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REVIEW ARTICLE

Genotoxicity of Silver Nanoparticles: Mechanisms, Implications, and Future Perspectives for Human and Environmental Health

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ABSTRACT: The widespread integration of silver nanoparticles (AgNPs) into consumer products, biomedical applications, and industrial processes has raised significant concerns regarding their potential cytotoxic and genotoxic effects on human health and the environment. AgNPs exhibit unique physicochemical properties, including high surface area-to-volume ratio, antimicrobial activity, and catalytic efficiency, which drive their commercial and scientific utility. However, their increasing prevalence necessitates a rigorous assessment of their genotoxic potential, encompassing DNA damage, chromosomal aberrations, and epigenetic modifications. This review synthesizes current literature on AgNP-induced genetic toxicity, elucidating molecular mechanisms such as oxidative stress, reactive oxygen species (ROS) generation, mitochondrial dysfunction, and inflammatory responses. By integrating findings from *in vitro* and *in vivo* studies, we highlight the dose-dependent and size-specific effects of AgNPs across diverse biological systems. Additionally, we discuss critical factors influencing genotoxicity, including surface coatings, aggregation dynamics, and exposure pathways. The environmental persistence of AgNPs and their bioaccumulation in aquatic and terrestrial ecosystems further underscore the need for comprehensive risk assessment frameworks. This review aims to bridge existing knowledge gaps by proposing standardized toxicity evaluation protocols, advancing mechanistic understanding, and advocating for sustainable nanomaterial design. Ultimately, our analysis informs regulatory policies, promotes safer AgNP applications, and encourages the development of mitigation strategies to minimize adverse health and ecological impacts.

Keywords: Silver nanoparticles (AgNPs), Engineered nanomaterials (ENM), Genotoxicity, Oxidative stress, Nanotoxicology, Environmental health, Risk assessment.

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1. INTRODUCTION

Silver nanoparticles have gained significant attention in various fields due to their unique physicochemical properties and diverse applications. AgNPs range in size from 1 to 100 nanometres, consisting of silver atoms arranged in a crystalline lattice structure [1]. The unique characteristics of metal nanoparticles are determined by factors such as size, composition, crystallinity, and morphology. They exhibit size-dependent properties that differ significantly from those of bulk materials. Their large surface area-to-volume ratio due to being ultramicroscopic and distinctive optical, electrical, and antimicrobial properties make them highly desirable for a wide range of industrial, biomedical, and

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consumer applications. When reduced to the nanoscale, their chemical, mechanical, electrical, structural, morphological, and optical properties are notably altered. These adaptations enable NPs to interact uniquely with cellular biomolecules, facilitating their penetration into cellular interiors. Furthermore, the high proportion of surface atoms in nanostructured materials contributes to enhanced surface reactivity. This distinctive feature has positioned nanomaterials as a focal point in both fundamental and applied research, particularly in the field of bionanotechnology [2, 3]. In recent years, there has been a remarkable increase in the production and utilization of AgNPs across numerous sectors. AgNPs find applications in areas such as electronics, textiles, cosmetics, food packaging, and medicine, owing to their antimicrobial, catalytic, and optical properties [4-6].

Their incorporation into various products has led to a significant rise in consumer exposure to AgNPs, raising concerns about their potential impact on human health and the environment. Despite the numerous advantages associated with AgNPs, there is a growing need to understand their potential adverse effects, particularly in terms of genotoxicity. Genotoxicity refers to the ability of a substance to cause damage to the genetic material within cells, potentially leading to mutations, chromosomal aberrations, or DNA damage [7]. Understanding the genotoxicity of AgNPs is crucial for assessing their safety and establishing appropriate guidelines for their usage. Several studies have investigated the genotoxic potential of AgNPs, exploring their interactions with DNA and their ability to induce DNA damage in various cell types [8, 9]. However, there is still a need for a comprehensive review that consolidates the existing knowledge on AgNP genotoxicity, including the underlying mechanisms, factors influencing genotoxicity, and potential implications for human health and the environment.

The goal of this review is to present a thorough analysis of the knowledge that is currently available regarding the genotoxicity of AgNPs. To clarify the genotoxic potential of AgNPs, it will review the pertinent literature, assess the methodologies used, and synthesise the results. The implications for human health and the environment will also be covered, along with mechanistic insights into AgNP-induced genotoxicity and the variables influencing genotoxicity outcomes. The data presented will aid in a better comprehension of the potential dangers connected to exposure to AgNP and guide future studies aimed at creating secure and long-lasting nanomaterials.

2. SYNTHESIS OF AgNPs

Silver nanoparticles can be synthesized using various methods, each offering unique control over size, shape, and properties. AgNPs can be synthesized through chemical, physical, and biological methods. Chemical synthesis typically involves reducing agents that facilitate nanoparticle formation, often requiring stabilizers to prevent aggregation. Physical techniques, such as evaporation-condensation and pulsed laser deposition, provide a controlled environment for producing uniform nanoparticles without chemical additives. In contrast, biological synthesis harnesses natural biomolecules from microorganisms and plant extracts, promoting an environmentally friendly and cost-effective route to nanoparticle production. Each method influences the final properties of AgNPs, making method selection critical for specific applications (Figure 1).

Chemical reduction methods involve the reduction of silver ions (Ag^+) to metallic silver (Ag^0) using chemical reducing agents in an aqueous or organic medium. The process typically involves three key components: a silver salt precursor (e.g., silver nitrate), a reducing agent, and a stabilizing or capping agent to prevent nanoparticle aggregation. Silver ions are reduced with sodium citrate using the well-known Turkevich method to produce uniform AgNPs [10]. Common reducing agents include sodium borohydride ($NaBH_4$), hydrazine, ascorbic acid, and citrate. These agents donate electrons to silver ions, enabling their reduction to metallic silver. Polymers (e.g., polyvinyl alcohol), surfactants, or organic molecules (e.g., citrate) are often added to stabilize the nanoparticles, control their growth, and prevent aggregation. The synthesis parameters, such as pH, temperature, and concentration of reagents, play a crucial role in determining the size, shape, and dispersion of the nanoparticles [11-14]. Physical methods like laser ablation and spark discharge allow precise control over nanoparticle size and shape. For instance, the gamma-ray irradiation technique involves the use of high-energy gamma rays to induce the reduction of silver ions into nanoparticles, providing a controlled and efficient approach to nanoparticle synthesis, showcasing the potential of physical methods. The process eliminates the need for chemical reducing agents, making it an eco-friendly and clean alternative. Additionally, gamma-ray irradiation facilitates the production of uniform nanoparticles with desirable properties, such as enhanced stability and controlled size distribution. This method has demonstrated potential for large-scale production and is particularly advantageous in biomedical and catalytic applications where purity and precision are crucial. The ability to fine-tune the synthesis parameters further highlights the versatility and significance of gamma-ray irradiation in the field of nanotechnology [15-17]. Biological methods for nanoparticle synthesis have gained significant attention in recent years due to their eco-friendly and sustainable nature. These methods employ natural sources such as plant extracts, bacteria, fungi, and algae for the synthesis of nanoparticles [18]. Plant extracts, for instance, contain biomolecules like proteins, carbohydrates, and phenolic compounds that can act as reducing and stabilizing agents, facilitating the formation of nanoparticles [19]. Similarly, microorganisms like bacteria and fungi can be engineered to produce nanoparticles through enzymatic reactions [20]. For instance, geranium leaves and endophytic fungi were used to bioreduce chloroaurate ions and produce gold nanoparticles [21].

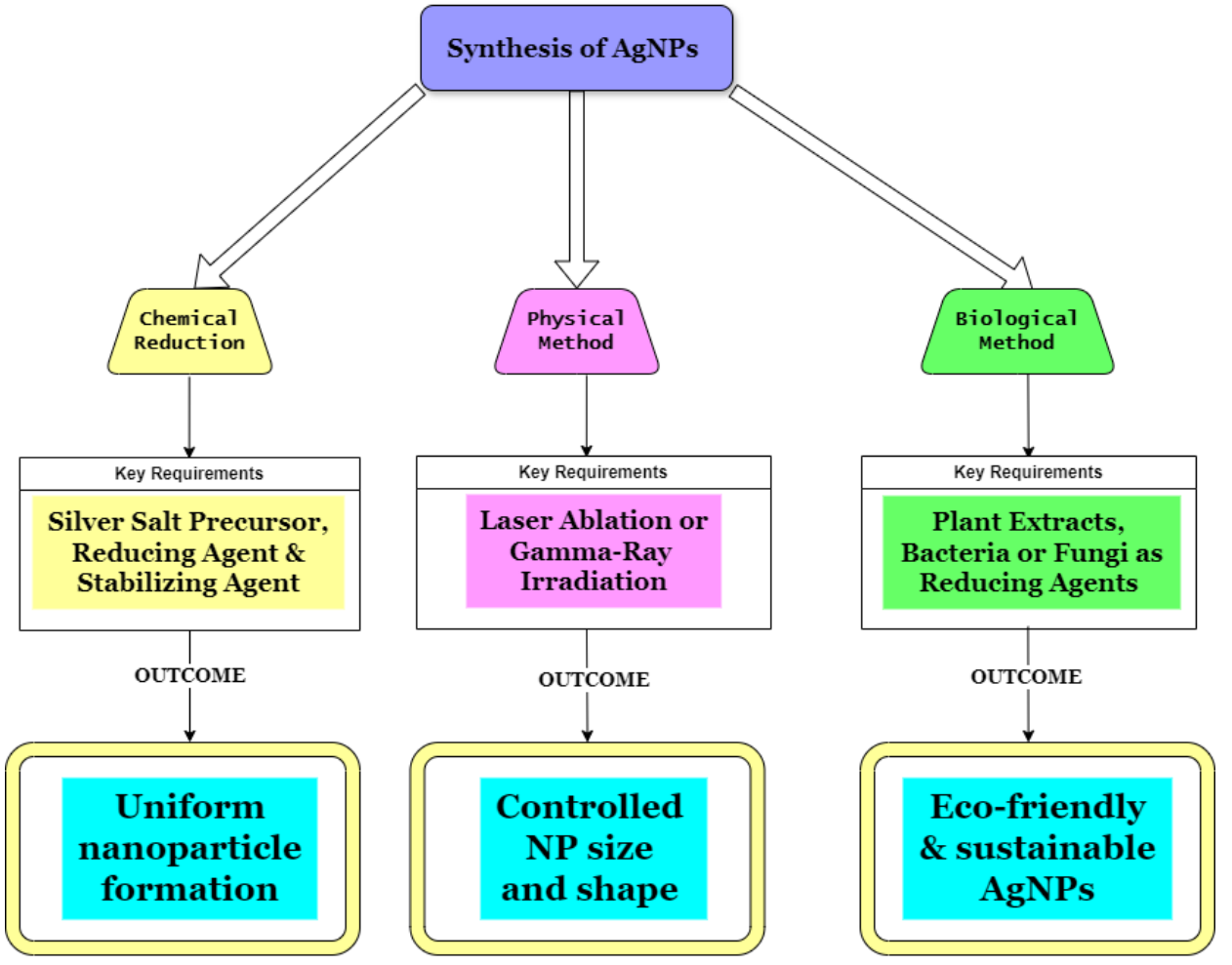

Fig. 1. Methods of AgNPs synthesis: Chemical, Physical, and Biological Approaches.

3. CHARACTERIZATION OF AgNPs

Characterizing AgNPs is crucial for understanding their properties and applications. With the rapid advancement of nanotechnology, various characterization techniques have been developed to analyse the physical, chemical, and biological properties of nanoparticles. Each technique has its unique advantages and limitations, making it essential to understand the strengths and weaknesses of each method to select the most suitable technique for a particular application (Table 1). Transmission Electron Microscopy (TEM) enables direct visualization of nanoparticle morphology and size. For instance, in a 2002 study, TEM was utilized to study the shape-controlled synthesis of gold and silver nanoparticles [22]. Scanning Electron Microscopy (SEM) provides information about nanoparticle shape and aggregation behaviour. For instance, SEM coupled with energy-dispersive X-ray spectroscopy (EDS) was employed in multiple studies to determine elemental composition [23, 24]. X-Ray Diffraction (XRD) reveals the crystalline structure of AgNPs. By analysing the diffraction patterns, XRD identifies the characteristic peaks corresponding to the face-centered cubic (FCC) structure of a nanoparticle. The average crystallite size of a nanoparticle can be estimated using the Debye-Scherrer equation, based on the peak broadening in the diffraction pattern. XRD also confirms the absence of impurities, ensuring the synthesis of pure nanoparticles. A study discussed the green synthesis of silver nanostructures and their characterization using XRD [25]. UV-Visible Spectroscopy is widely used for AgNP characterization, revealing surface plasmon resonance (SPR), a distinctive optical property of AgNPs, which arises from the collective oscillation of conduction electrons in response to light and providing insights into size and shape. AgNPs typically exhibit an SPR band in the range of 400–450 nm, depending on their size, shape, and surrounding medium. By analysing the SPR peak, valuable information can be obtained about the nanoparticles' formation, stability, and size distribution. The intensity and position of the SPR peak can also indicate changes in aggregation or surface modifications of AgNPs, making UV-Visible spectroscopy a vital tool for nanoparticle synthesis and functionalization studies [26, 27]. Fourier-Transform Infrared Spectroscopy (FTIR) identifies functional groups on nanoparticle surfaces, aiding in understanding capping agents. FTIR spectra provide insights into the biomolecules or capping agents that act as reducing and stabilizing agents during nanoparticle synthesis. Peaks corresponding to functional groups such as hydroxyl (-OH),

carbonyl (C=O), and amine (-NH) indicate the presence of organic compounds that interact with AgNP surfaces. These interactions play a critical role in nanoparticle stability and biocompatibility. Additionally, FTIR analysis helps confirm the successful functionalization of AgNPs for specific applications, such as drug delivery or catalysis. By revealing the chemical composition of the nanoparticle surface, FTIR contributes significantly to understanding their synthesis mechanisms and potential applications [28, 29]. Dynamic Light Scattering (DLS) is widely used technique for characterizing the size distribution and hydrodynamic diameter of AgNPs in colloidal solutions. This method measures the fluctuations in the intensity of scattered light caused by the Brownian motion of particles in suspension. The hydrodynamic diameter obtained through DLS includes not only the core nanoparticle size but also the layer of capping or stabilizing agents surrounding the nanoparticles. DLS provides valuable insights into the polydispersity index (PDI), which indicates the uniformity of the particle size distribution [30, 31].

4. MECHANISMS OF AgNPs TOXICITY

The remarkable physicochemical properties of AgNPs have attracted substantial interest in them which has led to their use in various industrial and biomedical applications. However, the increasing use of AgNPs has raised concerns about their potential adverse effects to living organisms and the environment. So, in order to harness their benefits and mitigate their risks, it is of utmost importance to understand the intricate mechanisms through which AgNPs exert their toxic effects.

AgNPs interact with cell membranes and alter their fluidity and permeability. This allows them to disrupt the lipid bilayer, compromising the selective permeability and integrity of the cell membrane. This disruption is influenced by the size, shape, and surface charge of AgNPs [32]. AgNPs have been reported to target mitochondria and disrupt their function and impair the production of ATP. The mitochondrial dysfunction is linked to the impaired electron transport chain activity due to altered mitochondrial membrane potential, and increased ROS production within the organelles. This disruption compromises the cellular energy metabolism and contributes to the overall cellular stress and damage [33]. The leakage of ions and cellular contents triggers signaling cascades that may ultimately result in apoptotic cell death [34, 35].

Reactive Oxygen Species (ROS) are produced in the body as a part of normal physiological process and are, infact, required at moderate levels. However, the imbalance created due to the production of excessive ROS during the oxidative stress leads to the collapse of the antioxidant defense system [36]. Antioxidants prevent nanoparticle toxicity by neutralizing ROS through their ability to scavenge them by donating electrons or hydrogen atoms. Antioxidants act as molecular shields, intercepting ROS and preventing them from causing oxidative stress and damage to cellular components like DNA, proteins, and lipids [37]. AgNPs are also known to cause DNA damage as they can directly interact with DNA molecules, causing structural modifications and impairing DNA replication processes. In addition to that, the ROS generated due to the imbalance created during oxidative stress contribute to the DNA damage by causing oxidative modifications to DNA bases [38].

Table 1. Advantages and disadvantages of characterization techniques of nanoparticles.

Technique	Advantages	Disadvantages
Transmission Electron Microscopy (TEM)	High-resolution imaging, morphological information, and crystal structure analysis	Sample preparation is challenging, expensive, and limited to thin samples
Scanning Electron Microscopy (SEM)	High-resolution imaging, surface topography, and composition analysis	Sample preparation is required, and charging effects can occur
Dynamic Light Scattering (DLS)	Fast, non-invasive, and provides hydrodynamic size and size distribution	Limited to dilute suspensions, and results can be affected by sample polydispersity
X-ray Diffraction (XRD)	Provides crystal structure and phase information	Requires crystalline samples, and amorphous materials may be undetectable
Fourier Transform Infrared Spectroscopy (FTIR)	Provides molecular structure and functional group information	Sample preparation is required, and results can be affected by sample moisture and contamination
Zeta Potential Analysis	Provides surface charge and stability information	Requires dilute suspensions, and results can be affected by sample polydispersity and electrolyte concentration
UV-Visible Spectroscopy	Fast and provides information on optical properties, size & concentration	Limited to UV-Vis active samples, and results can be affected by sample scattering and absorption

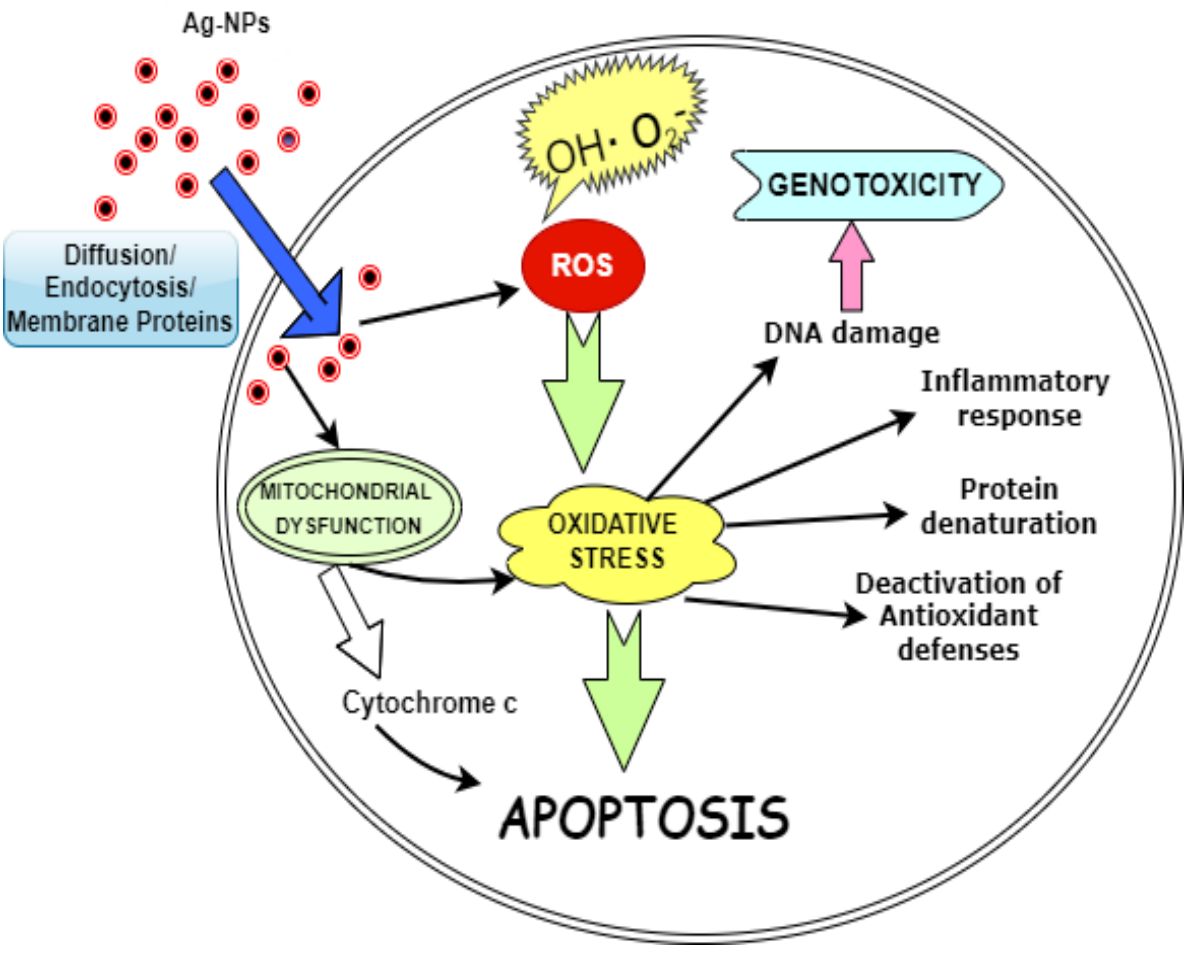

Fig. 2. Proposed cellular uptake and toxicity pathways of AgNPs across various cell types: A summary of key findings from multiple studies.

Another aspect of AgNP toxicity involves the triggering of an inflammatory response by activating immune cells like macrophages and promoting the release of pro-inflammatory cytokines. The recognition of AgNPs as foreign entities by immune cells, such as macrophages, leads to the secretion of pro-inflammatory cytokines which can lead to tissue damage and chronic health effects [39]. This facet of the AgNP toxicity adds another layer of complexity to their toxicity profile (Figure 2). The oxidative stress is usually accompanied by increased expression of proinflammatory genes and activation of neutrophils and macrophages [40].

AgNPs impact cellular function even without direct entry into the cell. They influence key signaling pathways, including serine/threonine protein kinase (PAK), mitogen-activated protein kinase (MAPK), and phosphatase 2A. Additionally, exposure to 20 nm AgNPs triggers cellular stress responses, such as increased reactive oxygen species (ROS) production and protein carbonylation. Furthermore, proteins associated with SUMOylation exhibit upregulation following AgNP exposure, suggesting a role in cellular stress adaptation. Furthermore, the MAPK signaling pathway activated in response to AgNP exposure comprises of ERK, JNK, and p38, and it regulates various cellular processes, including apoptosis, proliferation, and differentiation (Figure 3). Activation of the MAPK pathway can lead to the induction of apoptosis, thereby contributing to the suppression of ROS-generated damage [41, 42]

The apoptosis-inducing signaling pathway mediated by p53, AKT, and MAPK activation plays a crucial role in mitigating the oxidative stress triggered by AgNPs (Figure 4). Upon exposure to AgNPs, cells experience a significant increase in reactive oxygen species (ROS) production, leading to cellular damage and instability [41]. In response to AgNP-induced ROS generation, the tumour suppressor protein p53 is activated, triggering a cascade of downstream signaling events. Activated p53 induces the expression of pro-apoptotic genes, such as Bax and PUMA, while simultaneously inhibiting anti-apoptotic genes, like Bcl-2 [42]. Concomitantly, the AKT signaling pathway is modulated in response to AgNP exposure. AKT activation can have dual roles, promoting cell survival or inducing apoptosis, depending on the cellular context. In the case of AgNP-induced ROS generation, AKT activation is thought to contribute to the apoptotic response [43].

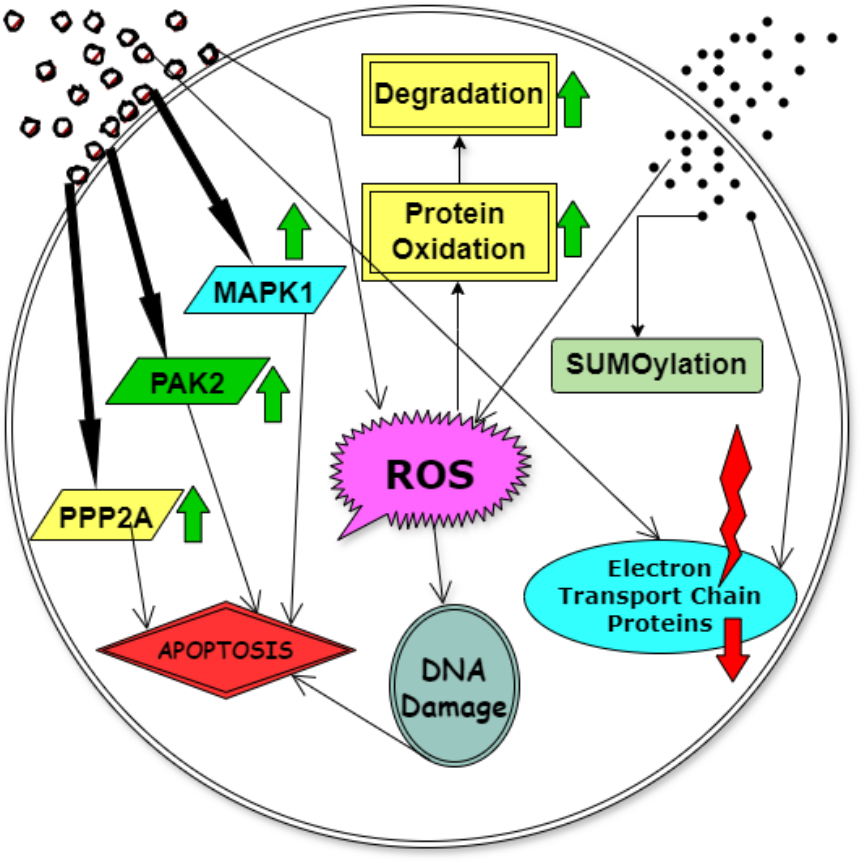

Fig. 3. Silver nanoparticles can induce toxic effects both through direct entry into cells and by influencing cellular signaling pathways from the outside. These pathways include key signal transduction mechanisms that mediate cellular stress and damage.

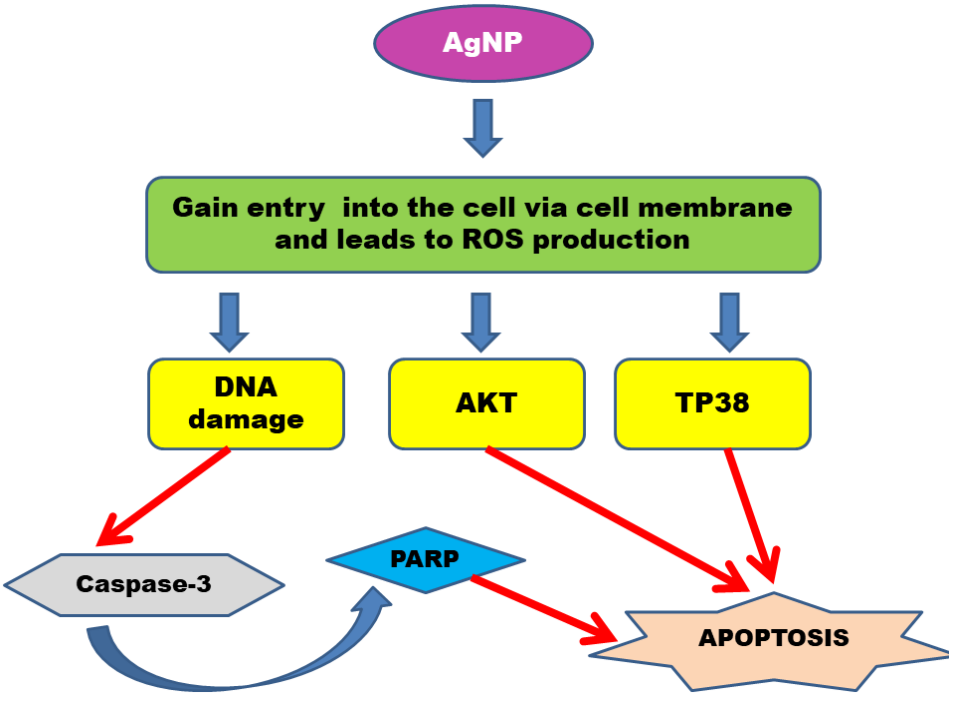

Fig. 4. AgNP-triggered ROS suppression: A signaling network regulated by p53, AKT, and MAPK activation.

Furthermore, the mitogen-activated protein kinase (MAPK) signaling pathway is also activated in response to AgNP exposure. The MAPK pathway, comprising ERK, JNK, and p38, regulates various cellular processes, including apoptosis, proliferation, and differentiation. Activation of the MAPK pathway can lead to the induction of apoptosis, thereby contributing to the suppression of ROS-generated damage [44, 45]. Collectively, the p53, AKT, and MAPK signaling pathways form a complex network that regulates the cellular response to AgNP-induced ROS generation. Elucidating the molecular mechanisms underlying this response is crucial for understanding the potential toxicological effects of AgNPs and for developing strategies to mitigate their adverse impacts [46].

5. *In Vivo* TOXICITY STUDIES

Multiple *In vivo* studies investigating the toxicity of AgNPs have revealed valuable insights into their potential impact on living organisms. Such studies have been conducted in organisms ranging from vertebrates to invertebrates to higher plants. Central to this aspect of nanotoxicity evaluation are the biodistribution studies which help us in exploring the complex journey of AgNPs within organisms. These studies have revealed the accumulation patterns of AgNPs in various organs, casting an emphasis on the liver and spleen as key sites of deposition. Table 2 exhibits the summary of some recent *In-vivo* nanotoxicological studies of AgNPs in mammalian models.

An *In-vivo* study on zebra fish embryos demonstrated that the AgNPs exhibited dose-dependent toxicity, causing mortality, bradycardia (slow heart rate), oedema, malformations, and hatching delays. The embryos treated with AgNPs displayed a slimy coating, possibly indicating skin injury from nanoparticle penetration. AgNPs were found to accumulate within nuclei, potentially leading to DNA damage and chromosomal aberrations. Deposition in the nervous system raised concerns about cardiac rhythm, respiration, and body movements [47].

In another study performed in the same year, the *in vivo* toxicity of various metallic nanoparticles on aquatic organisms was investigated and it was found that the silver and copper nanoparticles were toxic to all tested species, with substantial differences in sensitivity between species. Nanosilver and nanocopper toxicity did not solely result from particle dissolution. Filter-feeding organisms (daphnids, algae) were more susceptible than vertebrates (zebra fish). Nanoparticles generally showed lower toxicity compared to soluble metal forms, but particle aggregation and sedimentation complicated exposure assessment, highlighting challenges in accurately assessing *in vivo* nanoparticle toxicity [48].

A study investigated the developmental toxicity of waterborne AgNPs on embryos of *Clarias gariepinus*, a tropical fish species. Exposure of the embryos to different concentrations of AgNPs led to dose-dependent increases in mortality and various morphological malformations including notochord curvature and oedema were observed. The mortality rate, malformations, and DNA fragmentation increased with AgNP concentration and embryonic stage. Antioxidant enzymes and biomarkers related to oxidative stress were affected, indicating the role of oxidative damage in the observed toxicity. Histopathological analysis revealed distorted and wrinkled notochord and somite disorganization in AgNP-exposed embryos. According to the study, oxidative stress and genotoxicity are what cause AgNPs to be toxic to *C. gariepinus* embryos, and AgNP bioaccumulation is concentration-dependent in the embryos [49].

A scientific work published in 2013 investigated the *in vivo* toxicity of AgNPs on aquatic ecosystems, focusing on three Daphnia species over multiple generations across different exposure scenarios. The research investigates acute, chronic, and long-term effects of nanosilver exposure, shedding light on the potential ecological impacts of nanosilver use. Variations in toxicity within the Daphnia genus are observed, emphasizing the need for long-term studies to comprehend nanosilver's effects accurately. The influence of particle characteristics like size, surface coatings, and solubility on toxicity suggests a complex relationship between these factors and the biological responses observed [50]. The Micronucleus assay, Pig-a assay, and Comet assay were used in a mouse study to assess the clastogenicity and mutagenicity of AgNPs with varying sizes and coatings. AgNPs were administered intravenously to mice, and their distribution was confirmed in bone marrow and liver using analysis techniques like ICP-MS and TEM. While cytotoxicity was observed in bone marrow due to PVP-coated AgNPs, no significant increase in mutant frequencies or micronucleated reticulocytes was found. However, oxidative DNA damage was brought on by both types, PVP and silicon-coated AgNPs, in the liver [51].

In one of the research studies, investigation into the *in vivo* toxicity of AgNPs (20–100 nm-size) was done using intravenous administration in rats. The study reveals that while the highest dose (6 mg/kg body weight) is well tolerated, both sizes of AgNP cause growth retardation and substantial enlargement of the spleen due to increased cell numbers. Spleen, liver, and lymph nodes show accumulation of AgNP with brown and black pigmentation. Liver damage is indicated by clinical chemistry changes and there is a dramatic suppression of natural killer (NK) cell activity in the spleen, and other immune parameters are affected, including cytokine production. The liver damage was signified by the increased phosphatase, alanine transaminase, and aspartate transaminase [52].

To summarize, *in-vivo* toxicological studies remain a pivotal area of research. They paint a comprehensive portrait of the potential implications arising from AgNP exposure. By intertwining nanoparticle characteristics, exposure pathways, and organ-specific reactions, these investigations provide a roadmap for informed decision-making. This multifaceted approach equips us with the insights needed to responsibly harness the potential benefits of AgNPs across diverse applications, while remaining vigilant to their associated risks.

Table 2. Some recent *In vivo* nanotoxicological studies of AgNPs in mammalian models.

Size of AgNPs	Test System	Dose & Mode of Exposure	Assay Type & Results	Ref.
8 nm in diameter; spherical	Swiss albino mice	250, 500, and 1000 mg/kg; All treatment groups received a single oral dose	Bone marrow micronucleus assay revealed that no toxic effects in the bone marrow were observed in both the male and female rats at different AgNP doses.	[53]
Crystalline AgNP with size ranging between 7.77-28.4 nm	Male wistar rats	Low and high doses of 1 and 2mg/kg *i.p.* respectively, for 30 days	Nanotoxicity by AgNPs and its amelioration by eugenol evaluated using various biochemical assays and histopathological alterations confirmed the oxidative stress and eugenol as a potent attenuator of AgNP toxicity.	[54]
Average diameter of ≈10 nm	Sprague-Dawley rats	Ionic silver (Ag^+- 40 µg/mL) and silver nanoparticle species ($AgNP^+$-40 µg/mL) for 18 days orally	Histological and pathological analyses confirmed significant histological changes and $AgNPs^+$ appeared to be more toxic than Ag^+, especially in spleen.	[55]
Silver nanocomposites; Ag-NSP (silicate nanoplatelet) and Ag-SMA (Styrene co-malein anhydride)	Mice	Mice were fed 5000 mg/kg body weight of AgNP and peripheral-blood cells were collected at 36h,48h, and 72h.	No effect on genotoxicity was found in the form of micronucleated polychromatic erythrocytes for both groups fed with AgNP.	[56]
13 ± 3 nm in diameter	Male albino rats	2 and 4mg/kg were daily injected intraperitoneally for 28 days	Comet assay on lymphocyte cells revealed a highly significant increase in tail length, intensity, moment, and tail migration.	[57]
Average diameter of 22 nm	*Chinchilla lanigera* (Biological material was collected Post Mortem); Isolated cells extracted from the bone marrow derived from the femurs of chinchillas	Unstable and sodium citrate-stabilized silver nanoparticles; 5, 10 and 20 µg/L of silver nitrate after 3, 6 and 24 hours	Genotoxicity assessed comet assay revealed a dose dependent statistically significant relation between the comet tails and the increasing concentrations; Sodium citrate-stabilized silver nanoparticles exhibited maximum toxicity.	[58]
Mean particle size of 98.3 nm in suspension	Male wistar rats	3 mg/kg intraperitoneal injection was given to 3 groups for 1,2 and 3 weeks, respectively	DNA damage evaluated with the alkaline comet assay indicated that chronic exposure, even at a low dose may affect animal health.	[59]
Diameter of 20.7 ± 3.0 nm	Mice	50, 150 and 300 mg/kg dose per day administered orally by gavage for 3 consecutive days	Alkaline comet and micronucleus assays detected no statistically significant increase in DNA damage as compared to vehicle control given distilled water only.	[60]
Average size of 31.83 nm	Male wistar rats	5 and 10 mg/kg oral administration for a 28-day period on alternate days.	Serum biochemical parameters AST, ALT & LDH and Inflammatory markers (TNF–α and IL-6) were found increased as compared to control rats.	[61]
Mean diameter of 100 ± 25 nm (AgNPs); 200 ± 25 (Ag-PEG) and 150 ± 25 nm (Ag-BSA)	Swiss albino mice	500, 1000, and 2000 µg/kg injected subcutaneously at 3-day interval for 15 days.	Evaluation of oxidative stress markers and antioxidant enzymes level in PBMCs revealed high intracellular ROS which decreased on the surface modification.	[62]

Continued research is imperative to unravel the mechanisms underpinning these toxic effects and to guide the judicious use of AgNPs across various applications

6. *In vitro* TOXICITY STUDIES

In vitro toxicity studies play a crucial role in evaluating the potential adverse effects of AgNPs on biological systems. These studies provide valuable insights into the cellular and molecular mechanisms underlying AgNP-induced toxicity and help in elucidating their dose-response relationships. Such studies offer controlled experimental settings, revealing intricate mechanisms of toxicity, and reducing the need for animal testing. In spite of their benefits, the simplified environment requires careful interpretation when extrapolated to *in vivo* scenarios, which helps in understanding their safety profile before *in vivo* experiments, aiding in understanding their safety profile prior to *in vivo* experiments. *In vitro* data also direct regulatory choices and moral considerations, in addition to accelerating screening and risk assessment. Table 3 exhibits some recent *in vitro* nanotoxicological studies of AgNPs in different cell lines.

In order to assess the potential biological impacts, a diverse array of cell lines and cell types has been employed in the realm of *in vitro* studies investigating the toxicity of silver nanoparticles. These cell lines encompass a range of tissue origins and functionalities, such as Human Epidermal Keratinocytes, Skin Keratinocytes, Monocytic cells, Macrophages, Lymphocytes, Peripheral Blood Lymphocytes, Alveolar Epithelial Cells, Stem Cells and Porcine Kidney Cells. The selection of these diverse cell lines allows researchers to capture distinct cellular responses, enabling a comprehensive understanding of AgNP-induced toxicity across different physiological contexts. This section highlights key findings from notable *in vitro* studies that contribute to our understanding of AgNP toxicity.

A study on the Human Embryonic Kidney 293T cells (HEK293T cells) *in vitro* employs a combination of advanced atomic force microscopy (AFM) biomechanical techniques and conventional biological assays to assess the toxicity of AgNPs. The results reveal that AgNP exposure induces notable changes in both cellular biomechanics and biology. The biomechanical alterations, evidenced by reduced viscosity, coincide with significant DNA damage as demonstrated by increased tail DNA% and tail moment, indicative of genotoxicity. Additionally, gene expression analysis highlights shifts in the expression of apoptosis-related genes [63].

A different study examines the *in vitro* toxicity of AgNP on Human Skin Keratinocytes (HaCaT cells), using metabolomics to determine how AgNP characteristics affect cellular metabolism. By analysing AgNPs of various sizes and coatings, the study reveals that nanoparticle characteristics significantly dictate their impact on cell health, with smaller AgNPs prone to agglomeration and surface coatings like polyethylene glycol (PEG) and bovine serum albumin (BSA) showing reduced toxicity compared to citrate-coated AgNPs. Comparisons were made between the effects of AgNPs and ionic silver (Ag+) on cellular metabolism. While both AgNPs and Ag+ ions induced metabolic changes, some differences were observed, particularly in the effects on pathways like the Krebs cycle and energy metabolism, indicating that certain pathways' alteration by AgNPs isn't exclusively ROS-mediated [64].

Another study investigated the cytotoxicity of 20-nanometer AgNP utilizing Human Liver Hepg2 Cells and Human Colon Caco2 Cells as *in vitro* models. The research assesses cell viability, DNA damage, oxidative stress, and mitochondrial injury as indicators of toxicity. Results indicate that nanosilver exposure leads to concentration-dependent cytotoxicity, DNA damage, and mitochondria membrane potential reduction in both cell types, with HepG2 cells being more sensitive. Notably, the study challenges the common assumption that cellular oxidative stress is the primary mechanism behind nanoparticle-induced cytotoxicity, as no significant oxidative stress was detected. Instead, mitochondrial injury emerges as a potential mechanism [65].

In yet another *in vitro* toxicity study, the potential adverse effects of AgNPs using two human cell lines of Alveolar Basal Epithelial A549 Cells and Hepatoblastoma Hepg2 Cells to evaluate cytotoxicity, oxidative stress, and mitochondrial injury. The study reveals that AgNPs induce dose-dependent decreases in cell viability, accompanied by heightened oxidative stress, as indicated by increased reactive oxygen species (ROS) levels, lipid peroxidation, and induction of stress-responsive proteins.

The decline in mitochondrial membrane potential (MMP) is observed alongside cellular AgNPs uptake, both correlating with oxidative damage and mitochondrial impairment. These findings suggest a sequence where oxidative stress triggers MMP collapse and DNA damage, culminating in mitochondrial dysfunction and cell death [66].

In an additional investigation, assessment of the cytotoxicity, inflammatory response, and skin penetration of AgNPs in human epidermal keratinocytes (HEKs) and porcine skin was done. Unwashed AgNPs significantly reduced HEK viability in a dose-dependent manner and induced proinflammatory cytokine release (IL-1β, IL-6, IL-8, TNF-α). In contrast, washed and carbon-coated AgNPs showed no significant toxicity. AgNPs were localized in HEK cytoplasmic vacuoles, while porcine skin exhibited focal inflammation and AgNP deposition in the upper stratum corneum. Despite no visible irritation, microscopic changes suggest potential skin penetration and inflammatory risks of unwashed AgNPs in consumer products. [67].

Using an array of *in vitro* models, including THP1 Cells, Human Whole Blood, and Enriched Peripheral Blood Monocytes in a separate research, the immunotoxic and immunomodulatory effects of AgNPs were assessed. The research reveals that AgNPs induce dose-dependent proinflammatory cytokine production, enhance immune responses to classical stimuli like LPS, and impact B cell activation. While AgNPs do not significantly affect certain T cell responses, they reduce IL-10 release.

Table 3. Some recent *in vitro* nanotoxicological studies of AgNPs in different cell lines.

Size and type of AgNPs	Test System and Dose	Assay Type & Results	Ref.
16.3 nm- Citrate AgNP and 5.9nm- (polyvinyl alcohol) PVA AgNP	HepG2 (Human hepatoblastoma cell line); 0-100 mg/L citrate stabilized AgNP and 10mg/L PVA-AgNP	MTT assay detected that silver ions and citrate-coated AgNPs reduced cell viability in a dose-dependent manner.	[75]
Hydrodynamic diameter of 70 ± 5 nm	NIH-3T3 (Fibroblasts); A-549 (Human lung adenocarcinoma epithelial cell line); PC-12 (Rat adrenal pheochromocytoma cell line); HEP-G2 (Human hepatocellular carcinoma cell line)	MTT assay detected that AgNPs are considerably more toxic than silver ions. PC-12 and NIH-3T3 are comparatively more sensitive expressing a toxic response.	[76]
Citrate-coated AgNPs; 61.2 ± 33.9 nm	PK15 (Porcine kidney cell line); Concentrations- 50mg/L AgNPs and 50mg/L Ag^{+}	MTT assay detected a dose-dependent decrease in the number of viable Pk15 cells. Comet assay revealed that AgNP in higher concentrations was able to induce genotoxicity in Pk15 cells.	[77]
13.2±4.72 nm	HeLa (adhesive cells) and U937 (suspension cells); AgNP concentration- 0.5, 1.0, 2.0, 4.0, and 8.0µg/mL	MTT assay results confirmed that AgNP exhibited strong toxicity toward both tumor cells types in the concentration range studied.	[78]
27 nm	SVK14- (Human keratinocytes); NIH3T3- (Mouse fibroblasts cell line)	MTT assay detected higher sensitivity to AgNPs and Ag^{+} in NIH3T3 than in SVK14 cells. 'Annexin V-Cy3' evaluation detected comparatively increased number of apoptotic and necrotic cells in the NIH3T3 cell line and have larger DNA damage.	[79]
12–50 nm	Hep-G2- (Hepatic cancer cell line); AgNP concentrations- 0.01; 0.1; 0.2; 0.5 and 1.0 µM	Assessment of the cytotoxic effect of AgNPs on Hepatic cancer cells (Hep-G2) by cellular density measurement using a Neubauer chamber revealed that the surface modified AgNPs (using Mora leaf) showed no cytotoxicity at the concentration ranging from 0.01 µM to 1.0 µM on the Hep-G2 cell line.	[80]
30 nm citrate coated AgNPs and 30nm poly(ethylene glycol) coated PEG-AgNP	HaCaT- (Human keratinocyte cell line); AgNP concentrations- 0, 0.5, 5, 10, 25, 50, 75 and 100mg/mL	The results showed that the coating molecules per se were not cytotoxic & citrate-coated AgNPs and PEG-coated AgNPs decreased cell proliferation and viability and that the former is more toxic to the cells than the latter.	[81]
30.71 nm	HepG2- (liver hepatocellular adenocarcinoma); AgNP concentrations- 0, 25, 50, 75 and 100 µg/mL	MTT results revealed cytotoxic effects due to ROS production with decreased cell viability in a dose-dependent manner and the induction of cell apoptosis as well.	[82]
10 nm	HGF-1 (Human gingival fibroblast cells); AgNP Concentrations- 5, 10, 20, 40, 60, 100 µg/mL; AgNP types used; uncapped (AgNPs-UC), Lipoic acid capped (AgNPs-LA) and polyethylene glycol capped (AgNPs-PEG)	MTT assay results revealed that AgNPs-LA and AgNPs-PEG demonstrated lower cytotoxicity against HGF-1 as compared to AgNPs-UC.	[83]

The study emphasizes the role of culture conditions and protein corona formation in influencing AgNP-induced immune responses. The models proposed in the study offer insights into NP targeting the immune system and suggest that the whole blood assay is the closest representation of *in vivo* conditions for evaluating immunotoxic effects [68]. AgNPs exhibit concentration- and time-dependent inhibition of HaCaT Keratinocyte Cell viability, causing reduced proliferation. While internalization is limited, a short exposure to AgNPs induces long-lasting antiproliferative effects, suggesting potential skin toxicity concern for products containing AgNPs [69].

The impact of AgNPs on NIH 3T3 Mouse Embryonic Fibroblast Cell was evaluated and it was found that it induced oxidative stress, reactive oxygen species (ROS) generation and upregulation of Heme oxygenase 1 (HO-1) expression. The research demonstrates that AgNPs trigger both apoptosis and autophagy, with autophagy potentially playing a significant role in their response to nanoparticle toxicity. The findings highlight that monitoring autophagy could serve as a useful indicator for nanoparticle-induced cellular damage [70].

Exposure to AgNPs harmed Human Umbilical Vein Endothelial Cells (HUVECS), potentially contributing to early atherosclerosis. AgNPs caused reduced cell growth, membrane damage, and increased apoptosis. These nanoparticles also triggered inflammation, adhesion molecule expression, and chemokine release in the cells. Inflammatory cytokines, adhesion molecules, and chemokines were upregulated, promoting monocyte adhesion to endothelial cells. Dysfunctional endothelial cells were linked to the activation of the IKK/NF-kB pathway through oxidative stress. Antioxidant treatment mitigated the AgNPs-induced effects [71]. AgNPs affect human dermal and cervical cancer cells differently. Potential differences in cellular responses to AgNPs were analysed based on factors like cell origin and physicochemical characteristics of the nanoparticles. Different cytotoxicity assays show that HeLa cells are more sensitive to AgNPs than HaCaT cells. AgNPs induce cell damage, leading to reactive oxygen species (ROS) production and apoptosis [72]

AgNPs cause DNA damage and micronucleus formation in mammalian cells. Both nanosized Ag particles and released Ag+ ions contribute to this damage. Cysteine reduces some toxic effects, highlighting the role of Ag+ ions [73]. AgNPs are cytotoxic at higher doses than polystyrene nanoparticles (PS-NPs) and Ag_2CO_3. Even noncytotoxic doses promote cell proliferation. AgNPs cause DNA damage, especially micronuclei formation, due to nanosized Ag particles and ionic Ag+ release. While the toxicity of AgNPs is partially related to the release of ionic Ag+, the nanoparticles themselves also have unique toxic effects [74].

7. RISK ASSESSMENT AND REGULATION

Some nanoparticles arise naturally and others accidentally as a result of human activity while the manufactured nanoparticles are those that are purposefully produced by man and referred to as the Engineered Nano-Materials (ENM).The ENM brought about by the rapid development and integration of nanotechnology possess unique physicochemical properties that confer novel functionalities. The explosive growth of AgNPs in various industries has raised concerns about their potential impact on human health and the environment. The risk of occupational exposure to these substances in working environments is anticipated to increase with the increased use of ENM. Such exposure raises the concern of these nanomaterials entering and accumulating within the human body, potentially leading to harm or even fatalities. This is particularly worrisome for employees who might have prolonged and close encounters with these nanoparticles. Despite these dangers, research on determining the extent of exposure to humans and the environment from these nanomaterials is still in its early stages. As a result, developing effective exposure assessment strategies is a key focus area in the field of environmental health and safety (EHS), with a focus on real-time monitoring of the nanoparticles that people are exposed to. As a result, rigorous risk assessment and effective regulation have become essential to ensure the safe use of these nanomaterials. Among all types, the ENM are the primary cause of concern. Similar to how other chemical substances are assessed for risk, manmade nanomaterials also go through the following risk hazards chart mentioned in Figure 5. The risk assessment associated with AgNPs is a multifaceted process that involves evaluating their inherent characteristics, modes of exposure, and toxicological implications. AgNPs exhibit distinct properties owing to their small size, large surface area and high reactivity and accordingly have varied effects [84, 85]. These attributes can influence their interactions with biological systems and trigger biological responses.

7.1. Human health risks

Human exposure to AgNPs can occur through inhalation, dermal contact, and ingestion [86]. Figure 6 exhibits a schematic for the AgNPs exposure routes and associated nanotoxicity. Occupational exposure is particularly relevant in industries such as electronics, textiles, and medicine, where AgNPs are employed for their antimicrobial properties and catalytic activities [87]. The toxicological effects of AgNPs are dependent on factors including size, shape, surface coating, and concentration [88-90]**.** The evaluation of these effects involves assessing parameters such as cytotoxicity, genotoxicity, immunotoxicity, and oxidative stress. Studies have demonstrated that AgNPs can induce cellular damage by generating reactive oxygen species and disrupting cellular functions [91, 92]. The potential for AgNPs to accumulate in various tissues and organs raises concerns about long-term effects on human health [93, 94]. Biodistribution studies have demonstrated the ability of AgNPs to translocate to distant organs after exposure [95].

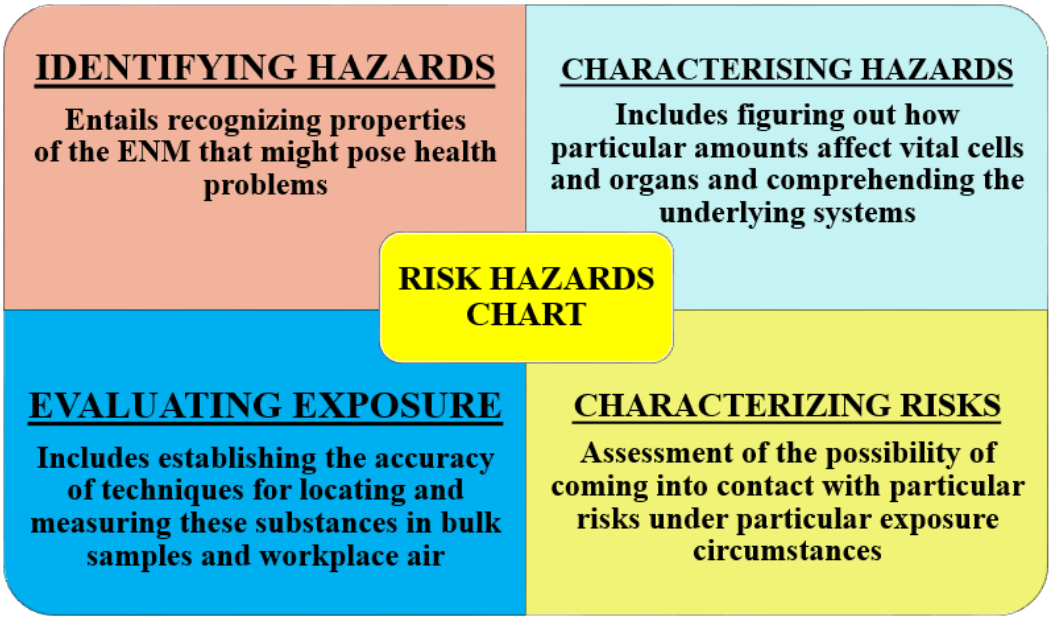

Fig. 5. Risk Hazards Chart.

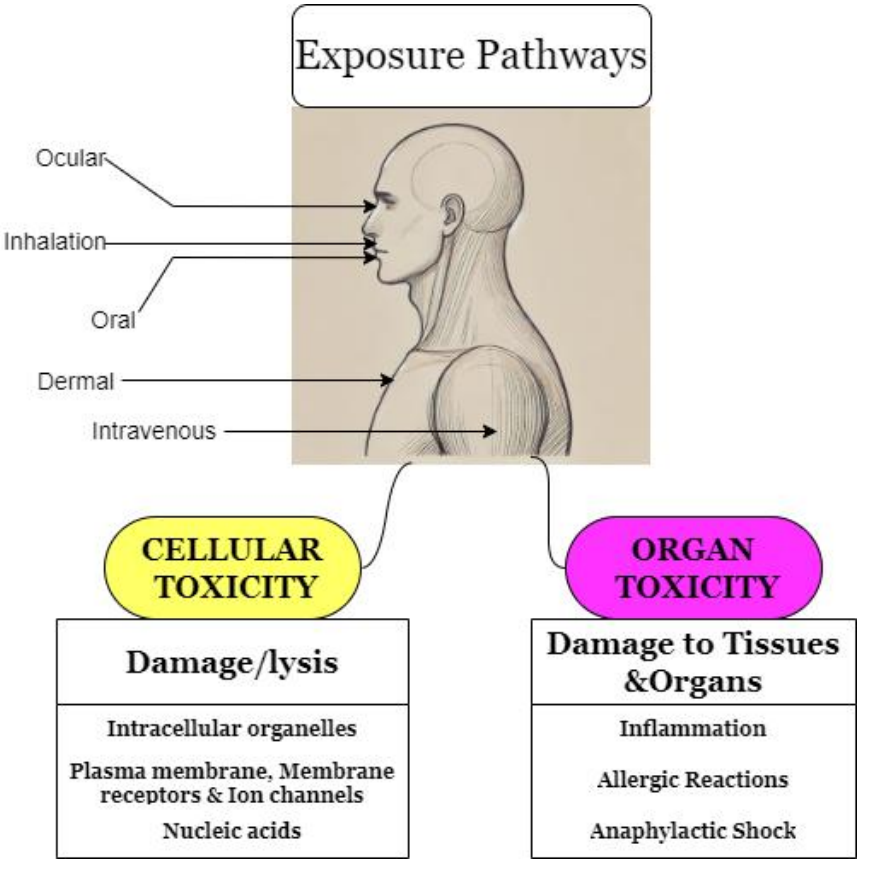

Fig. 6. Illustrative depiction of AgNP exposure routes and associated nanotoxicity.

7.2. Environmental risks

Because of unique physicochemical of AgNPs and their antimicrobial properties in particular, these are incorporated into a vast array consumer products [96] and, thus, have become a major concern for the environment regulators and consumer advocates. AgNPs can enter the environment through various routes, such as industrial effluents, sewage,

and agricultural runoff [97, 98]. These can accumulate in aquatic ecosystems and soil, potentially affecting non-target organisms [99]**.** Their release into water bodies can result in long-term ecological impacts. AgNPs' interact with aquatic organisms which has raised concerns about their potential ecotoxicity. Organisms at different trophic levels, from algae to fish, may be exposed to AgNPs, leading to potential alterations in ecosystems [100, 101]. They can affect aquatic life, disrupting various biological processes such as growth, reproduction, and behavior [102].

To gain a thorough understanding of AgNPs' effects in the environment, it's crucial to conduct comprehensive investigations into their absorption, distribution, metabolism, and excretion across different species, especially those from major phyla. Most of the research on the toxicity of AgNPs to aquatic and terrestrial organisms predominantly relies on controlled laboratory experiments involving a single strain of bacteria or fungi. Studies have demonstrated the toxicity of AgNPs to a range of organisms including bacteria, fungi, annual grass, green alga, vertebrates like zebra fish, and earthworms [103-106]. While some research has targeted fish, broader species-focused studies are needed. Evaluating the environmental risks of ENM like AgNPs can utilize established tiered approaches and regulatory frameworks, but adjustments in methodology considering the unique properties of these materials are necessary.

The risk management of AgNPs involves a multifaceted approach that encompasses regulations, exposure assessment, toxicity testing, risk communication, and the exploration of safer alternatives. Government agencies and international organizations play a pivotal role in developing regulations and guidelines that govern the production, use, and disposal of AgNPs. These regulations aim to ensure safe handling practices, appropriate labelling, and adherence to permissible exposure limits. The current regulatory framework for chemical risk assessment and management in the European Union, known as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), also covers ENM. One particular risk assessment challenge connected to ENM is the introduction of a sizable number of these qualitatively new compounds to be dealt with by the current risk assessment process, a challenge so big that the existing approach cannot deal with it. Therefore, new methods that are also trustworthy enough must be developed. Although the physicochemical properties of ENM are carefully taken into account by REACH when defining substances under the law, however, the true nature of ENM and the relationship between its properties and biological effects are not fully understood [107]. Research into alternative nanomaterials promotes safer substitutes [108]. By integrating these strategies, it's possible to harness the benefits of AgNPs while minimizing their potential adverse effects.

8. FUTURE DIRECTIONS

The rapid proliferation of AgNPs in consumer and industrial applications demands a forward-looking research agenda to address unresolved questions about their long-term safety. First, standardized methodologies for genotoxicity testing must be established to harmonize data across studies. Current assays (e.g., comet assay, micronucleus test) often yield variable results due to differences in experimental conditions, AgNP characterization, and cell models. Collaborative efforts between regulatory bodies and researchers should define protocols for particle characterization, exposure durations, and endpoint measurements to ensure reproducibility.

Second, mechanistic studies must delve deeper into the molecular pathways of AgNP-induced genotoxicity. While ROS generation and oxidative stress are well-documented, the roles of epigenetic alterations, mitochondrial DNA damage, and bystander effects in neighboring cells remain underexplored. Advanced techniques such as single-cell sequencing and high-resolution microscopy could unravel these complexities. Additionally, the interplay between AgNPs and cellular repair mechanisms, such as base excision repair (BER) and homologous recombination (HR), warrants investigation to assess cumulative genetic damage.

Third, longitudinal *in-vivo* studies are critical to evaluate chronic exposure effects, particularly in vulnerable populations (e.g., immunocompromised individuals, pregnant organisms). Research should prioritize environmentally relevant exposure scenarios, including low-dose, multi-generational studies in model organisms, to mimic real-world conditions. Concurrently, ecological risk assessments must expand beyond laboratory settings to field studies, tracking AgNP fate in soil, water, and food chains.

Fourth, the development of safer-by-design AgNPs is imperative. Surface functionalization with biocompatible coatings (e.g., polyethylene glycol, chitosan) and hybrid nanocomposites could reduce ROS generation while retaining antimicrobial efficacy. Computational modeling and structure-activity relationship (SAR) studies may accelerate the discovery of low-toxicity variants.

Finally, robust regulatory frameworks must evolve alongside scientific advancements. Policymakers should integrate nano-specific considerations into existing chemical safety guidelines (e.g., REACH, EPA regulations), emphasizing lifecycle analysis and occupational exposure limits. International collaboration is essential to align standards and promote transparency in AgNP usage across industries. By addressing these priorities, the scientific community can balance innovation with precaution, ensuring the sustainable advancement of nanotechnology.

9. CONCLUSION

This review consolidates compelling evidence that AgNPs pose measurable genotoxic risks to humans and ecosystems, mediated through oxidative stress, DNA strand breaks, and chromosomal instability. While their antimicrobial and catalytic properties offer transformative benefits, the duality of AgNPs necessitates a precautionary approach to mitigate unintended consequences. Key findings underscore that

genotoxicity is influenced by nanoparticle size, surface chemistry, and exposure route, with smaller, uncoated AgNPs exhibiting heightened biological reactivity. The accumulation of AgNPs in vital organs, such as the liver and spleen, and their persistence in environmental matrices amplify concerns about long-term exposure. Despite progress, critical knowledge gaps persist. The extrapolation of *in vitro* data to *in vivo* systems remains challenging due to dynamic bio-nano interactions in complex organisms. Furthermore, the environmental transformation of AgNPs—through sulfidation, aggregation, or protein corona formation—may alter their toxicity profiles in unpredictable ways. These uncertainties highlight the need for interdisciplinary research integrating toxicology, materials science, and environmental chemistry. To translate evidence into action, we advocate for a tiered risk management strategy. Immediate steps include enforcing stringent labeling of AgNP-containing products and prioritizing occupational safety measures in manufacturing sectors. Mid-term goals should focus on developing rapid detection tools for environmental monitoring and public databases to track AgNP usage and incidents. Long-term vision entails fostering international consortia to harmonize regulatory standards and fund independent toxicity studies. Ultimately, the responsible deployment of AgNPs hinges on a dual commitment to innovation and vigilance. By advancing mechanistic research, adopting safer nanomaterials, and embedding precautionary principles into policy, society can harness the benefits of AgNPs while safeguarding health and ecological integrity. The path forward demands collaboration across academia, industry, and governance to navigate the complexities of nanotechnology's promise and perils

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Authors' contributions

All authors contributed equally to this work.

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