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REVIEW ARTICLE

Silver nanoparticles: Toxicity and inhibitory effects against Aflatoxins

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Abstract

Among the numerous nanomaterials, metal nanoparticles, like silver nanoparticles (AgNPs), are the most employed. Significant focus has been given to their dual role due to their versatile properties. Beneficial, on the one side, as potent antimicrobial properties determine different applications in medicine, agriculture and food safety, to potentially harmful on the other side. Mycotoxins, secondary metabolites produced by toxigenic strains of fungi, are highly toxic substances recognized for their influence on processes of mutagenesis and carcinogenesis, hepatotoxicity, immunosuppression and estrogenic properties in animals and humans, posing severe threats to health through contaminated food and feed. Thus, this paper explores the toxicity mechanisms of AgNPs and their inhibitory effects on aflatoxins, a class of mycotoxins produced mostly by *Aspergillus* species that pose significant health risks. The interaction between AgNPs and aflatoxins is examined, highlighting the potential of AgNPs in mitigating aflatoxin contamination. The article gives a summary of the synthesis, properties and dual roles of AgNPs in the toxicity and inhibition of aflatoxins, concentrating on their possible uses and safety concerns at the end. It is found that, there are elements that affect AgNP's toxicity, like particle solubility, surface area, surface charge, size, concentration, formulation, tendency to agglomerate and exposure duration. Therefore, assessing the safe levels of AgNP exposure and developing guidelines for their use in different fields are crucial for minimizing the risks. It can be summarized that the biosynthesized AgNPs generated through green synthesis, owing to their biocompatibility and low toxicity, could be applied in harmless concentrations as strong antifungals and anti-mycotoxins. This can offer significant potential for enhancing food safety due to their strong antimicrobial properties, which can inhibit the growth of foodborne pathogens and extend shelf life. However, the potential for nanoparticle migration into food must be considered, which raises critical concerns about human health, regulatory challenges and environmental impact.

Keywords

Aflatoxin; AgNPs, fungi; metal nanoparticles; silver nanoparticles; toxicity.

Introduction

Nanotechnology has revolutionized various scientific fields, offering novel solutions to complex problems. It is an interdisciplinary domain that encompasses the production, manipulation and deployment of substances at scales less than 100 nm. This field focuses on materials at the molecular level and has recently expanded to a large number of uses (1). Among the numerous nanomaterials, metal nanoparticles (NPs) are among the most employed. Numerous systems have been thoroughly studied with metals like gold and silver incorporated, as well as metal oxide containing NPs like SiO_2, TiO_2 and cupric oxide (CuO) (2-6).

Silver nanoparticles (AgNPs) are the focus of more thorough research in the fields of infection and inflammation prevention concerning their exceptional properties. AgNPs dissolve quickly and, because of their small stature and great surface area, have a low likelihood of causing drug resistance (4, 7). However, AgNPs can be cytotoxic and genotoxic because they interact in human cells with electron transport chain enzymes and DNA, resulting in disrupted production of ATP, DNA damage and generation of various types of reactive oxygen species (ROS) (8). It's intriguing, though, that AgNPs do not show cytotoxicity *in vitro* when they are used as antimicrobial coat/biofilm on surfaces of medical devices (implants, catheters) and wound dressings. Instead, AgNPs show strong antibacterial activity against typical pathogenic bacteria (9). Regarding the aforementioned, AgNPs are still being thoroughly researched as antimicrobial materials, but in this context, their ability to inhibit aflatoxin generation is not well characterized.

Mycotoxins are secondary metabolites that can contaminate a variety of food products when hazardous strains of fungi/molds produce them. Representatives of *Aspergillus*, *Penicillium* and *Fusarium* genera produced the most frequently detected mycotoxins. Given the significant harm that mycotoxins cause to the health of both humans and animals, it is especially problematic when they are found in cereals, nuts, milk and other food products (10). Aflatoxin B1, Ochratoxin A and fumonisin B1 are the three mycotoxins that are particularly harmful to mammals (11). Aflatoxins, which are a subgroup of the mycotoxins group, mostly produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, are difuranocoumarin derivatives. These are highly toxic substances recognized for causing hepatotoxicity, immunosuppression, mutagenicity, carcinogenicity and estrogenic effects in mammals, posing severe threats to health through contaminated food and feed (12-14). Aflatoxin B1 and B2 and aflatoxin G1 and G2 are the main aflatoxins that are found naturally (Fig. 1). The term "aflatoxicosis" refers to the illnesses brought on by consuming aflatoxin. Aflatoxin B1 stands out as one of the most powerful natural carcinogens among all aflatoxins (15). Hepatocellular carcinoma is the main illness linked to aflatoxin B1 (16). The toxicity of aflatoxin B1 varies depending on the species, gender and age.

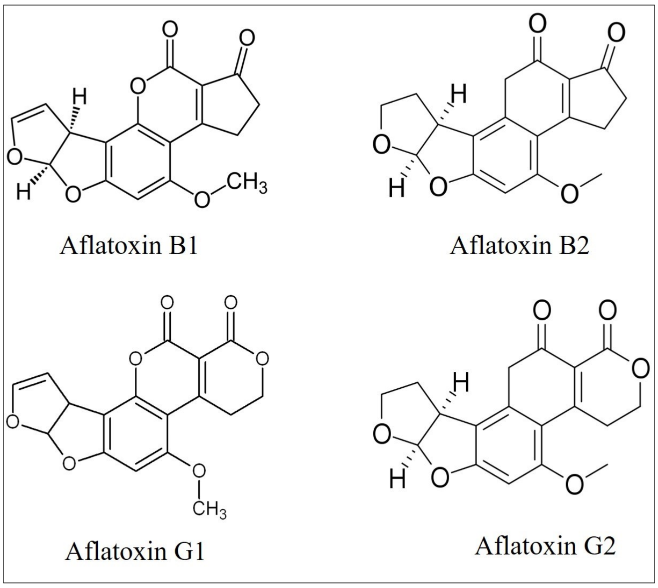

Fig. 1. Structural formulae of naturally occurring major aflatoxins.

Given previous findings, it is crucial to develop methods and techniques (physical, chemical or biological) for detoxifying mycotoxins. Traditional methods of mycotoxin mitigation, such as chemical treatments and thermal processing, often have limitations, including incomplete removal, nutrient loss, or environmental concerns. One of the interesting and promising approaches to address this issue is the use of NPs. AgNPs offer a novel and effective alternative in the fight against mycotoxins. Their use as components in food packaging materials and coatings, provides innovative solutions to reduce contamination risks and improve food quality. It is recognized for its ability to disrupt various processes in the cell of microorganisms, including metabolism, altering the composition and capabilities of cell membranes and inhibiting protein expression related to ATP production. The three main mechanisms of action in mycotoxin mitigation by AgNPs are (a) disruption of cell membranes, interference with enzymatic processes and induction of oxidative stress; (b) adsorption of mycotoxins directly from the contaminated food or feed; and (c) synergistic action with mycotoxin-degrading enzymes (10, 17).

In mammalian tissues, silver, which exists naturally and has low toxicity, is regarded as a powerful antibacterial agent, leading to the widespread use of AgNPs in applications such as water purification, washing machines, textiles, cosmetics, cooking utensils, toys, in consumer goods that are antiseptic and disinfecting, as well in wound dressings and implant surfaces (18 -21). The incorporation of AgNPs into food-related products must consider the potential for nanoparticle migration into food, which could pose health risks through the ingestion route. Long-term exposure to AgNPs through ingestion of food remains poorly understood and there is a need for comprehensive toxicological studies to establish safety thresholds. Additionally, the environmental release of AgNPs during manufacturing, usage, or disposal could disrupt ecosystems, particularly microbial communities, which could be essential for soil- and water-ecosystem health (4, 7, 11, 18). Thus, this review aims to elucidate the dual role of AgNPs in exerting toxicity towards fungal pathogens and inhibiting aflatoxin production.

Synthesis and Properties of AgNPs

AgNPs are usually produced through either top-down or bottom -up techniques. The first strategy is a physical method that entails disassembling bulk materials into NPs by using techniques like evaporation-condensation, laser ablation or ball milling (22–24). These techniques, however, are energy-intensive and costly. In contrast, the second approach synthesizes AgNPs through chemical reactions where atoms self-assemble into nuclei and grow into nanoscale particles. In this method, chemical reactions with reducing substances (such as sodium borohydride, N, N-dimethyl formamide) are primarily used (25). While chemical synthesis is common, it poses environmental risks due to the hazardous chemicals involved, which can be carcinogenic, genotoxic and cytotoxic.

Nowadays, chemical reduction, in which Ag^+ ions are reduced to metallic silver by the action of reducing agents and green synthesis, which creates environmentally benign NPs using plant extracts or microbes, are the most widely used techniques for synthesizing AgNPs (26-29). Therefore, green synthesis presents an eco-friendly alternative, utilizing bacteria, fungi, algae, or plant extracts to produce NPs without harmful chemicals. Biosynthesized NPs, generally using reduction ability of natural plant extracts, are declared to require less modifications and to be more biocompatible (30). The precise process by which green synthesis biosynthesizes metal NPs remains unclear. However, it is suggested that biomolecules such as proteins, polyphenols, polysaccharides, vitamins and enzymes which are present in microbial or plant extracts, have the ability for reactions of reduction with silver salts and subsequent conversion of the latest to AgNPs (31). The resulting AgNP's physicochemical characteristics, including morphology, size, surface charge and shape, have a major impact on their biological activity.

General mechanism of antifungal toxicity of metallic NPs

Eukaryotic fungi can be frequently found in the environment and there are lot of representatives of them that function as opportunistic pathogens. There are a plethora of research works where the main subject of investigation is the efficacy of NPs as possible antifungal agents to address life-threatening fungal infections (10, 20, 32-34). These investigations have included mainly gold, silver, copper and titanium dioxide NPs. Metallic NPs interact with fungi in ways that, according to the type of NPs, the fungus species and the surrounding circumstances, may be advantageous or detrimental (35).

It has been discovered that a variety of NPs can act against fungal species that are resistant by causing alterations in the cell wall, including modifications of the surface, cell aggregation, formation of pores and total deformation of the fungal cell. Fig. 2 illustrates the complex antifungal toxicity of NPs.

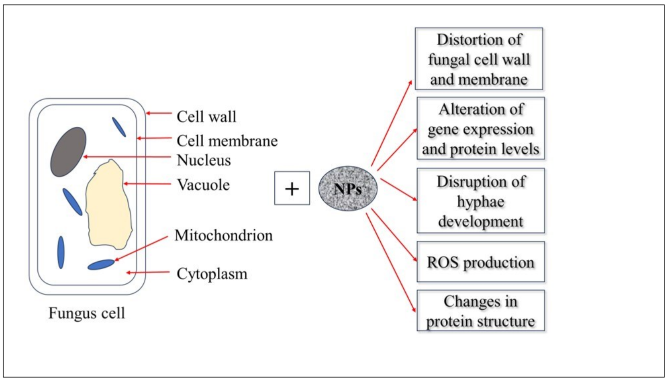

Fig. 2. Mechanism of antifungal toxicity of metallic NPs.

Research indicates that during adsorption, NPs have the ability to embed themselves in the cell walls of fungi, changing their morphology (36, 37). Fungal cells' inner membranes are also deformed, resulting in modifications to the arrangement of organelles. These include a process of increasing the number of vesicles and vacuoles in the cell, as well as the decrease of the content of cytoplasm, which causes the contents of the cell to leak out. Smaller NPs can avoid serious cell wall damage by entering cells through fluid-phase endocytosis. NPs exposure can also alter gene expression and protein levels and some NPs can interact with nucleic acids inside the cell (38). Fungal hyphae and spores are highly susceptible to the effects of NPs. The hyphae may undergo deformation and shrinkage and the growth patterns may be altered, resulting in thinning and clumping of the hyphae fibers. Interruption of hyphal development is essential for colonization and pathogenesis for which biofilm formation and adherence are required. In this direction, NPs can prevent the production of biofilms (39). NPs can also affect existing biofilms by depositing extracellular polysaccharides, which are vital for structural integrity and may cause damage to the cells and oxidative stress by inducing the generation of ROS in fungal cells (40, 41).

Toxicity of AgNPs against fungi producing aflatoxins

The low cost and ease of manufacture of AgNPs, along with their antibacterial, antioxidant and anticancer capabilities, make them appealing as therapeutic agents. Ongoing discussions surround their toxicity and biocompatibility, though.

Ag^+ ions and AgNPs have the ability to modify the transcriptomes, epigenomes and metabolomes of fungal cells, resulting in notable alterations of the cell functions. An important part here is the gene down-expression, especially of genes linked to lipid metabolism, ergosterol production, redox metabolism and the tricarboxylic acid cycle, which results in structural alterations, especially in the membranes of fungal cells (42). The exact genes affected by AgNPs are not defined yet.

AgNPs have demonstrated strong antifungal properties which can differ due to their structural characteristics. Predominantly spherical and small-sized nanoparticles are particularly effective against various strains of fungi which are pathogenic for plants. AgNPs are incredibly tiny, which makes it easy to pass through the membranes. The toxic potential of those NPs is partially linked to the generation of ROS in the exposed cell. Dimensions between 10 and 30 nm have shown relatively potent antifungal activities (43, 44). Three-nm-sized AgNPs were found to be very effective against forty-four distinct strains belonging to six different fungus species, including 30 strains of *T. mentagrophytes* and 14 strains of *Candida species* (45, 46). Research has shown that the concentration, chemical composition and dimensions of AgNPs have a major impact on the filamentous fungus *Penicillium verrucosum* development and mycotoxin production. AgNPs ranging from 0.65 to 5 nm can adhere to the mycelial surface, enter fungal cells and form aggregates in the cytoplasm and various organelles (47).

Fungal-NP interactions are significantly influenced by the concentration of NPs used, which affects different strains of the fungus. Through electrostatic attraction, AgNPs stick to the fungal surface and release Ag^+ ions that seep inside the cell (48). Prior research has aimed to establish the ideal concentration required for achieving effective antifungal activity, but the exact concentration of AgNPs affecting fungal cells is not established yet. *Aspergillus niger* and *Penicillium chrysogenum* exhibit altered enzymatic profiles, reduced synthesis of organic acids (citric, maleic and oxalic acid) and up to 80% less generation of mycotoxins when 45 ppm of AgNPs are applied (49). It's interesting to note that there are findings that claim that potency has often increased at lower doses in comparison to greater concentrations. Good examples are AgNPs (in concentration of 20 ppm) formed by using extracts from two plants, *Momordica charantia* and *Psidium guajava*. When applied to different fungal strains, including *Aspergillus flavus*, *Aspergillus niger* and *Fusarium oxysporum*, AGNPs efficiently suppressed their growth and mycotoxin production (50). The contradictory results (antifungal activity increased with greater concentrations) were obtained in a separate investigation where

AgNPs produced from *Camellia sinensis* leaves extracts (green and black tea) in concentrations of 10, 25, 50 and 100 ppm, were examined for their effectiveness against *Aspergillus parasiticus* and *Aspergillus flavus*. Besides AgNPs in this study CuNPs (copper nanoparticles) and FeNPs (iron nanoparticles) were synthesized and compared concerning their antibacterial and antifungal activity. Their adsorbent capacity for aflatoxin B1 in solution was evaluated also. AgNPs, compared to CuNPs and FeNPs, showed enhanced antibacterial and antifungal properties and decreased the generation of aflatoxins with the maximum level of inhibition in the concentration of 100 ppm (51). Opposite were the results for the adsorbent capacity of three types of NPs in solution with aflatoxin B1 impurities, where AgNPs showed the lowest adsorbent properties. A study that looked into how AgNPs affected *A. parasiticus* development and aflatoxin production found that the minimum inhibitory concentration (MIC) was 180 µg/mL. Moreover, at a dosage of 90 µg/mL, AgNPs reduced aflatoxin production by 50% (52). The mechanism by which these NPs engage in interaction with fungus cells at non-growth-inhibitory concentrations remains unclear.

Against the pathogenic fungus *Candida albicans*, the formulation of AgNPs stabilized with sodium dodecyl sulfate (SDS) at a concentration of 0.05 mg/L showed the most growth inhibitory impact (32). Additionally, the study shows that the stabilizing agent utilized affects how effective AgNPs are against fungal growth. Mallmann et al. in 2015, also produced biosynthesized AgNPs, employing SDS as a stabilizer and ribose as an agent for reduction (53). The study found that the antifungal drug amphotericin B had similar and comparable efficiency to the highest antifungal activity of 80 µg of the biosynthesized AgNPs against *Candida* strains (*C. albicans* and *C. tropicalis*). Notably, stabilized silver nanoparticles did not exhibit cytotoxicity to human fibroblast cells, in contrast to ionic silver. Another study investigated a gel formulation containing AgNPs with a particle size of 7-20 nm, intended for topical use for indications such as wounds and burns. It was found that the evaluated formulation repressed the growth of *C. albicans* (MIC of 25 µg/mL) and *A. niger* (antifungal index 55.5%) (54).

Concerning the all above, it can be summarized that, the AgNPs toxic potential is influenced by elements like particle solubility, surface area, surface charge, size, concentration, formulation, tendency to agglomerate and exposure duration. Usually, NPs smaller in size, are more reactive and have a bigger surface area, which makes them more hazardous. As aflatoxins pose significant risks in different types of products, necessitating effective mitigation strategies. AgNPs have shown promising inhibitory effects on aflatoxin production and fungal growth. The above-mentioned toxicity properties determine the mechanism of AgNPs anti-fungal action through multiple pathways, including:

- ***Interaction with cellular membrane:*** AgNPs cause cellular membranes to rupture, which increases permeability and allows cell contents to seep out (17, 55, 56). The dissolution of AgNPs releases silver ions (Ag^+), which are highly reactive and can interact with other cellular components, disrupting normal cellular functions.
- ***Protein and DNA Interaction:*** AgNPs have the ability to attach to proteins and DNA, interfering with phosphate groups in the latter and sulfhydryl groups in proteins and enzymes. This can disrupt cellular activities and replication (56-58). AgNPs interfere with the biosynthetic pathway of aflatoxins by downregulating key enzymes and genes involved in their production. A significant decrease in aflatoxin levels when AgNPs are present has been demonstrated in multiple studies (59, 60).
- ***ROS production:*** AgNPs cause oxidative stress in cells by producing ROS, which damages cells and causes DNA breakage and apoptosis. (41, 61).
- ***Synergistic effects:*** Combining AgNPs with other antifungal agents or preservatives can enhance the inhibitory effect on aflatoxins, providing a more effective strategy for managing aflatoxin contamination (58).
- ***Inhibition of mitochondrial functions:*** AgNPs exposure could trigger cytotoxicity primarily damaging mitochondria, their structure and morphology (induction of membrane potential collapse and swelling in the mitochondria), raising intracellular ROS levels and interfering with the dynamics and biogenesis of the mitochondria. Those events were shown in HepG2 cells (62) and also in mouse hippocampal HT22 cells exposed to AgNPs (63).

AgNPs - Toxicological concerns and safety

AgNPs have attracted a lot of attention because of their special qualities and variety of uses, especially in the industrial and medicinal domains (5, 6). Their beneficial role can be found in:

- ***Antimicrobial applications:*** Their antimicrobial activity (including antifungal) is due to the release of silver ions (Ag^+), which disrupt microbial membranes, denature proteins and interfere with DNA replication. This property is applicable in wound dressings, coatings for medical devices, water purification and food packaging.
- ***Catalysis:*** Their large surface area and high reactivity enhance reaction rates.
- ***Biomedical applications:*** Due to their biocompatibility and ability to target specific cells or tissues AgNPs are used in drug delivery, imaging and cancer therapy.
- ***Sensors and diagnostics:*** Due to their optical and electrical properties, such as surface plasmon resonance AgNPs are employed as biosensors and diagnostic tools.

Despite their benefits, the use of AgNPs raises concerns regarding their toxicity to non-target organisms and the environment. Their harmful role can be found in:

- ***Cytotoxicity:*** This toxicity poses risks to tissues and organs if exposure is not carefully controlled.
- ***Environmental impact:*** AgNPs can affect ecosystems when released into the environment.
- ***Antimicrobial resistance:*** Resistant strains of microorganisms can be obtained when prolonged exposure to AgNPs is on site. This can reduce the long-term efficacy of AgNPs.

Research suggests that AgNPs may build up in human organs and tissues and may have negative effects (64, 65). Due to their many uses, people are exposed to them more frequently, which raises the possibility of short- and long-term toxicity.

Table 1. Toxicity of AgNPs in different modes of exposure

Rute of exposure	Source and possible effects	References
Dermal	AgNPs in wound dressings and cosmetical personal care products can be absorbed through the skin, potentially causing local (inflammation and oxidative stress) and systemic effects	68-74
Inhalation	Inhalation of AgNPs, especially in occupational settings, can lead to respiratory issues and systemic toxicity	75-79
Ingestion	Consumption of AgNP-contaminated food and water can affect the gastrointestinal tract (inflammation) and other organs	71, 80-82

AgNPs are harmful to human cells emanating from the different tissues and organs (liver, lungs, skin, brain, reproductive organs and vascular network), according to several investigations in *in vitro* models (65-67). Concerning human health, the possible exposures that pose the highest risk, are given in Table 1.

The dual role of AgNPs necessitates careful consideration of their design, usage and disposal. Strategies such as surface modification, dose optimization and eco-friendly synthesis methods help mitigate risks while maximizing benefits.

Therefore, assessing the safe levels of AgNPs exposure and developing guidelines for their use in different fields are crucial. For example, the determination of specific exposure levels for food applications versus medical applications could be valuable. Establishing comprehensive regulations and regulatory measures to control the production, usage and disposal of AgNPs is mandatory to minimize their environmental and health impacts. Risk assessment is also an important part of safety considerations, consequently, conducting thorough risk assessments, including toxicity studies and exposure evaluations, can help identify potential hazards and establish safe usage guidelines.

Green-synthesized AgNPs are gaining significant attention due to their environmentally friendly and sustainable production methods. Green synthesis involves the use of plant extracts, microorganisms, or natural polymers as reducing/stabilizing agents. These biomolecules often impart biocompatibility to the AgNPs and eliminate harmful chemical residues present in conventionally synthesized NPs. Green synthesis methods allow for better control over the size and morphology of AgNPs, which are critical factors in determining their interaction with biological systems. Biomolecules used for green synthesis often added additional stability to the obtained AgNPs, preventing agglomeration and ensuring consistent biological activity. This stability reduces the risk of unintended interactions that might lead to toxicity. Their reduced toxicity compared to chemically synthesized counterparts makes them an excellent choice for biomedical and industrial applications (28). Promoting green synthesis methods using biological materials can reduce the reliance on hazardous chemicals, mitigating the toxicological risks associated with AgNPs production.

Last but not least, increase of public awareness by educating consumers and industries about the potential risks of AgNPs and encouraging responsible usage, can help prevent misuse and overexposure.

Conclusion

AgNPs present a promising solution for controlling aflatoxin contamination due to their potent antifungal properties and ability to inhibit aflatoxin production. Besides the fact that AgNPs hold great promise in aflatoxin mitigation, their widespread application requires addressing certain challenges such as toxicological concerns (the potential health impact of AgNPs residues in food or feed), environmental impact (the fate and sustainability of AgNPs in agricultural and ecological systems) and regulatory approval (clear guidelines and safety standards particularly under varying environmental conditions and prolonged exposure).

Beyond their established role in food safety, extending their impact to critical industries such as medicine and environmental management holds immense promise. The broader implications of AgNPs demand a balanced approach that integrates robust regulatory frameworks, sustainable production methods and continued research into their long-term effects on human health and the environment.

In order to balance effectiveness and safety, their application needs to be carefully monitored. Understanding the mechanisms of toxicity and implementing safety measures are essential to harness the advantages of AgNPs while minimizing their risks.

It is imperative that future research concentrates on creating safer synthesis procedures, thorough toxicity assessments and efficient regulatory structures to guarantee the sustainable and secure application of AgNPs. The biogenic AgNPs generated via green synthesis have the potential to be employed as effective antifungals and anti-mycotoxins at nontoxic levels due to their low toxicity and biocompatibility. By leveraging their multifunctional properties through eco-friendly production methods, AgNPs exemplify the future of nanotechnology as a sustainable, versatile and transformative solution across diverse fields. This broader perspective underscores the importance of continued research and innovation in the development of AgNPs for a greener and healthier future.

AgNPs represent an innovative approach to aflatoxin mitigation by addressing both fungal growth and toxin detoxification. By integrating AgNPs into existing food safety and regulatory strategies, the burden of mycotoxins in food systems on a global level can be significantly reduced. The process of advancing their practical applications requires a multidisciplinary effort including nanotechnology, toxicology and regulatory sciences to ensure AgNPs safe and sustainable use.

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Authors' contributions

DGA and TKP participated in the design and conceptualization of the study. DGA contributed to writing, original draft preparation and editing. TKP played a key role in writing, review and editing. All authors have read and approved the final version of the manuscript.

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