs can induce oxidative DNA fragmentation, lipid peroxidation, and mitochondrial dysfunction in zebrafish models, leading to apoptosis and neurotoxicity [53]. Additionally, zebrafish embryos exposed to Fe$_2$O$_3$ NPs exhibit inflammatory responses and oxidative stress in gill and liver tissues due to ROS-induced damage [50,54].

Cadmium Selenide Quantum Dots (CdSe QDs) have optoelectronic applications due to their fluorescent properties. Studies have demonstrated that CdSe QDs induce DNA fragmentation, lipid peroxidation, and apoptosis in zebrafish models, leading to developmental abnormalities and neurotoxicity [55]. Exposure to CdSe QDs has also been linked to mitochondrial dysfunction, as the nanoparticles interfere with electron transport chain activity, leading to increased ROS generation and cellular damage [56].

Zinc Sulfide Quantum Dots (ZnS QDs) have photonic applications due to their unique optical properties. Studies have shown that ZnS QDs can induce lipid peroxidation, oxidative DNA damage, and apoptosis in zebrafish embryos, particularly under illumination conditions [42,57]. Exposure to ZnS QDs has also been linked to mitochondrial stress and inflammatory responses, contributing to oxidative toxicity [43].

These findings reinforce the idea that NP size plays a crucial role in determining oxidative stress outcomes and toxicity levels. Future research should focus on optimizing NP size to balance efficacy in pharmaceutical applications while minimizing oxidative damage.

2.2. Surface Charge and Coating Effects

The charge and surface modifications of NPs significantly influence their cellular uptake, bioavailability, and oxidative stress potential. Positively charged (cationic) NPs tend to exhibit greater cytotoxicity due to enhanced cellular uptake and stronger electrostatic interactions with negatively charged cell membranes [58]. Conversely, surface coatings such as polyethylene glycol (PEG) can modify NP interactions and mitigate oxidative stress [59]. Figure 2 represents a schematic of the importance of the surface charge and the coating effects.

Cationic NPs show stronger ROS production and cytotoxicity compared to neutral or anionic NPs [60]. These nanoparticles tend to interact more aggressively with negatively charged cell membranes, facilitating their internalization and causing significant oxidative stress. Studies have demonstrated that cationic NPs exhibit increased genotoxicity and inflammatory responses due to their ability to disrupt cell membranes, induce mitochondrial dysfunction, and activate apoptosis

pathways [61]. For example, research on AgNPs has shown that positively charged silver nanoparticles are more cytotoxic than their neutral or negatively charged counterparts, leading to increased ROS production and DNA damage in zebrafish embryos [11,62]. Similarly, CuNPs exhibit a strong capacity to generate oxidative stress, leading to lipid peroxidation and cell membrane rupture [63]. Furthermore, cationic NPs have been linked to neuroinflammation and cardiovascular dysfunction in zebrafish models, highlighting the need for careful assessment of surface charge when designing nanoparticle-based applications in biomedical and pharmaceutical sciences [64–66].

Anionic and Neutral NPs tend to show reduced oxidative stress responses, as their charge properties limit cellular interactions and membrane permeability, leading to lower toxicity [67]. Studies have previously demonstrated that negatively charged NPs have lower cytotoxicity due to their repulsion from negatively charged cell membranes, reducing internalization and subsequent ROS production. Neutral lipid-based nanoparticles like liposomes have shown that they do not significantly induce ROS generation, making them promising candidates for drug delivery applications [68–70]. In another study, negatively charged Au NPs exhibited reduced inflammatory response and minimal oxidative damage compared to positively charged counterparts [71]. The reduced interaction of anionic and neutral NPs with cellular components suggests their potential as safer alternatives in biomedical applications, particularly in drug delivery and antioxidant therapy.

Surface coatings and modifications of NPs play a crucial role in influencing their interactions with biological systems, particularly in mitigating oxidative stress [61]. Functionalizing NPs with polymers such as polyethylene glycol (PEG) or albumin can reduce ROS production by decreasing NP aggregation and limiting cellular internalization. For instance, PEGylation of AuNPs has been shown to diminish oxidative stress and inflammatory responses [72,73]. Similarly, coating AgNPs with biocompatible polymers has been demonstrated to reduce their ROS generation and improve stability in biological environments [74]. These findings underscore the importance of controlled NP surface modifications to optimize biocompatibility and minimize oxidative stress-related toxicity in biological systems.
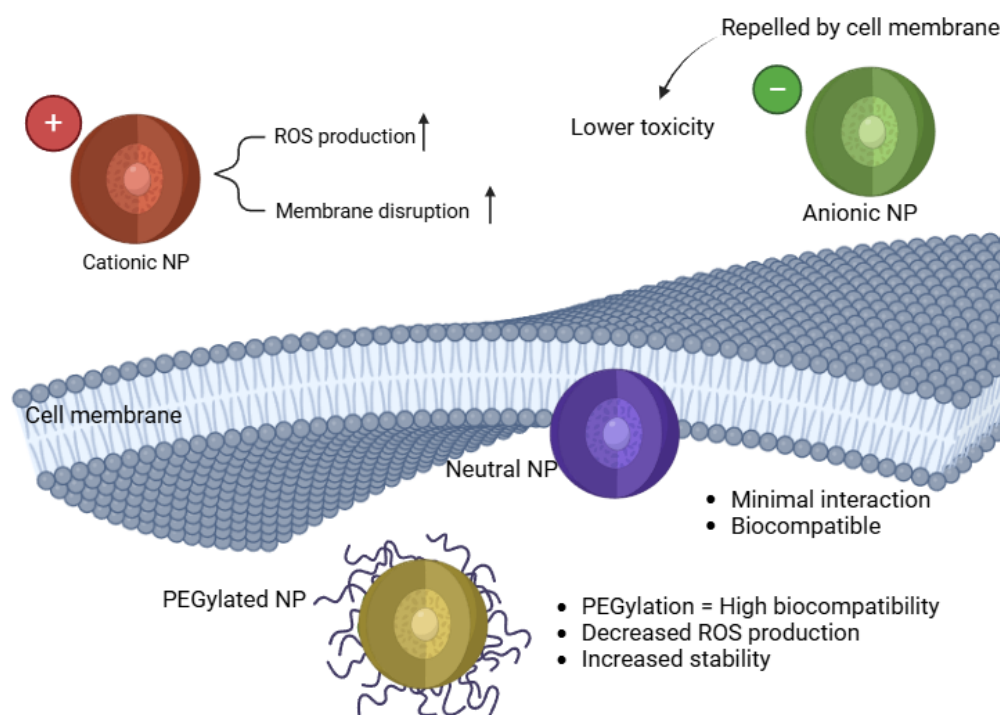

Figure 2. Impact of NP surface charge on cellular uptake and toxicity.

3. Oxidative Stress Pathways Activated by Nanoparticles in Zebrafish

NP-induced oxidative stress in zebrafish occurs either through direct ROS generation or secondary mechanisms like inflammation [16]. Excess ROS production disrupts cellular homeostasis, leading to biomolecular damage. One significant effect of NP exposure is the inhibition of key antioxidant enzymes, such as SOD, CAT, and GPx, weakening the cellular defense system [29]. Exposure to TiO_2 NPs has been shown to suppress antioxidant defenses in zebrafish liver and gill tissues, resulting in oxidative damage and inflammation [24]. Similarly, AgNPs significantly reduce SOD and CAT activity in zebrafish larvae, increasing oxidative stress and lipid peroxidation [44]. CuO NPs further disrupt glutathione homeostasis, impairing detoxification pathways and exacerbating ROS accumulation [75]. Also, ZnO NP exposure depleted antioxidant enzyme levels in zebrafish embryos, correlating with mitochondrial dysfunction and apoptosis [36]. These effects are synthetized in Table 2.

Table 2. Effects of Nanoparticles on Oxidative Stress Mechanisms in Zebrafish.

Nanoparticle Type	Affected Organ/System	Mechanism of Toxicity	Key Biomarkers/ Cytokines	Observed Effects	Case Study	Reference
TiO_2 NPs	Liver, Gills	ROS Overproduction, Antioxidant Enzyme Suppression	↓ SOD, ↓ CAT, ↑ MDA	Oxidative stress-mediated toxicity	TiO_2 NPs reduced SOD and CAT activity in zebrafish liver cells	[24,48]
CuO NPs	Brain	Mitochondrial Dysfunction, Apoptotic Pathways	↑ p53, ↑ Caspase-3, ↓ ATP	ROS-induced neurotoxicity	CuO NPs triggered apoptosis in zebrafish brain tissue via mitochondrial damage	[48,75,76]
Fe_2O_3 NPs	Gills, Immune System	NF-κB Activation, Inflammatory Response	↑ IL-8, ↑ IL-1β, ↑ NF-κB	Respiratory dysfunction, chronic inflammation	Fe_2O_3 NPs caused NF-κB-mediated inflammation in zebrafish gills	[29,77]
AgNPs	Liver	Hepatotoxicity, Cytokine Overexpression	↑ TNF-α, ↑ IL-6	Liver inflammation, oxidative damage	AgNPs upregulated TNF-α and IL-6 in zebrafish liver cells	[29,77,78]
ZnO NPs	Muscle, Liver	DNA Damage, Lipid Peroxidation	↑ DNA Strand Breaks, ↑ MDA	Chromosomal instability, apoptosis	ZnO NPs induced DNA double-strand breaks in zebrafish embryos	[8,34]
CdSe QDs	Brain, Muscle	Genotoxicity, Lipid Peroxidation	↑ XRCC1, ↑ p53	DNA fragmentation, neuronal dysfunction	CdSe QDs caused DNA strand breaks in zebrafish larvae	[9,26,79]

3.1. Mitochondrial Dysfunction and Apoptotic Pathways

Mitochondria are highly susceptible to oxidative stress caused by NP exposure. ROS accumulation in mitochondria disrupts electron transport chain (ETC) function, reduces ATP production, and activates pro-apoptotic signaling pathways [5,36]. NP-induced oxidative damage can trigger p53 tumor suppressor activation, leading to caspase-3-mediated apoptosis. A study on zebrafish embryos exposed to CuO NPs demonstrated significant mitochondrial swelling, cytochrome c release, and p53 activation, which triggers caspase-3-mediated apoptosis. This process has been associated with neurotoxicity in zebrafish brain tissue [80]. Another study found that AgNP exposure led to mitochondrial membrane potential collapse in zebrafish neurons, accelerating neurotoxicity through p53 upregulation and caspase-3 activation [81]. Additionally, TiO_2 NPs were reported to induce oxidative damage in zebrafish liver cells, impairing mitochondrial respiration and promoting apoptotic cell death [82]. Furthermore, CdSe quantum dots disrupted mitochondrial

membrane integrity in zebrafish cardiomyocytes, contributing to cardiac dysfunction and increased apoptotic cell death [83].

3.2. Inflammation and Immune Dysregulation

NP exposure in zebrafish has been linked to immune system dysregulation through the activation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nuclear factor-kappa B (NF-κB) [29]. These cytokines are key mediators of inflammation, leading to immune overactivation and subsequent tissue damage. Chronic immune activation contributes to prolonged oxidative stress, affecting multiple organ systems, including the gills, liver, and nervous system [54]. Studies indicate that NP-induced inflammation can also disrupt hematopoiesis and immune cell homeostasis, further exacerbating toxicity. Recent studies have shown that Fe_2O_3 NPs not only activate NF-κB pathways in zebrafish gills, leading to oxidative stress and impaired respiratory function but also increase the expression of IL-8 and IL-1β, leading to macrophage recruitment and localized inflammation [10]. Another study demonstrated that AgNPs cause upregulation of TNF-α and IL-6 in zebrafish liver cells, promoting hepatotoxicity and oxidative damage [84]. Additionally, exposure to TiO_2 NPs in zebrafish larvae resulted in increased leukocyte infiltration and cytokine secretion, demonstrating NP-induced immune activation in early developmental stages [85].

3.3. Genotoxicity and Lipid Peroxidation

Oxidative stress induced by NPs can lead to severe genotoxic effects, including DNA fragmentation, chromosomal aberrations, and mutagenic potential, which may contribute to long-term developmental and reproductive toxicity in zebrafish. DNA damage is commonly assessed through comet assays and micronucleus tests, which have revealed increased genotoxic markers upon exposure to metal-based and semiconductor NPs. Additionally, lipid peroxidation—caused by excessive ROS attacking membrane lipids—compromises cellular integrity, disrupts signaling pathways, and initiates apoptotic cascades [8]. Studies have shown that oxidative stress-driven lipid peroxidation is a key mechanism in NP-mediated toxicity, particularly in organs with high lipid content such as the brain and liver. CdSe QDs were found to induce DNA strand breaks in zebrafish larvae, leading to chromosomal fragmentation and developmental abnormalities [86]. A study using fluorescent in situ hybridization (FISH) confirmed increased nuclear damage following CdSe QD exposure, indicating their potential to cause long-term genetic instability [87]. Another study demonstrated that exposure to ZnO NPs in zebrafish embryos resulted in both DNA double-strand breaks and increased lipid peroxidation in liver and muscle tissues, further confirming the dual genotoxic and oxidative stress-related impacts of these NPs. Histopathological examination showed mitochondrial swelling and nuclear fragmentation, consistent with apoptotic progression [88].

4. Experimental Approaches for Assessing Oxidative Stress in Zebrafish

4.1. Biochemical Assays for ROS and Antioxidant Activity

Biochemical assays are fundamental for quantifying oxidative stress markers in zebrafish tissues, providing insights into the impact of nanoparticles on redox balance. The most commonly used assays include:

Lipid Peroxidation Assay: Measures malondialdehyde (MDA) levels, a key indicator of lipid peroxidation. Elevated MDA levels correlate with membrane lipid degradation and oxidative stress in NP-exposed zebrafish. Studies have shown increased MDA production in zebrafish embryos exposed to AgNPs and ZnO NPs, linking lipid damage to NP-induced toxicity [89].

Total ROS Quantification: Fluorometric and chemiluminescent methods detect ROS accumulation in tissues. The DCFDA assay is widely used for quantifying intracellular ROS levels.

In zebrafish models, increased ROS production has been documented following CuO NP exposure, with ROS levels peaking at 24 hours post-exposure [90].

Enzyme Activity Assays: Assess antioxidant defense mechanisms through measurement of SOD and CAT enzyme activity. Studies indicate that exposure to TiO_2 NPs leads to a significant reduction in SOD and CAT activity, suggesting antioxidant depletion and impaired oxidative defense in zebrafish liver and gill tissues [82].

4.2. Gene expression Analysis of Oxidative Stress Pathways

Examining gene expression changes in oxidative stress-related pathways provides insights into NP-induced molecular alterations. Quantitative PCR (qPCR) and RNA sequencing (RNA-seq) are commonly used to analyze differential gene expression in zebrafish exposed to various nanoparticles [10]. Key oxidative stress-related genes include:

nrf2: Master regulator of antioxidant response, upregulated in response to oxidative stress. Studies have shown increased nrf2 expression in zebrafish larvae following exposure to AgNPs, correlating with an upregulation of downstream detoxifying enzymes [3,91].

sod1: Encodes superoxide dismutase 1, responsible for neutralizing superoxide radicals. Zebrafish embryos exposed to ZnO NPs demonstrated sod1 overexpression as an early defense mechanism against ROS accumulation [34].

gpx1a: Encodes glutathione peroxidase, a key enzyme in detoxifying hydrogen peroxide and lipid peroxides. Research has demonstrated that CdSe quantum dots induce gpx1a suppression in zebrafish liver, leading to increased oxidative damage and apoptosis [92].

Studies comparing metal oxides, polymeric NPs, and quantum dots have revealed distinct gene expression profiles. For example, TiO_2 NPs predominantly upregulate nrf2 and sod1, while AgNPs induce a more systemic stress response, affecting inflammatory and oxidative stress-related genes. Polymeric NPs tend to induce a more moderate response, with lower activation of detoxification genes, suggesting reduced toxicity compared to metal-based NPs [93].

4.3. Histopathological and Imaging Techniques

Advanced imaging techniques enable direct visualization of oxidative stress-induced damage caused by nanoparticles in zebrafish tissues (Table 3). These approaches are essential for assessing nanoparticle localization, oxidative damage, and cellular alterations at the histopathological level.

Table 3. Imaging and Histopathological Techniques for Oxidative Stress Assessment.

Technique	Purpose	Application in Zebrafish	Key Findings	Reference
Fluorescent ROS Detection (DCFDA Assay)	Measures intracellular ROS levels	Used to quantify oxidative stress in zebrafish tissues	AgNP-exposed zebrafish embryos show significant ROS increase in brain and liver	[87,94,95]
Transmission Electron Microscopy (TEM)	Ultrastructural analysis of NP localization and organelle damage	Identifies mitochondrial swelling, cristae disruption, and vacuolization	TiO_2 NPs accumulate in hepatic mitochondria, leading to apoptosis	[72,96]
Hematoxylin and Eosin (H&E) Staining	Histopathological assessment of tissue damage and inflammation	Detects necrosis, epithelial degeneration, and immune cell infiltration	CuO NP exposure causes epithelial damage and chronic inflammation in zebrafish gills	[97]
TUNEL Assay (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling)	Identifies apoptotic DNA fragmentation	Used to assess neurotoxicity and genotoxicity in zebrafish tissues	CdSe QD exposure leads to increased TUNEL-positive cells in zebrafish brain	[98,99]

4.4. Behavioral and Physiological Endpoints

Oxidative stress can manifest as behavioral and physiological abnormalities in zebrafish, providing crucial indicators of NP-induced toxicity. Behavioral changes and physiological responses serve as non-invasive biomarkers of oxidative stress and neurotoxicity.

Swimming Activity: Reduced motility, erratic swimming patterns, and hypoactivity in NP-exposed zebrafish indicate neurotoxic effects associated with oxidative stress. A study on AgNP exposure demonstrated decreased locomotion and anxiety-like behavior in zebrafish larvae, correlating with increased ROS levels in the brain [100].

Heart Rate Variability: Cardiotoxicity is evaluated by measuring bradycardia or tachycardia in NP-exposed embryos. Zebrafish exposed to CuO NPs exhibited significant alterations in heart rate, attributed to mitochondrial dysfunction and ROS accumulation in cardiac tissues [75,101].

Developmental Malformations: Craniofacial deformities, pericardial edema, and spinal curvature are common teratogenic effects linked to NP-induced oxidative stress. Research on TiO_2 NPs exposure in zebrafish embryos revealed increased malformations, with ROS-mediated DNA damage being a key underlying mechanism [10,81].

Respiratory Function and Gill Damage: Respiratory distress, evidenced by rapid opercular movement, was observed in zebrafish exposed to Fe_2O_3 NPs, correlating with histopathological gill damage and NF-κB-driven inflammation [10,29].

5. Pharmaceutical Applications of Nanoparticles Using Zebrafish (*Danio rerio*) Models

Zebrafish have become an essential vertebrate model in nanomedicine research due to their high genetic homology to humans (~70%), rapid organ development, and affordability in large-scale experiments. Their high fecundity enables large-scale pharmacological screenings, and their transparent embryos allow real-time tracking of NP biodistribution and interactions in vivo. Zebrafish have been successfully used for toxicity screening, drug efficacy evaluations, and nanoparticle-mediated therapeutic interventions [102]. Studies indicate that selenium nanoparticles (SeNPs), quercetin-functionalized AuNPs, and curcumin-loaded NPs significantly enhance antioxidant enzyme activity in zebrafish tissues, mitigating ROS-related toxicity [103–105]. Recent findings highlight the potential of nanoencapsulated polyphenols in promoting cellular defense mechanisms against oxidative stress [106]. AuNPs associated with curcumin have been shown to exert neuroprotective effects in zebrafish models of oxidative stress, reducing neuronal apoptosis by 40% and significantly upregulating antioxidant enzymes (SOD, GPx, and CAT) in brain tissues [107]. Another study demonstrated that curcumin-loaded polymeric nanoparticles increased zebrafish survival rates in oxidative stress models by 50% compared to free curcumin administration [108]. Additionally, lipid-based antioxidant NPs loaded with resveratrol demonstrated enhanced bioavailability and anti-inflammatory effects in zebrafish models of cardiovascular disease, reducing oxidative stress markers and preventing endothelial dysfunction [109,110].

5.1. Nanoparticles as Antioxidant Therapeutics in Pharmaceutical Sciences

Nanoparticles infused with antioxidants show significant potential in the treatment of oxidative stress-related diseases, including neurodegenerative disorders, cardiovascular conditions, and cancer. By acting as ROS scavengers, these NPs can mitigate oxidative damage, prevent inflammatory cascades, and improve cellular resilience. Numerous studies indicate that NP-based antioxidant systems efficiently neutralize ROS, thereby protecting cells from oxidative damage [8]. For example, cerium oxide (CeO_2) NPs exhibit intrinsic catalytic activity that mimics SOD and CAT, making them highly effective in oxidative stress models [111]. A study on zebrafish oxidative stress models found that CeO_2 NPs reduced lipid peroxidation by 45% and significantly improved neuronal survival rates. The catalytic nature of CeO_2 NPs helped restore normal antioxidant enzyme function, effectively reducing neurotoxicity in zebrafish larvae exposed to oxidative stress-inducing compounds [112].

Other research has demonstrated that selenium nanoparticles (SeNPs) and AuNPs functionalized with flavonoids provide neuroprotection in zebrafish models of Parkinson's disease and Alzheimer's disease by preventing oxidative damage and modulating inflammatory pathways [113,114].

5.2. Natural Antioxidants Used in NP Formulations

The incorporation of natural antioxidants into NP formulations enhances their therapeutic potential while reducing toxicity. These information are detailed in Table 4.

Table 4. Natural Antioxidants in Nanoparticle Formulations for Oxidative Stress Reduction.

Type of Antioxidant-Functionalized NP	Mechanism of Action	Effect in Zebrafish Models	Reference
Polyphenol-coated NPs (quercetin, catechins)	Enhance oxidative stress resistance by scavenging ROS	Reduce ROS levels and increase antioxidant enzyme activity in zebrafish tissues	[57,115,116]
Resveratrol-loaded liposomes	Improve bioavailability and stability of antioxidant compounds	Prevent lipid peroxidation and DNA damage in oxidative stress-exposed zebrafish embryos	[110,117]
Plant-derived antioxidant NPs	Provide biocompatible approaches for reducing oxidative toxicity	Decrease apoptosis and inflammatory response in zebrafish liver and brain tissues	[118,119]
Liposomal curcumin NPs	Target oxidative damage by decreasing ROS accumulation and restoring mitochondrial function	Reduce oxidative damage in zebrafish liver tissue and improve metabolic activity	[108,109]

5.3. Drug Delivery and Biocompatibility Testing

Advanced imaging techniques, such as fluorescence imaging, confocal microscopy, and bioluminescence tracking, allow for real-time visualization of NP distribution in zebrafish models. Studies using quantum dots (QDs) and radiolabeled NPs have demonstrated their ability to monitor NP uptake, accumulation, and clearance in different organs, offering valuable pharmacokinetic insights [120].

Zebrafish models have been extensively used to analyze NP absorption, circulation, metabolism, and excretion. Studies on AuNPs and polymeric nanocarriers have shown their prolonged circulation times and biodistribution in zebrafish embryos, mimicking pharmacokinetic profiles observed in mammalian models [121]. Research on lipid-based NPs has also indicated improved bioavailability, making them suitable carriers for hydrophobic drugs [122,123].

Polylactic-co-glycolic acid (PLGA) NPs loaded with antioxidants have exhibited controlled drug release in zebrafish models, demonstrating sustained therapeutic effects, enhanced bioavailability, and reduced systemic toxicity. A study on curcumin-loaded PLGA NPs reported prolonged circulation and targeted accumulation in zebrafish liver, leading to a 50% reduction in ROS levels compared to free curcumin administration. Furthermore, chitosan-coated PLGA nanoparticles encapsulating vitamin E exhibited sustained release and antioxidant protection, significantly improving zebrafish survival rates under oxidative stress conditions and restoring mitochondrial integrity [108,124,125].

5.4. Balancing Toxicity versus Therapeutic Potential

Zebrafish-based oxidative stress models allow researchers to assess how different nanoparticle compositions and dosages affect biological systems and provide insights into their long-term safety profiles.

Studies have shown that determining the safe concentration range for therapeutic nanoparticles (NPs) is crucial to minimize off-target effects. Research involving AuNPs and polymeric nanocarriers in zebrafish models has demonstrated that precise dosing can reduce cytotoxicity while enhancing drug delivery efficiency. For instance, a study on the toxicity of various nanomedicine materials, including gold and iron oxide nanoparticles, evaluated their effects in zebrafish embryos, highlighting the importance of assessing safe concentration ranges to minimize adverse effects [54].

Moreover, during our own experience with silver NPs we observed a striking contrast in the biological effects of plant extracts when administered alone versus when incorporated into silver nanoparticle formulations. When used independently, the extracts demonstrated significant antioxidant and neuroprotective effects without any detectable toxicity. However, upon integration within silver NPs, at the same dose and under identical experimental conditions, we noticed an unexpected increase in toxicity. These findings highlight the complexity of NPs-mediated substance delivery, where interactions between the nanocarrier and the bioactive compound can alter pharmacodynamics and toxicity. Based on such discrepancies, comprehensive pharmacokinetics studies are essential in order to elucidate how NPs formulations can influence absorption, distribution and metabolism of therapeutic compounds. Understanding these mechanisms is imperative in order to properly balance the therapeutic potential of NPs formulations while mitigating possible unexpected and unintended toxicological effects.

Other studies on chronic NP exposure in zebrafish revealed potential risks associated with prolonged usage. AgNPs and TiO_2 NPs have been found to accumulate in the liver and the brain of zebrafish, leading to oxidative damage and inflammatory responses over extended periods [24,126]. Research on lipid-based NPs suggests that their biodegradable nature may reduce long-term toxicity risks, making them promising candidates for pharmaceutical applications [127].

Furthermore, a study on iron oxide (Fe_3O_4) NPs demonstrated that chronic exposure in zebrafish induced significant oxidative stress and neurotoxicity, highlighting the need for careful evaluation of NP formulations before their use in clinical settings [128].

6. Conclusions

The findings of this review highlight the significant role of oxidative stress in NP-induced toxicity, particularly in zebrafish models. Studies have demonstrated that various nanoparticles, including metal-based (AgNPs, TiO_2 NPs, Fe_2O_3 NPs), polymeric, lipid-based, and quantum dots, can induce oxidative stress through ROS overproduction, mitochondrial dysfunction, inflammation, and genotoxicity. Zebrafish have emerged as a versatile and reliable model organism for evaluating NP-induced oxidative stress, offering advantages such as genetic similarity to humans, rapid development, and transparent embryos that allow real-time assessment of oxidative damage and pharmacological responses.

From a pharmaceutical perspective, NPs functionalized with antioxidants, such as curcumin, resveratrol, quercetin, and selenium nanoparticles, have shown promising therapeutic applications in neuroprotection, hepatoprotection, and cardiovascular disease management. Zebrafish models have been instrumental in screening NP formulations, assessing biodistribution, and optimizing drug delivery systems to balance efficacy with safety. Additionally, zebrafish-based toxicity studies have provided valuable insights into the long-term risks associated with NP accumulation, guiding the development of safer nanopharmaceuticals. Despite these advances, standardized methodologies for oxidative stress assessment in zebrafish models remain a critical need. Future research should focus on long-term NP exposure studies, multi-generational toxicity assessments, and the integration of omics technologies (transcriptomics, proteomics, and metabolomics) to deepen the understanding of NP interactions with biological systems.

Furthermore, advancing the biodegradability and biocompatibility of NP formulations will be essential in mitigating potential toxic effects while maximizing therapeutic benefits. In conclusion, zebrafish models provide an effective and high-throughput platform for evaluating both the toxicity and therapeutic potential of nanoparticles. Understanding NP-induced oxidative stress mechanisms will not only facilitate the design of safer nanomaterials but also accelerate the development of novel antioxidant-based nanomedicines for various biomedical applications.

Future studies should consider **expanding multi-generational studies** (long-term exposure studies on zebrafish should be conducted to assess how NP-induced oxidative stress affects successive generations, particularly in the context of epigenetic modifications and inherited toxicity), **personalized nanomedicine** (develop patient-specific nanotherapeutics, particularly for

neurodegenerative diseases, cancer, and metabolic disorders), **green nanotechnology approaches** (develop environmentally friendly and biocompatible NPs using sustainable materials to reduce toxicity risks while maintaining therapeutic efficacy)

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Abbreviations

The following abbreviations are used in this manuscript:

NPs	Nanoparticles
ROS	Reactive oxygen species
CAT	Catalase
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
DNA	Deoxyribonucleic acid
MDA	Malondialdehyde
DCFDA	2',7'-dichlorofluorescin diacetate
RNA	Ribonucleic acid
ATP	Adenosine triphosphate
IL	Interleukin
TNF-α	Tumor necrosis factor alpha
PCR	Polymerase chain reaction

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