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ADVANCES IN Toxicology and Toxic Effects OPEN ACCESS JOURNAL

DOI: https://dx.doi.org/10.17352/atte

Review Article

Silver nanoparticles forensic uses and toxicity on vital organs and different body systems

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Received: 20 June, 2023
Accepted: 09 May, 2024
Published: 10 May, 2024

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Keywords: Forensic uses; Potential toxicity; Silver nanoparticles; Vital organs; Different body systems

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Abstract

This study aimed to investigate the forensic uses and potential toxicity of silver nanoparticles on vital organs and different body systems. A systematic review methodology was used to identify and critically evaluate the literature on the forensic uses of silver nanoparticles in different fields and to assess their potential toxicity on various vital organs and body systems. The study found that silver nanoparticles have potential forensic uses, particularly in forensic biology and forensic toxicology, but there are concerns about their potential toxicity. The study recommends further research on the mechanisms of toxicity of silver nanoparticles and the development of safe and effective strategies for their use in forensic science. The study's strengths include its systematic review methodology and use of multiple databases, while limitations include a limited time frame and focus on English language publications. Future research should focus on investigating the potential risks of silver nanoparticle exposure for forensic professionals.

Introduction

Silver nanoparticles are synthesized by various methods, including chemical reduction, electrochemical synthesis, and green synthesis using biological sources such as plants, bacteria, and fungi [1]. Their unique physical and chemical properties, including high surface area-to-volume ratio, strong plasmonic resonance, and antibacterial activity, make them suitable for a wide range of applications [2].

AgNPs have gained significant attention due to their potential uses in commercial applications. They exhibit special properties relative to their bulk components due to their unique physicochemical properties, including their small size, greater surface area, surface chemistry, shape, particle morphology, particle composition, coating/capping, agglomeration, rate of particle dissolution, particle reactivity in solution, efficiency of ion release, and type of reducing agents used for the synthesis of AgNPs. AgNPs are widely used in household utensils, food storage, healthcare, environmental, and biomedical applications such as wound dressings, surgical instruments, and disinfectants [3].

Silver nanoparticles (AgNPs) have been widely used in various applications, including medicine, electronics, and textiles, due to their unique physical and chemical properties [4]. In forensic science, AgNPs have been utilized as a powerful tool for trace evidence analysis and crime scene investigation. These nanoparticles have been shown to enhance the visualization of latent fingerprints on various surfaces [5] and could potentially provide valuable information for the identification of unknown substances found at a crime scene [5].

However, despite their widespread use, there is growing concern regarding the potential toxicity of AgNPs on different body systems, including the respiratory, cardiovascular, and

Citation: ALAtawi MK, AlAsmari AA, AlAliany AD, Almajed MM, Sakran MI (2024) Silver nanoparticles forensic uses and toxicity on vital organs and different body systems. Adv Toxicol Toxic Effects 8(1): 015-029. DOI: https://dx.doi.org/10.17352/atte.000018

nervous systems, as well as the liver and kidneys [6,7]. This is especially important in the context of forensic science, where forensic scientists and investigators may be exposed to AgNPs during the collection and analysis of evidence.

In forensic science, AgNPs have been shown to improve the visualization of latent fingerprints on various surfaces, including glass, plastic, and metal [8]. The use of AgNPs in fingerprint analysis has been shown to provide better results than traditional fingerprint development methods, such as ninhydrin and cyanoacrylate fuming. In addition to fingerprint analysis, AgNPs have also been utilized for the detection of gunshot residue [9] and the identification of drugs and explosives [8].

Despite the potential benefits of AgNPs in forensic science, there is growing concern regarding their potential toxicity on different body systems. Several studies have reported that AgNPs can cause adverse effects on the respiratory, cardiovascular, nervous, and immune systems, as well as the liver and kidneys [10]. The toxicity of AgNPs is influenced by various factors, including their size, shape, concentration, and surface chemistry [11]. Furthermore, the potential for exposure to AgNPs during forensic investigations highlights the need for a better understanding of their potential toxicity [8].

The use of silver nanoparticles (AgNPs) has increased in recent years due to their unique physical and chemical properties, which make them valuable in various fields, including consumer products and medical devices [12]. However, there is limited information on the potential health effects of these nanoparticles, especially on the cardiovascular system [8].

Literature review

AgNPs have shown numerous applications in various fields, from food preservation to biomedicine. However, AgNPs' toxicity remains a significant concern and requires a thorough understanding of their interaction with biological systems. AgNP toxicity has been reported in many studies on different cell lines, organs, and animal models, leading to abnormal cellular processes, inflammation, oxidative stress, and apoptosis. This paper provides an overview of the toxic effects of AgNPs on various human cell lines and animal models.

Exposure to AgNPs causes toxicity in different cell lines and animal models. The effect of AgNPs on the nervous system was studied on human neural stem cells (NSCs) [13]. Results showed an increase in mitochondrial production of reactive oxygen species (ROS), leading to apoptosis and necrosis of NSCs. Moreover, AgNPs negatively affected mature neurons by triggering abnormalities in cytoskeleton formation, presynaptic and postsynaptic proteins, and mitochondrial function, leading to cell death [14]. Mouse neural cells exposed to AgNPs induced the secretion of pro-inflammatory cytokines and the deposition of amyloid beta (Aβ) [15]. Human neuronal SHSY5Y cells and human glial D384 cells exposed to AgNPs showed toxic effects at low doses (0.5 µg/mL) and short-term (4–48 h, 1–100 µg/mL) or long-term (up to 10 days, 0.5–50 µg/mL) exposure (Coccini, et al. 2014). Additionally, exposure to nanoparticles reduces the expression of postsynaptic proteins, changes the morphology of astrocytes, causes neurodegeneration, and reduces the growth of neurites (Repair, et al. 2018).

Moreover, broken skin or skin with cuts and wounds may give rise to easier and direct absorption of nanoparticles into the bloodstream and translocation in the body. The fate and effects of these particles on and within the skin and human body are not clearly understood [7]. Thus, this may be an avenue for further investigation into the potential cardiovascular effects of AgNPs [16].

Study rational

AgNPs have shown great promise for forensic applications, particularly in the analysis of trace evidence. However, the potential toxicity of these nanoparticles on different body systems cannot be overlooked. Therefore, it is essential to evaluate the potential toxic effects of AgNPs and develop strategies to minimize their potential harm in forensic science.

General objective

The general objective of this study is to investigate the forensic uses and potential toxicity of silver nanoparticles on vital organs and different body systems.

Specific Objectives

1. To identify and critically evaluate the available literature on the forensic uses of silver nanoparticles in different fields such as forensic biology, forensic chemistry, and forensic toxicology.
2. To assess the potential toxicity of silver nanoparticles on various vital organs and body systems through a systematic review of the literature.
3. To evaluate the possible adverse effects of silver nanoparticles on human health, including the mechanisms of toxicity, to provide insight into their safe use and handling.
4. To provide recommendations for future research on the forensic uses and toxicity of silver nanoparticles.

Study significance

This study will provide insights into the forensic uses and potential toxicity of silver nanoparticles, which will be useful for researchers, practitioners, and policymakers in various fields. The findings of this study may also contribute to the development of guidelines and regulations for the safe use and handling of silver nanoparticles in forensic applications.

Methodology

Type of the study

Review article.

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Study duration

The study was conducted during the period from 1st December 2022 to 31st April 2023.

Search strategy

The methods used in this study were adapted from a similar review article, which described the process of identifying relevant literature on the toxicological aspects, safety assessment, and green toxicology of silver nanoparticles. The search was conducted using various databases such as Scopus, Google Scholar, Web of Science, and PubMed. The search was limited to studies published in English between January 2015 and March 2023. The search was conducted on April 5, 2023. The primary terms used to identify relevant articles were forensic biology, forensic chemistry, forensic toxicology, silver nanoparticles, nanotechnology, and nanoparticle toxicity. Both scientific sources and "grey" literature such as internet forums were searched during the data collection process.

Selection criteria

In the first step of the selection process, titles and abstracts of the identified studies were screened to exclude irrelevant studies. Then, the full texts of the remaining studies were analyzed to assess their relevance to the research problem. Flawed and illogical analyses were critically identified, and studies that did not meet the validity criteria were rejected. The studies selected were analyzed in detail, and relevant data were extracted for further analysis.

Strategy for data synthesis

To classify and present the results, the researchers focused on studies that discussed the forensic uses and potential toxicity of silver nanoparticles. Specifically, they considered studies that investigated the toxicological aspects of silver nanoparticles, the safety assessment of silver nanoparticles in different fields, and the green toxicology of silver nanoparticles. The toxicological aspects of silver nanoparticles covered in vitro/in vivo toxicology studies, toxicity of silver nanoparticles against immune cells and normal human cell lines, adverse effects of silver nanoparticles, organ toxicity of silver nanoparticles, toxicity mechanisms, non-oxidative stress-related mechanisms, and complex toxicity evaluation of silver nanoparticles. Safety assessment of silver nanoparticles covered the use of silver nanoparticles in cosmetic products, while green toxicology covered the environmental impact of silver nanoparticles.

The extracted data were analyzed and presented in three paragraphs for each aspect to ensure proper readability. The first paragraph presented an overview of the studies and their findings, while the second paragraph focused on the implications of the studies for the forensic uses of silver nanoparticles. The third paragraph discussed the potential toxicity of silver nanoparticles on vital organs and different body systems and provided recommendations for future research.

Risk of bias assessment

To ensure the validity and reliability of the study, the articles were independently reviewed by each author at different times. Disagreements in the selection of articles were resolved through discussion until a consensus was reached. Additionally, the quality of the selected studies was assessed, and the relevant data were extracted by two independent reviewers to ensure consistency.

Study results

Synthesis of sliver nanoparticles

The silver nanoparticles are the most appealing due to their high surface area-to-volume ratio. The surface of the nanoparticles is so important and should be controlled since a change in the size of the surface can generate a change in the physical and chemical properties of the nanoparticles. Nanoparticles have been of great scientific value since they came to reduce the gap between the bulk materials and the atomic and molecular structures. The properties are directly correlated with size when the particles are between 1 and 100 nm in size. By adjusting the size and shape of the particles, one can control the properties of the particles, including temperature, redox potential, color, conductivity, chemical stability, electrical qualities, optics, and others [8].

Numerous studies have demonstrated that the experimental conditions of their synthesis, the kinetics of the reaction, the interaction of the ions with the reducing agents, and the absorption processes of the stabilizing agent used have a significant impact on the size, morphology, stability, and properties specifically of the nano silver particles. Thus that the particular control over the desired silver nano particle's shape, size, and dispersion rests with the synthesis technique that is chosen. The majority of chemical synthesis relies on reduction reactions of metallic silver salts, but before choosing a method, one must decide on the desired shape of the nanoparticle, such as spherical, triangular, cubic, pyramidal, rodshaped, or cylinder-shaped. This is because the speed of the reaction and the interaction with the stabilizers determine the shape of the nanoparticle [17].

Physical method

Evaporation-condensation, laser ablation, electrical irradiation, gamma irradiation, and lithography are the most significant physical processes used to create silver nanoparticles. In their unique preparation techniques for metal colloids in inorganic solvents without the use of chemicals like redox reagents, polymers, electrolytes, glue, or other types of colloid stabilizers, Kimura and Bandow explored the measurement of the optical spectra of numerous metal colloid solutions. To evaluate the synthesis of silver NPs, three different preparation techniques—matrix isolation, gas flow-cold trap, and gas flow solution trap—were employed (Tsuji, et al. 2020).

Another approach to research the production of silver nanoparticles (Ag-NPs) is the laser ablation process, which

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has a variety of applications. A new practical and effective way to create and generate metal colloids without the need for chemical reagents is through the laser ablation approach. By varying the number of laser pulses, this technique aids in controlling the size of colloidal particles [15].

NLS, or nanosphere lithography, is a quick and low-cost nanofabrication technique that may be used to create a wide range of nanoparticle (NP) forms and neatly organized 2D NP arrays. Jensen et al. investigated the impact of solvent on the optical extinction spectrum of four different samples of NP arrays and periodic arrays of surface-confined silver nanoparticles produced by NSL. Four different samples of nanoparticle arrays were examined by Jensen et al.; three samples yielded nanoparticles with truncated tetrahedral shapes but with different out-of-plane heights, and one sample included oblate ellipsoidal nanoparticles [15].

Chemical methods

The chemical, physical, optical, and electrical properties of nanomaterials are significantly influenced by their size, shape, and surface morphology. One of the most popular techniques for creating silver nanoparticles using both inorganic and organic reducing agents is chemical reduction. In general, various reducing agents are used to reduce the silver ions (Ag+) in aqueous or nonaqueous solutions, including sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly(ethylene glycol)-block copolymers, hydrazine, and ammonium formate [18].

Using reducing agents such as ascorbic acid, sodium citrate, NaBH4, thiosulfate, and polyethylene glycol, the spherical silver nanoparticles were created. Additionally, by interacting with particle surfaces, surfactants like citrate, Polyvinylpyrrolidone (PVP), Cetyltrimethylammonium Bromide (CTAB), and Polyvinyl Alcohol (PVA) help stabilize particle development and guard against sedimentation and agglomeration [19].

Silver nanorods were created by Zhang et al. via photoinduced synthesis. In the first step, silver nitrate, Bis(P-Sulfonatophenyl)-Phenyl Phosphine dihydrate dipotassium salt (BSPP), trisodium citrate, and sodium hydroxide solutions were exposed to 254 nm light to produce monodisperse spherical seed nanoparticles. Following the injection of silver seeds into a growth medium comprising silver nitrate and sodium citrate, silver nanorods were produced in the solution. A halogen lamp and a bandpass filter were then used to selectively tune the radiation for 24 hours. With this photomediated technique, the architectural characteristics of the produced silver nanostructures could be controlled elegantly [18].

The aqueous solution containing AgNO3 was made by Ajitha et al. using sodium citrate dihydrate as a stabilizer. The aforementioned solution was then abruptly mixed with a solution of sodium borohydride (a reducing agent). Light yellow was used as the new color of the solution. On a magnetic stirrer, the entire solution was heated while being continuously stirred. To dissolve CTAB, a solution was made by boiling and stirring on a magnetic stirrer. Next, a solution of ascorbic acid and AgNO3 were added. The seed solution was then added, and finally, a few drops of NaOH were added to maintain a consistent pH. Temperatures for the synthesis ranged from 30 °C to 70 °C [20].

The reduction of silver nitrate with ethylene glycol and Polyvinylpyrrolidone (PVP) led to the creation of cubic silver nanoparticles. Ethylene glycol with hydroxyl groups serves as a solvent and a reducing agent in the polyol process. A capping chemical called polyvinylpyrrolidone was utilized to create the cubic form. The form of the final product is determined by the molar ratio of PVP and silver ions.

A water, surfactant, and oil mixture, or water, surfactant, and co-surfactant mixture, is referred to as a microemulsion. For the creation of the microemulsion during the manufacture of the silver nanoparticles, a variety of surfactants are available. In general, many surfactants can be used to create microemulsions, including nonionic surfactants like Triton X-100, cationic surfactants like cetyltrimethylammonium bromide, and anionic surfactants like bis(2ethylhexyl) sulfosuccinate, sodium dodecyl benzene sulfonate, and lauryl sodium sulfate. Surfactant-coated water droplets serve as micro-reactors and provide a special microenvironment for the creation of nanoparticles [21].

In contrast to the traditional heating approach, the microwave synthesis methods allow the reduction of the silver nanoparticles with variable rate microwave radiation. Quick results are produced via microwave-assisted technology, which reduces the time it takes for chemical reactions from hours or days to minutes. Additionally, microwave irradiation facilitates the ripening of materials without aggregation and provides homogeneous heating for the creation of metallic nanoparticles.

Green method

Due to the increased demand for ecologically friendly material synthesis technologies, biosynthesis of the nanoparticles has attracted a lot of attention. To give an example, a lot of work has gone into developing green synthetic processes for inorganic materials, particularly metal nanoparticles, which use microbes and plant extracts. While the study of microorganisms like bacteria, algae, yeast, and fungi for the intra and extracellular synthesis of metal nanoparticles has continued to this point, the use of plant parts in analogous nanoparticle synthesis methodologies is a fascinating possibility that is currently being investigated. The manufacture of silver nanoparticles has been successfully carried out using a variety of bacterial species, including Bacillus amyloliquefaciens, Acinetobacter calcoaceticus, Pseudomonas aeruginosa, Escherichia coli, and Bacillus licheniformis.

The use of readily accessible plants for nanoparticle production has the advantage that they include a wide range of active functional groups that can encourage the reduction of silver ions. The majority of plant parts, including leaves, roots, latex, bark, stems, and seeds, are used in the creation

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of nanoparticles. Biomolecules like polysaccharides, tannins, saponins, phenolics, terpenoids, flavones, alkaloids, proteins, enzymes, vitamins, amino acids, and alcoholic components are important elements that ensure the decrease of nanoparticles [21].

The step-by-step process for creating plant-based nanoparticles begins with the collection of the desired plant part from the available locations, followed by thorough washings with tap and distilled water to remove any plant impurities and sterile distilled water to flush out any associated wastes. After being cleaned, dried, and kept in the shade for 10 to 15 days, the plant is then blended into a powder. An approximate quantity of the dry powder is cooked in deionized distilled water to make the plant broth (Mikac, et al. 2014).

The extraction that results is then extensively filtered until no insoluble material is left in the broth. Then, a few milliliters of the plant extract are added to the 1 mM-concentrated silver nitrate solution. The solution's color alteration is evidence that Ag+ has been converted to Ag0. Transmission electron microscopy or scanning electron microscopy, along with UV-visible spectroscopy, are used to confirm how it formed (Gebeyehu, et al. 2017).

To create Ag-NPs with various morphologies, a wide variety of plant extracts have been employed in the synthesis of silver nanoparticles. In a plant-mediated synthesis of Ag-NPs, where the plant extract acts as the reducing agent, TEM, and SEM experiments have demonstrated that the presence of a reducing agent shapes the nanoparticle during its growth. In addition to controlling size and form, Ag-NPs are given plant antibacterial characteristics by using medicinal plants in their manufacture. One-step hydrothermal preparation of silver nanoparticles that is efficient against both gram-positive (Streptococcus epidermis) and gram-negative (Pseudomonas aeruginosa) bacteria was described by Tippayawat et al (Tippayawat, et al. 2016).

General effect on body systems and organs

In the body of a person, silver particles can accumulate and result in argyria, a blue-gray discoloration. There are two recognized excretion pathways: urine and feces. Following an abortion method including the intrauterine delivery of 7 g silver nitrate (64 mg silver/kg body weight), acute human death has been noted. It has been documented that exposure to silver ions, metallic surfaces, and nanocrystalline silver can cause localized argyria. Ionic and nanocrystalline silver was used in people at cumulative dosages ranging from 70 to 1500 mg/kg body weight without causing generalized argyria. Skin irritation is seen to have a low possibility with silver. There have been reports of cases of allergic contact dermatitis and eye discomfort. AgNPs may be genotoxic, but further information is needed to determine whether it is also carcinogenic. There have also been reports of hepatic, renal, neurological, and hematological toxicity [21]

In the digestive system, AgNPs are absorbed through the gastrointestinal tract, enter the bloodstream, and accumulate in organs such as the brain, lungs, liver, kidneys, and testes [22]. Consistent oral administration leads to organ toxicity and inflammatory responses. Treatment with AgNPs by the oral route altered the function of the small intestine mucosa, leading to the devastation of the microvilli and reducing absorption through the intestinal epithelium, resulting in weight loss in mice [23]. Silver nanoparticles penetrated the cell membrane and the mitochondria, causing oxidative stress, and inflammation, and leading to apoptosis during incubation with human gingival fibroblast cells [24]. The liver of offspring exposed to AgNPs induced oxidative stress and apoptosis [25]. In vivo, studies showed that AgNPs induced changes in the architecture of histological sections of the liver such as vacuolization and swelling of hepatocytes, edema around the blood vessel, and apoptosis [23,26].

Ingestion of silver nanoparticles has been shown to cause organ toxicity and inflammatory responses, with smaller nanoparticles accumulating in organs such as the brain, lungs, liver, kidneys, and testes. Oral administration of AgNPs has been shown to alter the function of the small intestine mucosa, leading to weight loss in mice [23]. Moreover, the toxic effects of AgNPs on the nervous system have been observed in human neuronal cells, leading to neurodegeneration and reducing the growth of neurites (Repair, et al. 2018).

Studies have shown that nanoparticles, including AgNPs, can be quickly translocated from the lungs into the circulation and transported to secondary target organs such as the heart [27]. Therefore, there are concerns about the potential adverse effects of AgNPs on cardiovascular health, including arrhythmias, thrombosis, hypertension, and myocardial infarction [28].

Furthermore, a study compared the toxic effects of AgNPs and titanium dioxide nanoparticles on testicular cells and found that AgNPs caused more damage to the cells [29]. This finding supports the need for further investigation into the potential adverse effects of AgNPs on different body systems, including the cardiovascular system.

Another study investigated the effects of combustion-derived nanoparticles (CDNPs) on cardiovascular health and found that exposure to CDNPs is associated with multiple adverse cardiovascular effects in both healthy and susceptible individuals. This study highlights the potential harmful effects of nanoparticles in general on cardiovascular health, including AgNPs [19].

Effect on liver

A previous study investigated the toxicity of silver nanoparticles on the liver, kidneys, brain, and spleen in mice to explore the possible mechanisms behind it. The silver nanoparticles-treated group had significantly higher serum levels of liver transaminases, urea, and creatinine, as well as significantly higher levels of lipid peroxides and TNF-, with a significant decrease in serum levels of reduced glutathione, superoxide dismutase, and total antioxidant capacity. Histopathology of the organs demonstrated tissue damage in

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the AgNPs-treated group, as evidenced by disrupted organ architecture, congestion, increased inflammatory cells, and symptoms of necrosis [6].

A similar study investigated the cytotoxic effects of AgNPs on the liver primary cells of mice, as well as the human liver HepG2 cell. Silver nanoparticles had cytotoxic effects on the HepG2 cell line and primary liver cells of mice. The results illustrated that nano-silver had a 44 times stronger inhibitory effect on the growth of cancerous cells (HepG2 cell line) compared to the normal cells (primary liver cells of mice) [30].

A study investigated and compared the effect of silver nanoparticles' particle size in terms of their potential hazard, as well as their potential protective effect in an LPS-induced hepatotoxicity model. The LPS elicited marked hepatic tissue injury manifested by elevated cytokines and proinflammatory markers. Both small silver nanoparticles and large silver nanoparticles efficiently attenuated LPS hepatotoxicity, mainly by preserving the cytokines' level and diminishing the inflammatory pathways [31].

The fact that some manufactured nanoparticles are redox active is the main toxicological worry related to another study we examined the impact of silver nanoparticles (40 and 80 nm) on the bioenergetics of the mitochondria in the rat liver. The addition of either 40 or 80-mn silver nanoparticles causes changes in the membrane potential and respiration capabilities of Wistar rat liver mitochondria. The findings demonstrate that silver nanoparticles affect mitochondrial function, primarily through changes in the permeability of the mitochondrial membrane. As a result, the oxidative phosphorylation system is uncoupled. As a result, the toxicity brought on by exposure to silver nanoparticles may be mostly attributed to mitochondrial toxicity [32].

The harmful effects of nanosilver (Ag-NPs) on liver function and several blood parameters of male and female Mus musculus mice were investigated. The levels of red blood cells (RBC), hemoglobin (Hb), and hematocrit (Hct) did not differ substantially between the control and Ag-NP-treated animals. The levels of liver enzymes changed dramatically between the treatment and control groups, with mice treated with Ag-NPs having significantly higher levels of ALT and AST at both doses (p 0.05). The results were unaffected by sexuality. Oral administration of Ag-NPs caused changes in blood chemistry and hepatotoxicity, as evidenced by elevated serum activity levels of both AST and ALT and histological liver damage [33].

Effect on respiratory system and lungs

Regarding the respiratory system, research has shown that exposure to AgNPs caused aberrations of chromosomes and altered energy-dependent DNA repair mechanisms in human lung fibroblast cells (AshaRani, et al. 2009). Silver nanoparticles have also been shown to induce genes responsible for cell cycle progression, causing chromosomal damage, cell cycle arrest, and cell death in human BEAS-2B cells [34]. In addition, AgNPs have been shown to affect the cardiovascular system in zebrafish, causing abnormal heart morphology, cardiac arrhythmias, and slow blood flow [35].

In the respiratory system, normal human lung fibroblast cells (IMR-90) exposed to AgNPs indirectly induced ROS production or decreased ATP production, resulting in aberrations of the chromosomes and altering energy-dependent DNA repair mechanisms (AshaRani, et al. 2009). The toxicology research of AgNPstreated lung cell line showed that small (10 nm) silver nanoparticles compared to larger sizes were more toxic [36]. Exposure to 56 nm size AgNPs caused upregulation of pro-inflammatory cytokines (IL-1β and IL-6) in human lung epithelial cells (A549) [37]. Furthermore, silver nanoparticles induced genes responsible for cell cycle progression and, therefore, caused chromosomal damage, cell cycle arrest, and cell death in human BEAS2B cells [34].

The distribution of silver (Ag) into distant organs as a result of the application of Ag nanoparticles (Ag-NP) to the lung is still unknown and was studied in rats using imaging methods. Dose-finding tests were conducted with 50 nm or 200 nm Polyvinyl Pyrrolidine (PVP)-coated Ag-NP in vitro and female rats that received Ag-NP through intratracheal instillation. Following a 75 g lung dosage, Ag accumulation was considerable in the liver and other peripheral organ homogenates. The use of quantitative laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) in conjunction with enhanced dark field microscopy and autometallography revealed focal accumulations of Ag and/or Ag-NP in sections of peripheral organs: mediastinal lymph nodes contained AgNP, particularly in peripheral macrophages, and Ag in argyrophilic fibers. Ag had collected in the kidney's proximal tubuli, but there was no Ag in the renal filter structures. There were additional isolated localizations in immune cells from the liver and spleen [38].

A previous study summarised the last ten years' reports on the toxicity of AgNPs in cellular respiratory system models (e.g., mono-culture models, co-cultures, 3D cultures, ex vivo and in vivo) reported that, in vitro, Ag nanoparticles (Ag-NP) are cytotoxic to alveolar macrophages, causing inflammation and, at higher concentrations, genotoxicity in the lung, as evidenced by DNA double-strand breaks. Regardless of the dose-dependent quality of effects on the lung, Ag-NP in the lung causes Ag to be distributed to distant organs, where it can be detected as focal accumulations. Surprisingly, DFM did not corroborate the particle character of numerous silver depots, even though they were predominantly detected in macrophage-like cells [39].

A study evaluated the toxic effects of two different doses of 0.5, 10 mg/kg of AgNPs (35±8.5nm) on the lungs of adult male albino rats following 30 days of oral administration and also assessed the protective role of green tea extract (GT). In all groups, normal daily activity was observed. A statistically significant rise in mean body weight in AgNPs-treated groups, but a nonsignificant increase in GT and GT+ AgNPs-treated groups at the end of the trial compared to the starting value. AgNPs decreased considerably (CAT) while increasing SOD levels. GT considerably increased relative lung weight while AgNP groups showed no significant increase when compared to controls. Histological examination of lung tissue revealed histological alterations in groups treated with AgNPs, which

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were more pronounced in high doses (10mg), including alveolar wall thickening, alveolar destruction, dilated alveoli, mononuclear cellular infiltration associated with marked collagen deposition, and weak immunoexpression for surfactant protein B (Ahmed el al., 2016).

A549 lung epithelial cells were treated to 15 nm NM300K or 59 nm PVP-coated Ag-NPs under two distinct circumstances in another investigation. The cells received the same total sub-lethal concentration of Ag-NPs in both situations, but the dose was either supplied over 24 hours (acute exposure) or split over four consecutive days (repeated exposure). Repeated exposure of A549 cells to Ag-NPs had a greater effect on DNA integrity than acute exposure. Nonetheless, repeated exposure to a low concentration of Ag-NPs disrupted cell metabolism and stopped cell cycle progression, showing that both exposure regimes are harmful [40].

Alveolar macrophages respond with increased pro-inflammatory mediator production of pro-inflammatory mediators (TNF-α, MIP-2, and IL-1β) after exposure to AgNP [5]. The effect of human macrophages on 5 nm or 100 nm AgNPs showed that smaller nanoparticles induced stronger expression of proinflammatory cytokines (IL-8) and stress genes (heme oxygenase-1 and heat shock protein-70) than exposure to 100 nm AgNP [41].

[42] The pulmonary toxicity of silver nanoparticles (AgNP) may be influenced by lung inflammation as well as particle size and surface chemicals. In Brown-Norway (BN) and Sprague-Dawley (SD) rats, the effects of intratracheally delivered AgNPs were compared. While SD rats showed a neutrophilic response at day 1, which was strongest for the 20nm citrate-capped AgNPs, BN rats showed both an eosinophilic and neutrophilic response. On day 1, BN and SD rats had higher levels of eosinophilic cationic protein in their bronchoalveolar lavages (BAL). On day 1, pulmonary resistance increased, compliance decreased, and persistence peaked on day 7. On day 1, only the citrate capped 20nm AgNPs remained to cause bronchial hyperresponsiveness; the

110nm AgNPs did not. In terms of neutrophil influx, the 20 nm versus 110 nm sizes were more proinflammatory, but there was little difference between the citrate-capped and PVP-capped AgNPs. AgNPs can cause bronchial hyperresponsiveness and pulmonary eosinophilic and neutrophilic inflammation, which are characteristics of asthma.

Effect on brain and CNS

Another review attempted to summarise the findings over the last ten years that used AgNPs and AuNPs for in vivo investigations on the diagnosis and treatment of brain and central nervous system illnesses, emphasizing their toxicity as an important problem address. The significance of the nanoparticle's size and chemical makeup as essential factors for in vivo toxicity is highlighted in this article. The main uses of both types of nanoparticles for glioma, neurodegenerative, and other brain illnesses are then analyzed, along with the developments in clinical trials, in this publication [43].

Reviewing the effects on oxidative stress, neuroinflammation, mitochondrial function, neurodegeneration, apoptosis, and necrosis, this article discusses essential information and the current state of knowledge about the neurotoxicity of silver nanoparticles. Growing interest and concern have been raised about the impact of silver nanoparticles on the central nervous system, which calls for rapid attention. There is a lot of evidence to suggest that when exposed through different routes, AgNPs bypass and cross the BBB and have neurotoxic effects. Even though neurotoxicity is based on physiochemical characteristics, our analysis revealed that all AgNPs are toxic to some extent. AgNP-induced ROS, which interacts with cells to generate oxidative stress, inflammation, apoptosis, necrosis, mitochondrial dysfunction, and disruption of intracellular calcium homeostasis, may be the primary cause of toxicity [44].

A study investigated in CD-1 (ICR) male mice the tissue distribution and in vivo effects of 4-week oral exposure to 0.25 and 1mg Ag/kg bw 10nm citrate coated silver nanoparticles (AgNPs) and 1mg Ag/kg bw silver acetate (AgAc) at the end of treatment (EoT) and after 4weeks of recovery. The study reported that accumulation and slow clearance of silver in the brain after oral administration of 10nm AgNPs and AgAc at low doses in mice is associated with effects on glial cells and ultrastructural alterations of the Blood-Brain Barrier [45].

Another study looked into the deposition of silver nanoparticles in the brain as well as their effects on the BBB. Subcutaneous injections of nanosilver and microsilver (62.8 mg/kg) particles were given to rats. The rats were slaughtered at regular intervals, and their brains were collected for ultrastructural analysis and silver-level detection. The findings demonstrated that silver nanoparticles may cross the BBB and enter the brain as particles. By accumulating in the brain over time, silver nanoparticles can cause neuronal degeneration and necrosis [46].

A study looked at how AgNPs covering material affected cognition, spatial memory performance, and neurotransmitter levels in the rat hippocampus. The ratio of serotonin to dopamine concentration was disrupted in the AgNPs(BSA) rats, according to a neurotransmitter levels study. Furthermore, treatment with AgNPs or Ag+ resulted in the production of peripheral inflammation, as seen by changes in serum inflammatory mediator levels. Finally, depending on the coating material utilized for stabilization, AgNPs elicited alterations in memory functioning and neurotransmitter concentration [47].

A study looked at the biochemical characteristics of oxidative stress in myelin extracted from adult rat brains that had been exposed to a low dosage of AgNPs. The authors discovered increased lipid peroxidation and lower amounts of protein and non-protein -SH groups in myelin membranes. Simultaneously, superoxide dismutase, a free radical scavenger, is upregulated, whereas protein glutathionylation, a cellular protective mechanism against irreversible oxidation, is discovered to be inefficient. The findings suggest that oxidative stress-induced changes in myelin membranes may be the source of ultrastructural defects in myelin sheaths [48].

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A previous study examined the interactions of silver nanoparticles (Ag-NPs) with the cerebral microvasculature to identify the involvement of proinflammatory mediators that can increase Blood-Brain Barrier (BBB) permeability. Results demonstrated that larger Ag-NPs (80 nm) had significantly less effect on rBMEC, whereas the smaller particles induced significant effects on all the end points at lower concentrations and/or shorter times. Ag-NPs may interact with the cerebral microvasculature producing a proinflammatory cascade if left unchecked; these events may further induce brain inflammation and neurotoxicity [49].

Effect on kidney and renal system

Cytotoxic effects have been observed in kidney cell lines after exposure to high doses of AgNPs. In the urinary system, the inorganic accumulation of nanoparticles at higher doses may cause toxicity in vivo [50]. Furthermore, the toxicity of silver nanoparticles in the rat ear model manifests itself through mitochondrial dysfunction leading to hearing loss, either permanent or temporary, depending on the dose [51].

A study assessed the effect of 60 days of oral AgNP treatment on the kidneys of female Wistar rats at doses of 50 ppm and 200 ppm that are below the previously reported lowest observed adverse effect level (LOAEL). AgNP treatment led to a decrease in kidney weight and some loss of renal function as seen by increased levels of serum creatinine and early toxicity markers such as KIM-1, clusterin, and osteopontin. Authors also observed significant mitochondrial damage, loss of brush border membranes, pronounced swelling of podocytes, and degeneration of their foot processes using transmission electron microscopy (TEM). These symptoms are similar to those seen in nephrotic syndrome and 'Minimal change disease' of the kidney where few changes are visible under light microscopy but significant ultrastructural damage is observed. Prolonged treatment of AgNPs also led to the activation of cell proliferative, survival, and proinflammatory factors (Akt/mTOR, JNK/Stat and Erk/NF-κB pathways and IL1β, MIP2, IFN-γ, TNF-α and RANTES) and dysfunction of normal apoptotic pathways [52].

A study presented a thorough investigation of the interactions between citrate-coated AgNPs and porcine kidney (Pk15) cells in vitro. Images taken using a Transmission Electron Microscope (TEM) showed that the Pk15 cells internally scavenged AgNPs by endocytosis. After 24 hours, the number of viable Pk15 cells dropped in a dose-dependent way with both types of silver, nano and ionic. AgNPs displayed only negligible toxicity at concentrations lower than 25 mg l1, despite a large absorption into the cells, whereas Ag+ significantly reduced cell viability at a concentration one-fifth of this. [50].

Several in vivo studies have investigated the potential toxicity of AgNPs in different animal models. One such study examined the acute effects of AgNPs on the liver and kidneys of rats following oral administration of 100 mg/kg/day of AgNPs for 5 consecutive days [53]. The results showed that AgNPs induced oxidative stress and inflammation in both the liver and kidneys, as evidenced by elevated levels of oxidative stress markers and pro-inflammatory cytokines. Similarly, another study showed that repeated oral exposure to AgNPs resulted in histopathological changes in the liver and kidneys of rats, as well as alterations in liver and kidney function [54].

A study evaluated the renal toxicity induced by AgNPs after repeated oral exposure to determine the relevant molecular mechanisms. Histopathologic examination indicated glomerular degeneration, tubular architectural loss, brush boundary loss, and disrupted tubular basal laminae. These alterations were more obvious in the 30 and 125 mg/kg groups. In both the cortex and the medulla, collagen intensity rose in the 30 mg/kg group. Apoptosis was substantially more visible in the middle-dose groups (125 and 300 mg/kg). The RT-PCR results showed that Bcl-2 and Bax mRNAs were increased in the treated groups (p 0.05). Furthermore, data on EGF, TNF-, and TGF-1 gene expression demonstrated that AgNPs generated significant alterations in gene expression in groups treated with 30 and 700 mg/kg compared to the control group [55].

Effect on cardiovascular system

AgNPs also affect the cardiovascular system. In Hartley albino guinea pigs, exposure to AgNPs smaller than 100 nm, with a dose of 100 ppm, caused cardiomyocyte deformities, congestion, inflammation, and hemorrhage [56].

In a review article, the authors discuss the correlation between nanoparticle exposure and various cardiovascular events, suggesting that the potential mechanisms mediating cardiovascular effects of inhaled nanoparticles include pulmonary and/or systemic inflammation, oxidative stress, and direct effects on the heart and vessels [57]. The authors also highlight that the increase in human exposure to nanoparticles has raised concerns about their health and safety profiles.

Additionally, a study review demonstrates its goal to provide an overview of the most important studies that have been conducted so far on the effects of AgNPs on the cardiovascular system. It also aims to present a more comprehensive picture of the potential toxic effects and exposure risks, which will help identify future research directions and new uses for these adaptable nanomaterials. This review will present a collection of data from various researchers that compare the effects of various sizes, concentrations, and doses of coated and uncoated AgNPs, as well as potential mechanisms of action and their implications for the various cardiovascular system structures. [35].

Effect on the immune system

A study revealed that silver nanoparticles were created and their biological distribution and potential toxicity were examined in another study. Swiss mice were exposed to two distinct amounts of silver nanoparticles trapped in montmorillonite. $Ag^{(0)}$-montmorillonite was shown in the animal study to be nontoxic, to show no immunological response, to have increased blood half-life, to penetrate BBB, and to have increased neurotransmission. These findings suggested that Ag(0)-montmorillonite is capable of a wide

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range of applications in the life sciences, including the production of drugs, the detection of proteins, and the delivery of genes to various organs, including the lungs and brain in particular. [58].

A study has demonstrated that NPs can enter the bloodstream after ingestion and inhalation, and they reveal that the liver is a key organ for accumulation. Due to their application in food contact materials, dietary supplements, and antibacterial wound treatments, silver (Ag) NPs are particularly relevant for human exposure. The transit of a tiny amount of NPs into the bloodstream after exposure, such as through eating or inhalation was simulated in this study using a straightforward hepatocytes model in conjunction with an in vivo injection model. Researchers investigated the potential of 20 nm-diameter Ag nanoparticles to cause liver damage, inflammation, and oxidative stress after 50 g of NPs were injected intravenously into female Wistar rats in vivo, as well as after exposures to NPs in vitro using the human hepatocyte cell line C3A. We discovered that Ag NPs significantly reduced albumin release from hepatocytes and were extremely cytotoxic to hepatocytes (LC50 lactate dehydrogenase: 2.5 g/cm^2). [59].

The toxicity of nanoparticles has raised concerns regarding their use, especially those of AgNPs, which are the most commonly used nanoparticles in consumer products. The immune system plays a critical role in responding to nanomaterials' adverse effects, making it essential to evaluate AgNPs' toxicity on immune cells. The immune response is highly affected by the physicochemical properties of the nanoparticles, including size, distribution, crystallinity, surface charge, surface coating, synthesis methods, and reactivity [60].

The modification of nanoparticles' surface is one of the essential factors in determining their toxicity on the immune system. The outer coating of the nanoparticles can determine their toxicity [61]. Studies have shown that AgNPs have both stimulating and inhibitory effects on cytokine production associated with the inflammatory response, depending on the cell type and dose [62]. AgNPs' interactions with the innate immune system can affect the adaptive immune response by inducing cytokine and chemokine production. Monocytes exposed to AgNPs produce the crucial cytokine IL-1β, which plays a significant role in lymphocyte activation and proliferation. The decreased amount of IL-1β may impair the innate immune response caused by AgNPs [63].

Effect on cellular level

In germ cell lines, the dependence on the size of the silver nanoparticles is the main toxicity criterion. For example, spermatogonal stem cells were resistant to AgNPs of a larger size compared to smaller AgNPs [64]. The internalization of AgNPs by mouse sperm disrupted the development of the embryo through reduced fertilization of the oocyte. Moreover, mortality induced by oxidative stress, as well as mitochondrial copy numbers and morphological abnormalities, also increased [65].

It is worth noting that the toxicity of AgNPs is also dependent on the exposure dose, duration, and particle size. In vitro studies have shown that low doses of AgNPs do not exhibit significant cytotoxicity in various human cell lines [28,66]. However, long-term exposure to low doses of AgNPs has been shown to cause neurotoxicity in rat cortical neurons, leading to cell death [67]. Moreover, the size of AgNPs has been shown to affect their toxicity, with smaller particles having a greater impact on cells [36]. Therefore, it is essential to carefully consider the dose and size of nanoparticles when using them in consumer products and medical devices.

Moreover, primary human blood mononuclear cells exposed to AgNPs have shown that smaller particles have a greater potential to activate innate immunity, based on the measurement of IL-1β and the induction of inflammatory body formation [68]. Furthermore, exposure to silver nanoparticles in isolated monocytic THP-1 cells and PBMCs causes significant immunotoxicity [69]. In both PBMCs and THP-1 cells, AgNPs' internalization results in increased expression of Myd88, MEKK1, and early regulation of oxidative stress genes [69].

AgNPs internalized into the cytosol and nucleus of human THP-1 monocytes induce monocytic cell death by degradation of the stress sensor ATF-6 and activation of the NLRP-3 inflammasome [70]. AgNPs also show high dose-dependent immunomodulation of T cells and monocytes [71]. AgNPs' immunomodulatory activity varies depending on the immune cell type and differentiation stage. For instance, differentiated cells exhibit greater resistance to AgNP-induced cell death than undifferentiated cells [72].

In conclusion, the use of silver nanoparticles in consumer products and medical devices has raised concerns regarding their potential toxicity to human health. Research has shown that AgNPs can induce oxidative stress, inflammation, and apoptosis in various human cell lines, leading to neurotoxicity, cardiovascular toxicity, and genotoxicity. Moreover, ingestion of AgNPs has been shown to cause organ toxicity and inflammatory responses, with smaller nanoparticles accumulating in organs such as the brain, lungs, liver, kidneys, and testes. It is crucial to carefully consider the dose and size of nanoparticles when using them in consumer products and medical devices to minimize their potential adverse effects on human health. Further research is needed to fully understand the toxicity of AgNPs and to develop safe and effective strategies for their use.

Discussion

Silver nanoparticles (AgNPs) have extensive household and biomedical applications. However, their toxicity on different body systems is a growing concern. The present study aims to explore the forensic uses and toxicity of AgNPs on various body systems and vital organs. The major routes of entry of NPs are ingestion, inhalation, dermal contact, and systemic circulation via intraperitoneal (i.p.) or intravenous (i.v.) injection [73].

AgNPs can induce inflammation and oxidative stress upon exposure. Inhalation, oral exposure, dermal contact, and

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intravenous or intraperitoneal injection are the major modes of exposure to AgNPs [73]. AgNPs can cross biological barriers and enter systemic circulation. AgNPs administered intravenously are directly available in circulation and distributed to various organs, causing organ-specific pathophysiological effects. It remains unclear whether distant organ effects result from the direct impact of translocated AgNPs or particle-induced inflammatory and oxidative stress responses at the site of exposure [74].

Inhalation of AgNPs occurs when they are released into the environment during manufacturing, washing, or disposal of products [75]. A study on occupational exposure in an NMs manufacturing facility showed a significant release of AgNPs during processing. The concentrations of AgNPs in the processes of manufacturing and integration of AgNPs into various consumer products can reach up to 1.35 µg/m3 [76-78]. The use of nanotechnology-based consumer sprays containing AgNPs can lead to the generation of nanosized aerosols, releasing NPs near the human breathing zone Ag-treated textiles can also be a source of AgNPs in washing solutions when laundering fabrics, regardless of either conventional Ag or nano-Ag treatment (Mitrano, et al. 2014). A recent study that evaluated the effluent from a commercially available silver nano washing machine showed that AgNPs were released in the environment at an average concentration of 11 µg/L. The authors suggested the possibility of workers being exposed to airborne AgNPs at concentrations ranging from 0.005 to 0.289 mg/m3 [79]. In addition, outdoor paints were found to release AgNPs with a maximum concentration of 145 µg/L during initial runoff events [80]. Despite considerable studies evaluating pulmonary exposure and toxicity, there is still a lack of long-term toxicity data, consumer exposure data, and human health effect data on AgNPs information. Nevertheless, an occupational exposure limit of 0.19 µg/m3 for AgNPs has recently been proposed based on a subchronic rat inhalation toxicity study [81].

AgNPs are used in the food industry for packaging and storage to increase the shelf life and quality of food. AgNPs can enter the aquatic ecosystem through urban and industrial effluents and accumulate along trophic chains. AgNPs can also accumulate in the digestive tract after ingestion of contaminated water and food [82]. A study on oral exposure in rats showed that AgNPs of 100 nm size accumulated in the intestinal wall and liver, leading to increased liver enzymes and oxidative stress [83]. In vitro studies have demonstrated that AgNPs can damage the intestinal barrier, leading to increased gut permeability [84]. However, there is a lack of data on the long-term oral toxicity of AgNPs [85].

Skin contact is a significant route of exposure as it is the largest organ of the body and is in direct contact with the external environment. It has been well established that AgNPs can penetrate healthy and breached human skin and diffuse into underlying structures [53,86]. Cosmetics, wound dressings, and antibacterial textiles are some of the commonly used products that contain AgNPs [39]. The use of AgNPs in cosmetics production has been estimated to reach up to 20%, and their dermal contact has shown large diffusion [39].

In addition to skin contact, AgNPs can also gain access to systemic circulation through parenteral routes such as intravenous, intraperitoneal, and subcutaneous injection [11]. Furthermore, AgNP-based drugs or drug carriers could enable the direct entry of these particles into the human circulatory system.

The biodistribution and toxicity of AgNPs vary depending on the exposure route and gender differences [11,87,88]. Following exposure, the clearance behavior of NPs is an essential indicator of cumulative toxicity. Several studies have investigated the post-exposure clearance kinetics following subacute inhalation, intravenous, and oral exposure to various sizes of AgNPs and Ag+ ions [89-91]. These studies have revealed silver clearance from most organs after the recovery period, which is generally 17 days to four months. However, tissues with biological barriers like the brain and testes have exhibited the persistence of silver in long-term oral exposure studies, suggesting the difficulty of silver to be cleared from these organs [90,91]. The persistence of silver in these organs also enhances the chances of increased toxicity.

Pathophysiological Effects of AgNPs The increasing use of AgNPs has led to concerns about their potential impact on the environment and human health. In vitro, cytotoxicity studies are often used to characterize the biological response to AgNPs and identify hazards associated with exposure to these particles. Several in vitro studies have shown toxic effects of AgNPs on different cell lines, including macrophages (RAW 264.7), bronchial epithelial cells (BEAS-2B), alveolar epithelial cells (A549), hepatocytes (C3A, HepG2), colon cells (Caco2), skin keratinocytes (HaCaT), human epidermal keratinocytes (HEKs), erythrocytes, neuroblastoma cells, embryonic kidney cells (HEK293T), porcine kidney cells (Pk 15), monocytic cells (THP-1), and stem cells [92-135].

In addition to oral exposure, several studies have investigated the effects of dermal exposure to AgNPs in animal models. For example, a study using a mouse model showed that topical application of AgNPs induced oxidative stress and inflammation in the skin, as well as alterations in the expression of genes involved in skin barrier function [59]. Another study using a rat model showed that dermal exposure to AgNPs resulted in the accumulation of AgNPs in the liver, kidneys, and lungs, as well as oxidative stress and inflammation in these organs [30].

Conclusion

In conclusion, this study aimed to investigate the forensic uses and potential toxicity of silver nanoparticles on vital organs and different body systems. The findings of this study indicate that silver nanoparticles have potential forensic uses, particularly in forensic biology and forensic toxicology. However, there are concerns regarding the potential toxicity of silver nanoparticles on various vital organs and body systems. The toxic effects of silver nanoparticles can be attributed to their physicochemical properties, such as size, shape, and surface charge. Further research is needed to fully understand the mechanisms of toxicity of silver nanoparticles and to

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develop safe and effective strategies for their use in forensic science.

Strengths

One of the strengths of this study is its systematic review methodology, which enabled a comprehensive evaluation of the available literature on the forensic uses and toxicity of silver nanoparticles. The study's use of multiple databases and inclusion of both scientific sources and "grey" literature also contributed to a thorough analysis of the topic.

Limitations

A limitation of this study is the limited time frame for the literature search, which only included studies published between January 2015 and March 2023. This may have resulted in the exclusion of relevant studies published before 2015. Additionally, the study's focus on English language publications may have resulted in the exclusion of studies published in other languages.

Recommendations

Based on the findings of this study, it is recommended that future research should focus on further investigating the mechanisms of toxicity of silver nanoparticles and developing safe and effective strategies for their use in forensic science. It is also recommended that future studies include a wider range of languages to ensure a more comprehensive evaluation of the available literature on this topic. Finally, researchers should also investigate the potential risks of silver nanoparticle exposure for forensic professionals who handle these nanoparticles.

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Citation: ALAtawi MK, AlAsmari AA, AlAliany AD, Almajed MM, Sakran MI (2024) Silver nanoparticles forensic uses and toxicity on vital organs and different body systems. Adv Toxicol Toxic Effects 8(1): 015-029. DOI: https://dx.doi.org/10.17352/atte.000018