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The Effect of Silver Nanoparticles on Oxidative Stress Enzymes in Drosophila Melanogaster

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Abstract **Review Article**

The study focuses on the effects of silver nanoparticles (AgNPs) on oxidative stress enzymes in *Drosophila melanogaster*, a widely used model organism for toxicological research. AgNPs, known for their unique antimicrobial properties, pose potential risks due to their ability to generate reactive oxygen species (ROS), leading to oxidative stress. The document emphasizes how exposure to AgNPs disrupts the antioxidant defence system by altering the activity of key enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes play a crucial role in maintaining redox homeostasis by neutralizing ROS, but prolonged exposure to AgNPs depletes their activity, resulting in cellular damage, mitochondrial dysfunction, and DNA fragmentation. Additionally, the study highlights the involvement of the Nrf2-Keap1 pathway, a critical regulator of oxidative stress responses, which is dysregulated upon AgNP exposure. The consequences of AgNP-induced oxidative stress are far-reaching, affecting cellular integrity, inducing apoptosis, and impairing developmental processes. The study also discusses the potential environmental and health implications of AgNP exposure, suggesting that AgNPs can disrupt ecosystems and cause harm to non-target organisms. In response, future research aims to explore safer formulations of AgNPs with enhanced biocompatibility and reduced toxicity. This research is vital for assessing the safety of AgNPs, particularly in biomedical, industrial, and environmental applications, and for developing strategies to minimize their toxicological effects.

Keywords: Silver nanoparticles, oxidative stress, *Drosophila melanogaster*, reactive oxygen species, antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, Nrf2-Keap1 pathway, mitochondrial dysfunction, DNA damage, nanotoxicology, nanoparticle toxicity, environmental impact, biocompatibility.

INTRODUCTION

Nanotechnology has become an integral part of modern scientific advancements, particularly in the fields of biomedical and environmental research. Among the various nanoparticles (NPs), silver nanoparticles (AgNPs) have gained prominence due to their unique physicochemical properties, including antimicrobial activity, catalytic efficiency, and surface plasmon resonance (Mali *et al.*, 2025). However, the increasing application of AgNPs has raised significant concerns regarding their potential toxicity, particularly their ability to induce oxidative stress in biological organisms. This has necessitated extensive research into their impact on oxidative stress enzymes, particularly in model organisms such as *Drosophila melanogaster* (Demir *et al.*, 2022).

Drosophila melanogaster has been widely recognized as an effective model organism for evaluating the toxicological effects of nanoparticles due to its genetic similarity to humans, short life cycle, and well-mapped genome (Demir & Turna, 2022). Approximately 75% of human disease-related genes have homologs in *D. melanogaster*, making it a valuable model for studying cytotoxicity, genotoxicity, and oxidative stress responses (Mishra & Panda, 2021). Additionally, its short life cycle, high reproductive rate, and ease of genetic manipulation allow researchers to conduct rapid and cost-effective toxicity assessments (Rehman & Khan, 2023).

Several studies have demonstrated the toxic effects of AgNPs in *Drosophila melanogaster*, particularly regarding the modulation of oxidative stress enzymes. Exposure to AgNPs has been linked to an increased production of reactive oxygen species (ROS), resulting in oxidative damage in key tissues, including the gut, nervous system, and reproductive organs (Mali *et al.*, 2025). The fruit fly's antioxidant defense system, which includes enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), plays a crucial role in mitigating AgNP-induced toxicity (Demir *et al.*, 2022). However, high levels of ROS generated by AgNPs often overwhelm these defense mechanisms, resulting in oxidative stress, apoptosis, and tissue damage (Demir & Turna, 2022).

Oxidative stress occurs when an imbalance arises between ROS production and the cellular antioxidant defense system (Rehman & Khan, 2023). ROS, which include superoxide anions (O_2^-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2), are highly reactive molecules that can damage cellular components such as lipids, proteins, and DNA (Mishra & Panda, 2021). The exposure of *Drosophila melanogaster* to AgNPs has been reported to induce significant oxidative stress, leading to lipid peroxidation, protein carbonylation, and genotoxicity (Demir *et al.*, 2022).

Several mechanisms have been proposed to explain AgNP-induced oxidative stress:

- **ROS Generation and Cellular Damage** – AgNPs interact with cellular components, triggering excessive ROS production and oxidative damage (Mali *et al.*, 2025).
- **Mitochondrial Dysfunction** – AgNPs disrupt mitochondrial function by interfering with the electron transport chain, reducing ATP production, and triggering apoptosis (Mishra & Panda, 2021).
- **Enzyme Inhibition and Antioxidant System Disruption** – Exposure to AgNPs leads to dysregulation of key antioxidant enzymes, impairing the cell's ability to neutralize oxidative stress (Demir *et al.*, 2022).
- **DNA Damage and Genotoxicity** – AgNPs have been shown to induce DNA fragmentation, chromosomal aberrations, and impaired cell cycle regulation in *Drosophila* cells (Rehman & Khan, 2023).
- The primary defense against AgNP-induced oxidative stress in *Drosophila melanogaster* consists of antioxidant enzymes, including:
- **Superoxide Dismutase (SOD)**: Converts superoxide anions into less toxic hydrogen peroxide, preventing ROS accumulation (Demir & Turna, 2022).
- **Catalase (CAT)**: Breaks down hydrogen peroxide into water and oxygen, reducing oxidative damage (Mali *et al.*, 2025).
- **Glutathione Peroxidase (GPx)**: Detoxifies hydrogen peroxide and lipid peroxides, maintaining cellular redox balance (Mishra & Panda, 2021).

Studies have shown that AgNP exposure leads to altered expression and activity of these enzymes, either as an adaptive response or due to enzyme inhibition caused by excessive oxidative stress (Rehman & Khan, 2023). Increased SOD activity is often observed initially as a compensatory mechanism, but prolonged exposure leads to enzyme depletion and oxidative damage (Demir *et al.*, 2022). Similarly, CAT and GPx levels fluctuate in response to AgNP dose and exposure duration, further highlighting the complexity of nanoparticle-induced oxidative stress responses (Mali *et al.*, 2025).

The toxic effects of AgNPs extend beyond laboratory models, raising concerns about their environmental and biomedical implications (Rehman & Khan, 2023). Due to their widespread use, AgNPs are released into various ecosystems, where they pose potential risks to non-target organisms, including insects, aquatic species, and plants (Mishra & Panda, 2021). Bioaccumulation of AgNPs can disrupt biological pathways, leading to ecosystem imbalances and biodiversity loss (Demir *et al.*, 2022).

In the biomedical field, understanding AgNP-induced oxidative stress is crucial for developing safer nanoparticle formulations (Mali *et al.*, 2025). Strategies such as surface modifications, antioxidant coatings, and controlled-release formulations are being explored to mitigate the adverse effects of AgNPs while preserving their beneficial properties (Rehman & Khan, 2023).

The study of AgNP toxicity in *Drosophila melanogaster* provides valuable insights into the molecular mechanisms underlying nanoparticle-induced oxidative stress and enzymatic dysregulation. By investigating the interactions between AgNPs and oxidative stress enzymes, researchers can more accurately assess their safety profile and develop strategies to mitigate their harmful effects in both environmental and biomedical contexts (Mali *et al.*, 2025). Future research should focus on identifying safe exposure limits, optimizing nanoparticle formulations, and investigating long-term effects on biological systems (Demir & Turna, 2022). Advanced studies should aim to explore alternative nanoparticle formulations with reduced toxicity while maintaining their functional properties (Mishra & Panda, 2021).

Silver Nanoparticles: Synthesis, Properties, and Applications

Silver nanoparticles (AgNPs) are widely recognized for their unique physicochemical properties and broad spectrum of applications, spanning biomedical, environmental, and industrial sectors (Meher *et al.*, 2024). Due to their small size and large

surface-to-volume ratio, AgNPs exhibit enhanced catalytic, electrical, and antimicrobial properties, making them essential components in modern nanotechnology (Dhayalan *et al.,* 2021). The synthesis of AgNPs has undergone significant evolution, with researchers exploring diverse physical, chemical, and biological methods to optimize their stability, functionality, and environmental impact (Iravani *et al.,* 2014). Recent advancements emphasize green synthesis approaches, which utilize plant extracts, microorganisms, and biomolecules to enhance sustainability and biocompatibility (El-Batal *et al.,* 2014).

The synthesis of AgNPs can be categorized into three primary methods: physical, chemical, and biological. Physical synthesis techniques, such as laser ablation, thermal decomposition, and mechanical milling, provide precise control over nanoparticle size and shape; however, they often require high energy inputs (Tsuji *et al.,* 2003; Khayati & Janghorban, 2012). Chemical synthesis, the most widely used approach, involves the reduction of silver salts with agents like sodium borohydride, citrate, or hydrazine, yielding well-defined nanoparticles (Jamkhande *et al.,* 2019). However, concerns over chemical residues and toxicity have spurred the adoption of biological synthesis, which leverages plant-derived polyphenols, bacterial enzymes, and fungal metabolites for eco-friendly AgNP production (Philip, 2010; Sathishkumar *et al.,* 2012).

The physicochemical properties of AgNPs play a crucial role in their effectiveness across various applications. Factors such as size, shape, surface charge, and functionalization influence their reactivity and biological interactions (Xu *et al.,* 2020). Spherical AgNPs are the most common, but nanoparticles with triangular, cubic, and rod-shaped morphologies exhibit distinct optical and antimicrobial characteristics (Piras *et al.,* 2019). Surface modifications with biocompatible polymers, biomolecules, or ligands further enhance their stability and specificity for targeted applications, such as drug delivery and biosensing (Barman *et al.,* 2022). The ability of AgNPs to generate localized surface plasmon resonance (LSPR) upon exposure to light makes them highly effective in imaging and diagnostic applications (Zhang *et al.,* 2016).

AgNPs have revolutionized the biomedical field, demonstrating potent antimicrobial, antiviral, and antifungal activities. Their incorporation into wound dressings, medical coatings, and implant materials helps prevent infections and promote tissue regeneration (Babu *et al.,* 2018). In cancer therapy, AgNPs induce selective cytotoxicity in tumor cells via oxidative stress and DNA damage, making them promising candidates for nanomedicine (Gurunathan *et al.,* 2015). Additionally, their role in biosensors and imaging technologies enables the precise detection and monitoring of diseases (Tan *et al.,* 2021). However, concerns regarding nanoparticle accumulation and cytotoxicity necessitate further studies to ensure their safe and effective clinical use (Rafique *et al.,* 2017).

Figure 1.1: Application of AgNPs in Various Fields

Beyond healthcare, AgNPs find applications in environmental and industrial domains. They serve as antimicrobial agents in water purification systems, effectively removing bacteria and pollutants (Guimarães *et al.,* 2020). In agriculture, AgNP-based nanopesticides and nanofertilizers enhance crop yield while reducing chemical inputs (Raza *et al.,* 2016). Their integration into food packaging materials extends shelf life by preventing microbial contamination (Barman *et al.,* 2022). Additionally, AgNPs contribute to catalysis, electronics, and renewable energy sectors due to their superior conductivity and catalytic activity (Rak *et al.,* 2016). Silver nanoparticles are also used in other fields, such as the food industry, textiles, and medicine, as demonstrated in Figure 1.1:

Mechanisms of Nanoparticle Interaction with Biological Systems

Understanding the mechanisms of nanoparticle interaction with biological systems is crucial for optimizing their biomedical applications while mitigating potential toxicity risks. Silver nanoparticles

(AgNPs), due to their unique physicochemical properties, interact with cells through multiple pathways, affecting cellular uptake, bioaccumulation, and molecular responses (Haddad *et al.*, 2023). These interactions are governed by nanoparticle size, shape, surface chemistry, and mechanical cues within the cellular microenvironment (Elblová *et al.*, 2024). Recent studies have highlighted that nanoparticle-cell interactions are highly dynamic, influencing cellular metabolism, oxidative stress, and gene regulation (Majood *et al.*, 2023).

The uptake of AgNPs into cells primarily occurs through endocytosis, which encompasses phagocytosis, pinocytosis, and receptor-mediated pathways. Phagocytosis is predominant in immune cells such as macrophages, whereas clathrin- and caveolin-mediated endocytosis governs uptake in non-phagocytic cells (Wilhelm *et al.*, 2023). The efficiency of endocytosis depends on nanoparticle characteristics; smaller particles (<50 nm) are more likely to be internalized via receptor-mediated endocytosis, while larger aggregates enter through macropinocytosis (Frickenstein *et al.*, 2023). Once internalized, AgNPs localize in endosomes and lysosomes, where they undergo degradation or release ionic silver, contributing to cellular responses (Haddad *et al.*, 2023).

Table: Mechanisms of Nanoparticle Interaction with Biological Systems

Mechanism	Description	Key Effects	References
Cellular Uptake	Internalization through endocytosis pathways, including phagocytosis, pinocytosis, clathrin- and caveolin-mediated uptake	Uptake efficiency depends on size (<50 nm = receptor-mediated; larger = macropinocytosis)	Haddad *et al.*, 2023; Wilhelm *et al.*, 2023; Frickenstein *et al.*, 2023
Intracellular Localization	AgNPs accumulate in the cytoplasm, mitochondria, and nucleus	Mitochondrial dysfunction, ATP depletion, ROS overproduction, DNA damage	Elblová *et al.*, 2024; Lunova *et al.*, 2024; Dejneka *et al.*, 2024
Oxidative Stress and ROS Generation	ROS generated through Fenton-like reactions; antioxidant enzyme depletion	Lipid/protein/DNA damage, apoptosis, ER stress, inflammation	Jirsa *et al.*, 2024; Lunov *et al.*, 2024; Haddad *et al.*, 2023
Membrane Interaction & Mechanical Stress	AgNPs disrupt plasma membrane structure and function	Alters fluidity, permeability; induces immune response and inflammation	Elblová *et al.*, 2024; Majood *et al.*, 2023
Influence of Mechanical Cues	Substrate stiffness and shear stress modulate uptake	Increased uptake in stiffer environments or under flow conditions	Lunova *et al.*, 2024; Wilhelm *et al.*, 2023
Signaling Pathway Disruption	Alteration of NF-κB, MAPK, PI3K/Akt, p53, and others	Affects inflammation, apoptosis, gene expression, and cell survival	Jirsa *et al.*, 2024; Dejneka *et al.*, 2024
Epigenetic Modifications	DNA methylation and histone acetylation changes	Long-term changes in gene expression, differentiation, and stress responses	Haddad *et al.*, 2023; Jirsa *et al.*, 2024
Systemic Toxicity	Accumulation in liver, kidneys, spleen, brain; interaction with microbiota	Neurotoxicity, cognitive impairment, dysbiosis, organ damage	Wilhelm *et al.*, 2023; Frickenstein *et al.*, 2023; Elblová *et al.*, 2024
Design Strategies for Safer Application	Surface functionalization, targeted delivery, charge modulation	Enhances biocompatibility, reduces toxicity, and improves therapeutic targeting	Majood *et al.*, 2023; Haddad *et al.*, 2023
Role of Mechano-transduction	Influence of mechanical signals on nanoparticle processing	New insight into uptake behavior in various tissue environments	Lunova *et al.*, 2024

After cellular uptake, AgNPs distribute throughout the cytoplasm and organelles, potentially leading to bioaccumulation. Studies suggest that nanoparticles tend to accumulate in the mitochondria and nucleus, disrupting critical cellular processes (Elblová *et al.*, 2024). Mitochondrial accumulation leads to inhibition of the electron transport chain (ETC), ATP depletion, and overproduction of reactive oxygen species (ROS), thereby contributing to oxidative stress and apoptosis (Lunova *et al.*, 2024). The nuclear localization of AgNPs may lead to DNA fragmentation and chromosomal aberrations, raising concerns about genotoxicity and long-term cellular damage (Dejneka *et al.*, 2024). This can lead to further epigenetic modifications that alter gene expression profiles, thereby affecting cellular differentiation and stress responses (Jirsa *et al.*, 2024).

AgNP-induced toxicity is primarily mediated through oxidative stress and ROS generation. The

excessive accumulation of ROS disrupts redox homeostasis, leading to lipid peroxidation, protein oxidation, and DNA damage (Jirsa *et al.,* 2024). AgNPs catalyze Fenton-like reactions, generating hydroxyl radicals that further exacerbate cellular injury (Lunov *et al.,* 2024). The depletion of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) further amplifies oxidative stress, making cells more vulnerable to AgNP exposure (Haddad *et al.,* 2023). Furthermore, ROS-induced damage can disrupt the function of the endoplasmic reticulum (ER), leading to ER stress and activation of the unfolded protein response (UPR), which may contribute to cytotoxicity and inflammation (Majood *et al.,* 2023).

The interactions of AgNPs with biological membranes can also lead to mechanical stress and membrane disruption. Studies have shown that nanoparticle adhesion to the plasma membrane alters membrane fluidity, permeability, and receptor function (Elblová *et al.,* 2024). This can trigger inflammation and immune responses, as observed in macrophages and epithelial cells exposed to AgNPs (Majood *et al.,* 2023). Furthermore, mechanical cues such as substrate stiffness and shear stress influence nanoparticle internalization, with stiffer environments promoting higher uptake rates (Lunova *et al.,* 2024). In vascular endothelial cells, increased nanoparticle uptake under flow conditions suggests that mechanical forces play a significant role in regulating nanoparticle behavior in circulation (Wilhelm *et al.,* 2023).

AgNPs also interfere with cellular signaling pathways, affecting gene expression and metabolic functions. Exposure to AgNPs alters key signaling pathways, including NF-κB, MAPK, and PI3K/Akt, which regulate inflammation, apoptosis, and cell survival (Jirsa *et al.,* 2024). Additionally, p53-dependent DNA damage responses are activated in cells exposed to AgNPs, leading to cell cycle arrest and potential senescence (Dejneka *et al.,* 2024). Epigenetic modifications, such as DNA methylation and histone acetylation, have been reported in cells exposed to AgNPs, indicating long-term regulatory effects on gene expression (Haddad *et al.,* 2023).

The systemic effects of AgNPs extend beyond individual cells, impacting tissue homeostasis and organ function. In vivo studies suggest that AgNPs accumulate in the liver, kidneys, and spleen, potentially causing histopathological changes and systemic toxicity (Wilhelm *et al.,* 2023). The blood-brain barrier (BBB) poses a significant challenge for nanoparticle-based therapies. However, studies indicate that AgNPs may cross the BBB, raising concerns about neurotoxicity and cognitive impairments (Frickenstein *et al.,* 2023). Furthermore, nanoparticle interactions with gut microbiota can alter microbial composition, potentially leading to dysbiosis and metabolic disorders (Elblová *et al.,* 2024).

Future research should focus on refining nanoparticle design to enhance biocompatibility while minimizing adverse effects. Surface functionalization with biocompatible coatings, controlled-release formulations, and targeted delivery mechanisms can enhance the efficacy of AgNPs while mitigating toxicity risks (Majood *et al.,* 2023). Nanoparticle surface charge and ligand functionalization have been demonstrated to modulate uptake efficiency, underscoring the significance of nanoparticle engineering in therapeutic applications (Haddad *et al.,* 2023). Understanding the complex interplay between AgNPs and biological systems is essential for advancing their applications in nanomedicine, diagnostics, and environmental safety (Elblová *et al.,* 2024). Additionally, investigating the role of mechano-transduction in nanoparticle-cell interactions may provide novel insights into how cells process nanomaterials in various tissue environments, thereby further optimizing their biomedical applications (Lunova *et al.,* 2024). Figure 1.2 illustrates the mechanism of nanoparticle interaction with Biological Systems.

Oxidative Stress: A Key Mediator of Nanoparticle Toxicity

Oxidative stress plays a crucial role in nanoparticle (NP)-induced cytotoxicity, particularly in the case of silver nanoparticles (AgNPs). The generation of reactive oxygen species (ROS) leads to significant cellular damage, affecting lipids, proteins, and DNA (Portugal *et al.,* 2024). Studies have shown that mitochondrial dysfunction and oxidative stress are key drivers of NP toxicity, leading to cell apoptosis, chronic inflammation, and systemic toxicity (Horie & Tabei, 2021). The excessive production of reactive oxygen species (ROS) disrupts cellular homeostasis and has been implicated in various diseases, including cancer, neurodegenerative disorders, and cardiovascular dysfunction (Daiber *et al.,* 2020). Understanding the mechanisms of oxidative stress in NP toxicity is crucial for developing safer nanomaterials and minimizing their toxicological risks.

The primary source of ROS in NP-exposed cells is mitochondrial disruption. AgNPs interfere with the electron transport chain, causing an increase in superoxide anion (O_2•−) production (Balkrishna *et al.,* 2021). Additionally, the release of silver ions (Ag^+) catalyzes Fenton-like reactions, generating hydroxyl radicals (OH•), which intensify oxidative stress and DNA fragmentation (Apopa *et al.,* 2009). Mitochondrial impairment due to NP exposure triggers a cascade of cellular events, including ATP depletion, loss of membrane potential, and cytochrome C release, ultimately leading to apoptosis (Hill *et al.,* 2023). Furthermore, endoplasmic reticulum (ER) stress caused by NPs exposure activates the unfolded protein response (UPR), exacerbating ROS production and cellular apoptosis (Latvala *et al.,* 2016).

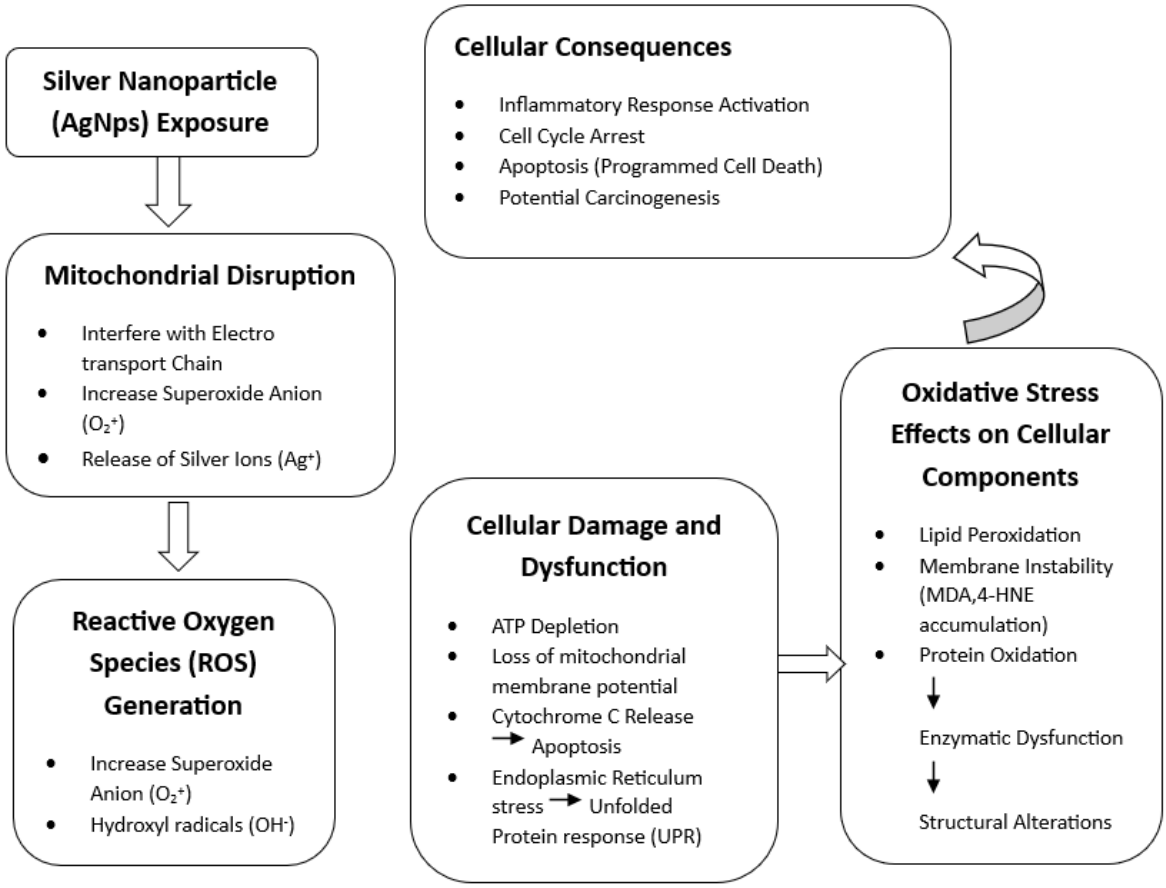

Figure 1.2: Mechanisms of Nanoparticle Interaction with Biological Systems

ROS-mediated damage triggers lipid peroxidation, leading to cellular membrane instability (Holme *et al.*, 2019). The accumulation of lipid peroxidation by-products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) further amplifies oxidative damage, affecting essential cellular functions (Kelly & Fussell, 2020). These by-products interact with proteins and DNA, forming adducts that interfere with normal cellular processes (Vallabani *et al.*, 2023). Additionally, protein oxidation disrupts enzymatic activity and signalling pathways, contributing to increased cytotoxicity and inflammatory responses (Oelwein *et al.*, 2019). Structural alterations in proteins resulting from oxidative modifications can impact receptor function, cell adhesion, and metabolic pathways, thereby exacerbating cellular stress (Szabó *et al.*, 2007). Oxidative DNA damage, characterized by elevated 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels, leads to genomic instability, increased mutagenesis, and carcinogenesis (Fahmy & Cormier, 2009). DNA strand breaks and base modifications caused by ROS lead to impaired DNA repair mechanisms, enhancing the risk of cell cycle arrest and apoptosis (Johnston *et al.*, 2010).

Table: Oxidative Stress – A Central Mechanism of Nanoparticle-Induced Toxicity

Aspect	Description	Key Molecules/Mechanisms Involved	References
Primary ROS Generation	Disruption of the mitochondrial electron transport chain (ETC) by AgNPs leads to ROS overproduction.	Superoxide anion ($O_2^{\bullet -}$), Hydroxyl radical (•OH), Ag^+-induced Fenton-like reactions	Balkrishna *et al.*, 2021; Apopa *et al.*, 2009
Mitochondrial Dysfunction	NP exposure causes ATP depletion, cytochrome C release, and loss of membrane potential.	Cytochrome C, ATP, Mitochondria	Hill *et al.*, 2023
Endoplasmic Reticulum (ER) Stress	ER stress activates unfolded protein response (UPR) and ROS production	UPR, ER, ROS	Latvala *et al.*, 2016

Aspect	Description	Key Molecules/Mechanisms Involved	References
Lipid Peroxidation	ROS attack lipids, causing membrane instability and cell dysfunction	MDA, 4-HNE	Holme *et al.*, 2019; Kelly & Fussell, 2020
Protein Oxidation	Alters enzymatic activity, disrupts signaling, and cell adhesion	Protein carbonyls, Oxidized enzymes	Ohlwein *et al.*, 2019; Szabó *et al.*, 2007
DNA Damage	8-OHdG formation leads to mutagenesis, genomic instability, and apoptosis	8-OHdG, DNA strand breaks	Fahmy & Cormier, 2009; Johnston *et al.*, 2010
Antioxidant Defense Impairment	NP exposure suppresses SOD, CAT, GPx, depletes GSH	SOD, CAT, GPx, GSH, Nrf2	Birben *et al.*, 2012; Itoh *et al.*, 1997; Itoh *et al.*, 2004
Inflammatory Response	ROS triggers cytokine release and activates inflammatory signaling	IL-6, TNF-α, NF-κB, MAPK	Huang *et al.*, 2019; Wills, 1971
Systemic Effects	Contributes to neurodegeneration, cardiovascular diseases, and cancer	Neurons, Endothelium, Atherosclerosis	Cho *et al.*, 2018; Daiber *et al.*, 2020
Mitigation Strategies	Antioxidant-functionalized NPs, green synthesis, surface modification	PEGylation, Polyphenols, Flavonoids	Hill *et al.*, 2023; Horie & Tabei, 2021
Future Directions	Safer nanomaterial design, regulatory frameworks, and interdisciplinary research	Long-term toxicity studies, Nano-biocompatibility	Portugal *et al.*, 2024; Ohlwein *et al.*, 2019

Cells rely on antioxidant defense mechanisms, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), to mitigate oxidative stress (Birben *et al.*, 2012). However, AgNP exposure suppresses these antioxidant enzymes and depletes glutathione (GSH), intensifying oxidative damage (Itoh *et al.*, 1997). The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which is crucial for regulating antioxidant responses, is also impaired by NP exposure, further diminishing the cell's ability to counteract reactive oxygen species (ROS) (Itoh *et al.*, 2004). The suppression of Nrf2 results in a reduction of phase II detoxifying enzymes, rendering cells more susceptible to oxidative stress (Saud Alarifi *et al.*, 2013). Moreover, AgNPs have been found to alter redox signaling by disrupting the balance between pro-oxidant and antioxidant responses, leading to a prolonged state of oxidative damage (Mishra & Panda, 2021).

Systemic effects of oxidative stress extend beyond cellular toxicity. NP-induced ROS production has been linked to the release of inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which contribute to chronic inflammation and tissue damage (Huang *et al.*, 2019). ROS-induced inflammation activates various intracellular pathways, including nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), which further enhance the expression of pro-inflammatory genes (Wills, 1971). Persistent oxidative stress is associated with the development of neurodegenerative diseases, cardiovascular disorders, and metabolic dysfunctions (Cho *et al.*, 2018). In neurodegenerative conditions, such as Alzheimer's and Parkinson's disease, excessive ROS disrupt neuronal function, leading to synaptic loss and neuronal apoptosis (Szabó *et al.*, 2007). Additionally, NP exposure has been shown to compromise endothelial function, leading to changes in vascular permeability and an increased risk of thrombosis (Portugal *et al.*, 2024). Oxidative stress-induced vascular dysfunction can accelerate the progression of atherosclerosis, hypertension, and other cardiovascular diseases (Daiber *et al.*, 2020).

To mitigate NP-induced oxidative stress, researchers are focusing on engineering safer nanomaterials. Functionalization of nanoparticles with antioxidants, biocompatible coatings, or biomolecules has shown promise in reducing ROS production and enhancing NP stability (Hill *et al.*, 2023). Various strategies, including surface modification with polyethylene glycol (PEG) and functionalization with biomolecules such as polyphenols, have been explored to improve NP biocompatibility (Horie & Tabei, 2021). Green synthesis approaches using phytochemical-functionalized nanoparticles have demonstrated improved biocompatibility and reduced oxidative damage (Balkrishna *et al.*, 2021). Plant-derived antioxidants, including flavonoids and polyphenols, have been found to neutralize ROS and enhance cellular antioxidant defense mechanisms (Kelly & Fussell, 2020). Additionally, optimizing nanoparticle properties such as size, surface charge, and shape can minimize oxidative stress while improving therapeutic efficacy (Vallabani *et al.*, 2023). Reducing NP aggregation and enhancing targeted delivery mechanisms can also contribute to lower cytotoxicity and improved biocompatibility (Holme *et al.*, 2019).

Understanding oxidative stress mechanisms in NP toxicity is essential for developing safer nanomaterials and minimizing their toxicological risks.

Future research should focus on designing novel antioxidant-functionalized nanoparticles and refining NP properties to ensure safe applications in nanomedicine and industrial use (Portugal *et al.*, 2024). Investigating the long-term effects of NP exposure on oxidative stress and its implications for human health is crucial for establishing regulatory guidelines (Ohlwein *et al.*, 2019). Moreover, interdisciplinary approaches integrating nanotechnology, toxicology, and biomedical research will play a key role in advancing the safe design and application of nanoparticles (Hill *et al.*, 2023). By developing innovative strategies to mitigate oxidative stress, researchers can enhance the therapeutic potential of nanoparticles while reducing their adverse effects on biological systems (Daiber *et al.*, 2020).

Effects of Silver Nanoparticles on Antioxidant Enzyme Activity in Drosophila melanogaster

Silver nanoparticles (AgNPs) have gained significant attention due to their widespread applications in medical, industrial, and consumer products. However, their increasing use has raised concerns about their potential toxicity, particularly their impact on biological systems. *Drosophila melanogaster*, a widely used model organism in toxicological studies, has been instrumental in understanding the biochemical and genetic implications of AgNP exposure. The toxic effects of AgNPs on Drosophila have been linked to oxidative stress, cellular damage, and disruptions in antioxidant enzyme activity, leading to significant physiological and developmental impairments (Mishra & Panda, 2021).

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play essential roles in mitigating oxidative damage caused by environmental stressors, including nanoparticle exposure. These enzymes work in coordination to neutralise reactive oxygen species (ROS), preventing oxidative damage to cellular macromolecules. However, studies have shown that AgNP exposure significantly alters the activity of these antioxidant enzymes in *Drosophila melanogaster*, leading to an imbalance in redox homeostasis and subsequent cellular toxicity (Demir & Turna Demir, 2022).

Oxidative stress is one of the primary modes of toxicity induced by AgNPs. When AgNPs enter biological systems, they generate excessive ROS, overwhelming the cellular antioxidant defense mechanisms. In *Drosophila melanogaster*, studies have shown that exposure to AgNPs results in a significant increase in oxidative stress markers, including lipid peroxidation byproducts such as malondialdehyde (MDA), protein carbonylation, and DNA fragmentation (Ellah *et al.*, 2024). These oxidative modifications result in impaired cellular function and increased susceptibility to environmental stressors. Additionally, excessive ROS production disrupts mitochondrial function, leading to ATP depletion, loss of mitochondrial membrane potential, and activation of apoptotic pathways (Martínez-Cisterna *et al.*, 2024).

The impact of AgNPs on antioxidant enzyme activity in *Drosophila melanogaster* varies depending on the dose, exposure duration, and physicochemical properties of the nanoparticles. Several studies have reported that low concentrations of AgNPs can initially induce an adaptive response, characterized by increased expression and activity of antioxidant enzymes, serving as a protective mechanism. However, prolonged or high-dose exposure overwhelms these defense systems, leading to enzyme inhibition and subsequent oxidative damage (Tortella *et al.*, 2024). AgNP-induced modifications in gene expression further exacerbate the disruption of antioxidant enzyme activity. Transcriptomic analyses have shown that genes encoding SOD, CAT, and GPx are differentially expressed in AgNP-exposed Drosophila, indicating nanoparticle-induced genetic reprogramming that affects the organism's ability to cope with oxidative stress (Demir & Turna Demir, 2022).

Another critical aspect of AgNP-induced oxidative stress is its effect on cellular signaling pathways that regulate antioxidant defense. The Nrf2-Keap1 pathway, a master regulator of oxidative stress response, plays a pivotal role in controlling the expression of antioxidant enzymes. Under normal conditions, Nrf2 is sequestered by Keap1 in the cytoplasm, preventing its activation. However, oxidative stress triggers Nrf2 release, allowing it to translocate to the nucleus and activate the transcription of antioxidant genes. Studies have shown that AgNP exposure interferes with this pathway by downregulating Nrf2 expression, thereby suppressing the cellular antioxidant response in Drosophila melanogaster (Ellah *et al.*, 2024). This suppression further exacerbates oxidative damage, as the organism becomes unable to upregulate essential defense mechanisms in response to AgNP-induced stress.

The toxic effects of AgNPs extend beyond oxidative stress and enzyme modulation, impacting broader physiological functions in Drosophila melanogaster. Developmental toxicity is a significant concern, as AgNP exposure has been linked to impaired embryonic development, delayed larval growth, and reduced adult lifespan. Studies have shown that AgNP-induced oxidative stress leads to defects in neurodevelopment, altered reproductive function, and increased incidence of morphological abnormalities in exposed Drosophila populations (Martínez-Cisterna *et al.*, 2024). These findings suggest that oxidative stress not only disrupts cellular homeostasis but also has long-term consequences on organismal health and survival.

Comparative studies investigating the effects of AgNPs in Drosophila melanogaster and other model organisms have provided valuable insights into the

conservation of oxidative stress mechanisms across species. Similar patterns of antioxidant enzyme inhibition, mitochondrial dysfunction, and apoptotic activation have been observed in mammalian models, indicating that findings from Drosophila studies can be extrapolated to higher organisms (Mishra & Panda, 2021). This highlights the relevance of Drosophila as a suitable model for evaluating nanoparticle toxicity and underscores the need for further research to develop safer nanoparticle applications.

One promising avenue for mitigating AgNP-induced oxidative stress is the application of green-synthesized nanoparticles, which have been shown to exhibit lower toxicity compared to chemically synthesized counterparts. Studies have demonstrated that AgNPs synthesized using plant extracts exhibit enhanced biocompatibility and reduced oxidative damage, likely due to the presence of bioactive compounds that modulate ROS production (Tortella *et al.,* 2024). Additionally, functionalization of AgNPs with antioxidant molecules such as polyphenols, flavonoids, and peptides has been proposed as a strategy to improve their safety profile and minimize oxidative stress-related toxicity (Ellah *et al.,* 2024).

The findings from Drosophila melanogaster studies have important implications for human health and environmental safety. Given the widespread use of AgNPs in consumer products, medical devices, and industrial applications, there is an urgent need to assess their long-term impact on biological systems. Regulatory frameworks must incorporate data from model organisms to establish safe exposure limits and guidelines for nanoparticle usage. Additionally, further research is needed to explore the potential for developing engineered nanoparticles with enhanced safety profiles and minimal environmental impact.

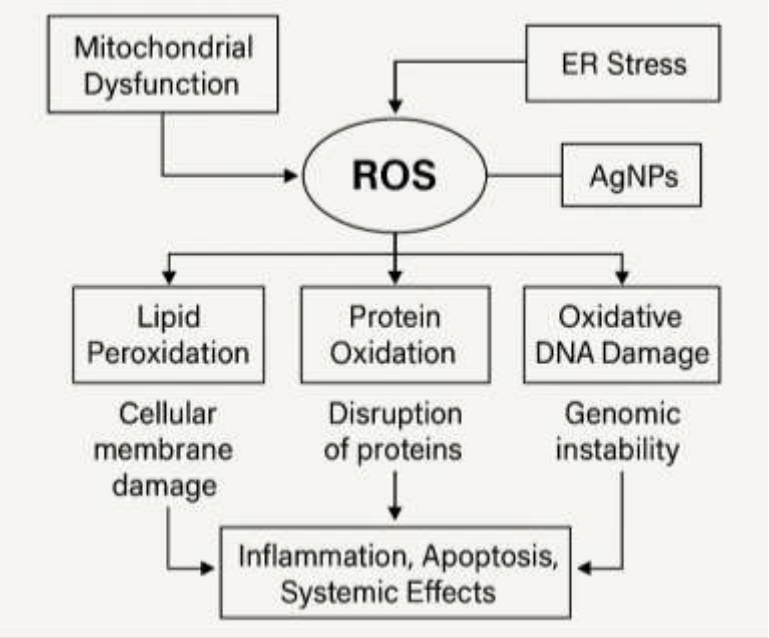

Figure: Oxidative Stress: A Central Mechanism of Silver Nanoparticle-Induced Cytotoxicity

In conclusion, AgNPs exert profound effects on antioxidant enzyme activity in Drosophila melanogaster, leading to oxidative stress, cellular dysfunction, and physiological impairments. The inhibition of SOD, CAT, and GPx activity disrupts the delicate balance of redox homeostasis, predisposing cells to oxidative damage and apoptosis. The interference of AgNPs with cellular signaling pathways further exacerbates their toxic effects, underscoring the importance of understanding nanoparticle interactions at the molecular level. Comparative studies across species reinforce the relevance of Drosophila as a valuable model for nanoparticle toxicology, providing critical insights into the broader implications of AgNP exposure. Moving forward, the development of safer nanoparticle alternatives and the implementation of regulatory measures will be essential in minimizing the potential risks associated with AgNP exposure while harnessing their technological benefits.

Molecular and Genetic Responses to Silver Nanoparticle Exposure in Drosophila melanogaster

Silver nanoparticles (AgNPs) have garnered extensive attention due to their applications in medicine, industry, and consumer products. However, concerns over their potential toxicity and environmental impact necessitate a deeper understanding of their biological effects, particularly in model organisms like *Drosophila melanogaster*. Studies have highlighted the molecular and genetic responses to AgNP exposure, including oxidative stress, epigenetic modifications, mitochondrial dysfunction, and developmental impairments (Eker *et al.*, 2024; Mali, 2024; Duman *et al.*, 2024).

Gene expression changes in response to AgNPs result in the differential regulation of genes associated with stress response, apoptosis, and oxidative damage. Studies indicate that AgNPs induce upregulation of stress response genes such as *hsp70* and *hsp83*, which play critical roles in cellular protection against nanoparticle-induced toxicity (Noga *et al.*, 2023; Mali, 2024; Eker *et al.*, 2024). Additionally, oxidative stress-related genes, including *sod2* (superoxide dismutase) and *cat* (catalase), exhibit increased expression, suggesting that AgNPs generate reactive oxygen species (ROS) that disrupt cellular homeostasis (Martínez-Cisterna *et al.*, 2024; Duman *et al.*, 2024). Increased ROS production leads to oxidative damage, impairing macromolecules such as DNA, proteins, and lipids. Moreover, apoptotic pathways involving *p53* and *bax* are activated, leading to programmed cell death in affected tissues (Rehman *et al.*, 2023; Akdașçi *et al.*, 2024). The dysregulation of these genes further suggests that AgNP exposure triggers cellular distress responses, potentially leading to genotoxicity and cytotoxicity in *Drosophila melanogaster* (Mali, 2024).

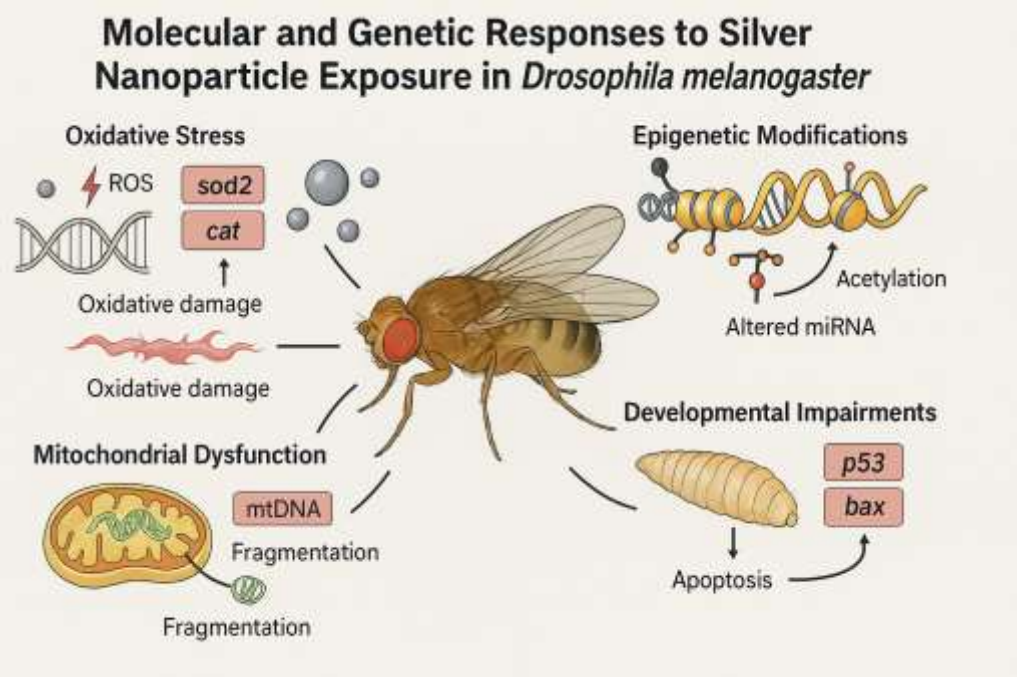

Figure: Molecular and Genetic Responses to Silver Nanoparticle Exposure in Drosophila melanogaster

Epigenetic modifications such as DNA methylation, histone modifications, and microRNA regulation are significant molecular responses to AgNP exposure. Studies show that AgNPs can alter DNA methylation patterns in *Drosophila melanogaster*, leading to transcriptional changes in key developmental genes (Eker *et al.*, 2024; Bechelany *et al.*, 2024). Histone modifications, including increased acetylation of H3K9 and decreased methylation of H3K27, have been observed in response to AgNPs, indicating chromatin remodeling that affects gene expression (Noga *et al.*, 2023; Mali, 2024). These modifications play a crucial role in controlling gene accessibility and expression, suggesting that AgNPs may have long-term effects on gene regulation beyond immediate exposure. MicroRNAs (miRNAs) such as *miR-277* and *miR-315*, which regulate developmental and stress-response pathways, show altered expression, further demonstrating the profound impact of AgNPs on the epigenome (Martínez-Cisterna *et al.*, 2024; Witkowska *et al.*, 2024).

AgNP exposure has been linked to mitochondrial dysfunction, characterized by altered mitochondrial genome integrity and increased ROS production. Studies demonstrate that mitochondrial DNA (mtDNA) is particularly susceptible to AgNP-induced damage, leading to mutations and deletions that compromise cellular energy production (Rehman *et al.*, 2023; Mali, 2024; Karav *et al.*, 2024). The mitochondria serve as the powerhouse of the cell, and any disruption in its function significantly affects cellular metabolism and homeostasis. Additionally, high ROS levels contribute to oxidative damage to nuclear DNA, resulting in increased fragmentation and activation of DNA repair pathways such as the *ATR* and *ATM* checkpoint kinases (Eker *et al.*, 2024; Duman *et al.*, 2024). These findings suggest that AgNPs pose a significant genotoxic risk, potentially leading to long-term genetic instability (Noga *et al.*, 2023). Mitochondrial dysfunction can also disrupt ATP synthesis, impair cellular respiration, and contribute to systemic metabolic imbalances, which may further impact survival and reproduction in *Drosophila melanogaster* (Mali, 2024).

The developmental and reproductive consequences of AgNP exposure in *Drosophila*

melanogaster are profound. Embryotoxicity is evident from increased lethality rates and developmental delays in larvae exposed to AgNPs. Genes involved in embryonic development, such as *dpp* (decapentaplegic) and *wg* (wingless), exhibit disrupted expression patterns, leading to morphological abnormalities (Martínez-Cisterna *et al.,* 2024; Akdașçi *et al.,* 2024). The disruption of these genes interferes with crucial signaling pathways involved in organogenesis and tissue differentiation. Fertility impairment is another major effect, with reduced egg-laying and hatchability observed in AgNP-exposed female flies (Rehman *et al.,* 2023; Karav *et al.,* 2024). This is likely due to oxidative stress affecting the reproductive organs and germ cells. Additionally, transgenerational effects have been reported, with offspring showing inherited defects in gene expression and mitochondrial function, suggesting potential heritable epigenetic alterations (Eker *et al.,* 2024; Mali, 2024). These findings indicate that AgNP-induced toxicity is not only limited to immediate exposure but can have persistent effects across multiple generations, necessitating further investigation into the long-term risks associated with nanoparticle exposure.

Comparative transcriptomic and proteomic analyses provide comprehensive insights into the molecular effects of AgNP exposure. Transcriptomic analysis reveals significant upregulation of detoxification genes such as *Cyp6g1* and *GstD1*, which are involved in xenobiotic metabolism (Noga *et al.,* 2023; Mali, 2024; Bechelany *et al.,* 2024). These genes play a role in mitigating nanoparticle toxicity by enhancing the breakdown and clearance of AgNPs from cells. Proteomic profiling identifies altered expression of proteins related to oxidative stress, energy metabolism, and apoptosis, further validating transcriptomic findings (Martínez-Cisterna *et al.,* 2024; Witkowska *et al.,* 2024). Proteomic data also highlight changes in cellular pathways related to protein folding, ubiquitination, and immune responses, suggesting widespread molecular disruptions induced by AgNP exposure. These analyses highlight the complex regulatory networks affected by AgNPs, emphasizing the need for further research into nanoparticle-induced toxicity mechanisms.

The molecular and genetic responses to AgNP exposure in *Drosophila melanogaster* underscore the potential risks associated with nanoparticle use. Gene expression changes, epigenetic modifications, mitochondrial dysfunction, and reproductive impairments collectively demonstrate the multifaceted impact of AgNPs. Comparative transcriptomic and proteomic studies further elucidate the intricate regulatory mechanisms underlying nanoparticle toxicity. Future research should focus on long-term and transgenerational effects to fully understand the implications of AgNP exposure in both environmental and biomedical contexts (Eker *et al.,* 2024; Mali, 2024; Duman *et al.,* 2024).

Table: Molecular and Genetic Responses to Silver Nanoparticle Exposure in *Drosophila melanogaster*

Biological Process	Genes/Proteins Involved	Effects/Outcomes
Oxidative Stress	hsp70, hsp83, sod2, cat, p53, bax	Induces ROS production, disrupts cellular homeostasis, causes DNA/protein/lipid damage
Epigenetic Modifications	DNA methylation, histone acetylation (H3K9), histone methylation (H3K27), miR-277, miR-315	Alters gene expression, chromatin remodeling, and has potential long-term effects
Mitochondrial Dysfunction	mtDNA, ATR, ATM, ROS, ATP, DNA repair proteins	Damage to mtDNA, impaired ATP production, and oxidative damage to nuclear DNA
Developmental and Reproductive Impairments	dpp, wg, Cyp6g1, GstD1	Embryonic defects, reduced fertility, altered gene expression across generations

Mechanisms of AgNP-Induced Oxidative Stress: ROS Generation and Cellular Damage

Silver nanoparticles (AgNPs) have been shown to induce significant oxidative stress by generating reactive oxygen species (ROS) within cells. These ROS include superoxide radicals, hydroxyl radicals, and hydrogen peroxide, which collectively lead to cellular damage. The generation of ROS can cause severe oxidative damage to proteins, lipids, and DNA, leading to disrupted cellular functions. ROS production is considered a major mechanism behind the toxicity of AgNPs. In particular, studies have highlighted the involvement of key antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), in modulating ROS levels in response to AgNP exposure.

These enzymes play critical roles in the cellular defense against oxidative damage, either by neutralizing ROS or by repairing oxidative damage (Mao *et al.,* 2018; Liu *et al.,* 2020), Fruit fly exposure to AgNPs has been shown to significantly alter the activity of these antioxidant enzymes. A study by Liu et al. (2020) found that AgNP exposure led to changes in the conformation and activity of CAT, which impairs its enzymatic function. In contrast, the interaction of AgNPs with SOD appeared to have minimal effect on its protein structure, although slight changes in its activity were observed. These modifications are crucial because they suggest that AgNP-induced oxidative stress can disrupt the balance of cellular redox homeostasis, making cells more

susceptible to damage (Liu *et al.,* 2020; Ahamed *et al.,* 2010).

Additionally, f 2-dependent antioxidant pathway, a key signaling pathway involved in cellular defense against oxidative stress. Activation of Nrf2 leads to increased expression of antioxidant genes, including those coding for SOD and CAT, which attempt to counteract the oxidative damage induced by AgNPs. This pathway has been shown to be upregulated in response to AgNP exposure in *Drosophila*, as seen in the enhanced GFP signal in Nrf2 reporter flies exposed to AgNPs (Mao *et al.,* 2018).

Overall, the studies confirm that CE a complex network of oxidative stress responses in *Drosophila melanogaster*, affecting key antioxidant enzymes and signaling pathways that govern cellular defense mechanisms. These findings underline the significant role of ROS in mediating AgNP toxicity and suggest that antioxidant enzymes are both biomarkers and targets in evaluating AgNP-induced oxidative stress.

More in-depth studies on the interactions between AgNPs and other cellular targets, such as mitochondrial function and genetic material, are essential for further insights into the molecular mechanisms and the long-term effects of AgNP exposure (Mao *et al.,* 2018; Ahamed *et al.,* 2010).

The Role of Antioxidant Enzymes in AgNP Toxicity

Silver nanoparticles (AgNPs) are widely used in various industrial and biomedical applications, but their increasing presence in the environment has raised concerns about their toxicity. One major mechanism through which AgNPs exert toxicity is by generating reactive oxygen species (ROS), which can overwhelm cellular antioxidant defense systems. The resulting oxidative stress plays a critical role in AgNP-induced cellular damage, and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are central to mitigating this damage (Mao *et al.,* 2018; Basak *et al.,* 2019).

AgNP exposure induces ROS production by interacting with cellular components. The generated ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, cause significant damage to cell membranes, proteins, and DNA. In response to this oxidative stress, cells activate antioxidant enzymes, which neutralize ROS and repair damaged molecules. However, the activity of these enzymes can be significantly impaired under prolonged exposure to AgNPs, leading to cellular dysfunction and damage (Mao *et al.,* 2018; Liu *et al.,* 2020).

SOD plays a crucial role in neutralizing superoxide anions (O_2^-) by converting them into hydrogen peroxide. Studies have demonstrated that AgNP exposure increases ROS production, leading to an initial upregulation of SOD as a compensatory response. However, long-term exposure to AgNPs leads to SOD enzyme depletion, which exacerbates oxidative damage (Mao *et al.,* 2018; Liu *et al.,* 2020).

Catalase is responsible for breaking down hydrogen peroxide (H_2O_2) into water and oxygen. AgNP-induced oxidative stress leads to the accumulation of H_2O_2, overwhelming the antioxidant defense system. Studies show that AgNP exposure reduces catalase activity, making cells more susceptible to oxidative damage (Basak *et al.,* 2019; Liu *et al.,* 2020). Catalase inactivation impairs the cell's ability to manage oxidative damage, leading to tissue and organ dysfunction.

GPx detoxifies hydrogen peroxide and lipid peroxides, contributing to redox homeostasis. AgNPs have been shown to reduce GPx activity in various organisms, including *Drosophila melanogaster*. This depletion of GPx exacerbates lipid peroxidation, further compromising cell integrity (Mao *et al.,* 2018). As a result, cells are more vulnerable to membrane damage and other forms of oxidative stress-induced damage.

The Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway is a key regulator of the antioxidant response. In organisms like *Drosophila melanogaster*, AgNP exposure activates the Nrf2 pathway, which increases the expression of antioxidant enzymes such as SOD, CAT, and GPx. While this pathway serves as a defence mechanism against oxidative damage, prolonged activation due to excessive AgNP exposure can lead to chronic oxidative stress and potential cellular damage (Mao *et al.,* 2018; Sadeghi & Ghaedi, 2020). The balance of Nrf2 activity is crucial in determining the extent of AgNP-induced toxicity.

Exposure to AgNPs generates ROS that overwhelms the cellular antioxidant defense system, which is characterized by enzyme depletion and failure to neutralize excess ROS. This disruption contributes to significant cytotoxicity, including DNA damage, lipid peroxidation, and protein oxidation. The cumulative damage from oxidative stress, in combination with the impaired antioxidant defence system, leads to severe consequences such as apoptosis, tissue degeneration, and developmental abnormalities (Akhtar *et al.,* 2016; Gupta & Manna, 2018).

The balance between ROS production and antioxidant defence mechanisms is critical in modulating the toxicity of AgNPs. Strategies aimed at enhancing the activity of antioxidant enzymes or preventing ROS overproduction may reduce AgNP-induced toxicity. Research into surface modifications of AgNPs and their ability to interact with cellular components offers potential approaches to mitigate oxidative stress (Wang *et al.,* 2019; Nagaoka *et al.,* 2021).

Genetic, Epigenetic, Developmental and Physiological Responses to AgNP Exposure in *Drosophila*

Silver nanoparticles (AgNPs) are commonly used in various consumer products due to their antimicrobial properties. However, developmental exposure to AgNPs may lead to long-term health consequences, particularly by disrupting the microbiome-gut-brain axis. Studies have shown that AgNPs can cross the placenta and blood-brain barrier, impacting offspring development (Li *et al.*, 2019; Lyu *et al.*, 2021).

1. Microbial Changes and Behavioral Effects:

Developmental exposure to AgNPs has been found to cause gut dysbiosis (imbalance in gut bacteria), affecting neurobehavioral outcomes in offspring. In rodents, exposure led to an increase in certain bacterial populations like *Bacteroides*, *Bacillus*, *Prevotella*, and *Streptococcus*, while reducing populations of *Bifidobacterium* and *Mucispirillum* (Li *et al.*, 2019; Lyu *et al.*, 2021). These microbial changes were correlated with neurobehavioral alterations such as increased repetitive behaviors and altered body composition, including higher fat content in offspring (Li *et al.*, 2019; Lyu *et al.*, 2021).

2. Genetic and Epigenetic Responses:

Developmental exposure to AgNPs can potentially alter gene expression and epigenetic regulation, which may contribute to neurodevelopmental disorders. Specific studies in *Drosophila* and other model organisms like zebrafish have shown that early AgNP exposure can result in behavioral changes, such as reduced progression through developmental stages and altered sensory-motor functions (Han *et al.*, 2014; Lyu *et al.*, 2021). This suggests that the genetic and epigenetic responses to AgNP exposure involve not just direct cellular damage, but also long-term alterations in gene expression linked to behavioral and metabolic outcomes (Lyu *et al.*, 2021).

3. Mechanisms and Pathways:

Exposure to AgNPs impacts the gut microbiome, leading to changes in bacterial diversity, which in turn affect brain function through the microbiome-gut-brain axis. Metagenomic analyses have shown that bacteria associated with metabolism, such as *Prevotella* and *Bacillus*, were significantly upregulated, while beneficial bacteria like *Bifidobacterium* were downregulated. These changes were linked to metabolic and cognitive disruptions (Borre *et al.*, 2014; Lyu *et al.*, 2021). Additionally, previous studies have pointed out that the increased abundance of *Bacteroides* spp. may be associated with obesity and other metabolic disorders (Borre *et al.*, 2014).

4. Potential Impacts on Neurodevelopmental Disorders:

Given the known association between certain gut bacteria and conditions like autism spectrum disorders (ASD) and anxiety, the shifts in microbiota caused by AgNP exposure might increase the risk of such conditions (Stilling *et al.*, 2014; Lyu *et al.*, 2021). The reduction in *Bifidobacterium* spp. is particularly concerning, as this bacterium is often used in probiotics to alleviate neurobehavioral symptoms in individuals with ASD (Rosenfeld, 2015; Lyu *et al.*, 2021).

The effects of silver nanoparticles (AgNPs) on *Drosophila melanogaster*, focusing on male reproductive toxicity. AgNP exposure led to dose-dependent accumulation, reduced viability, delayed development, and decreased fecundity, particularly in F1 males (Ong *et al.*, 2016). The key findings include:

1. **Germline Stem Cell (GSC) Reduction:** AgNP exposure significantly decreased the number of GSCs in the testis, which are essential for sperm production (Ong *et al.*, 2016).
2. **Oxidative Stress:** Increased reactive oxygen species (ROS) were observed, particularly in the GSC niche, which contributed to premature GSC differentiation and reduced self-renewal (Ong *et al.*, 2016).
3. **Disruption of Spermatogenesis:** The study showed premature differentiation of GSCs, indicated by earlier expression of differentiation markers like Bam, resulting in reduced GSC numbers and impaired fertility (Ong *et al.*, 2016).

Future research should focus on identifying safe exposure limits, optimizing nanoparticle formulations, and investigating long-term effects on biological systems (Demir & Turna, 2022).

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