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Title: Inorganic nanoparticles: Toxic effects, mechanisms of cytotoxicity and phytochemical interactions

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Running title:

Toxicity of inorganic nanoparticles

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Abstract

During the last few decades, nanotechnology has gained many applications in almost all fields of life because of the unique properties of nanoparticles. Nanotechnology has specially marked its name in the field of medicine. However, nanoparticles toxicity is detrimental to human health and is a prime concern in applied medicine. They can cause insomnia, vertigo, madarosis, epistaxis, hypokalemia, lymphopenia, Alzheimer's and Parkinson's diseases, etc. There is a gap in knowledge regarding the study of the toxicological effects of nanoparticles.

Mechanisms that are responsible for this toxicity are not fully understood yet. Phytochemicals have natural therapeutic effects of reducing metal nanoparticles' toxicity by acting as stabilizers and nontoxic reducing agents. However, the interaction between phytochemicals and nanoparticles is remained to be elucidated. This review will provide in-depth knowledge about the various types of inorganic nanoparticles and their associated toxicities, key parameters determining the toxic behaviour of nanoparticles, and the mechanisms behind their cytotoxicity. It also emphasizes the need for further research to understand the interaction between various phytochemicals and nanoparticles for therapeutic purposes.

Keywords: medicine, nanoparticles, phytochemicals, toxicity, therapeutic effects

Introduction

A lot of progress has been made in the area of nanotechnology over the last few decades. Nanoparticles (NPs) usually have a nano scale size, i.e., a diameter of less than or equal to 100 nm.[1] Because of their unique properties, they have applications in numerous fields including cosmetics, electronics and medicine.[2] Silver, Gold, Zinc oxide (ZnO), and Titanium oxide (TiO_2) nanoparticles are used in cosmetics because of their excellent drug delivery system, skin whitening and moisture retention properties. Their use in cosmetics is safe as they don't penetrate the skin. Therefore, they are not as harmful as long as they are used dermally.[3] NPs use in diagnostics and therapeutics is growing day by day. However, safety is needed to be ensured for their effective use in various fields like food, cosmetics, medicine, etc.[4] Plants possess abundant radical scavenging molecules like vitamins, phenolic compounds, terpenoids, etc. These molecules have antioxidant activity, thus enabling them to reduce toxicity caused by nanoparticles.[5] As NPs are toxic to health, workers dealing with them must wear personal protective equipment (PPE) such as respirators, nitrile gloves, lab coats, goggles and closed-toed shoes. Fume hoods, gloves, biosafety cabinets should be employed for handling nanoparticles.[6]

This review discusses different types of iNPs, their toxicity, factors affecting toxicity of iNPs, mechanisms behind their toxicity, strategies to avoid toxicity and the interaction between phytochemicals and inorganic nanoparticles.

Types of inorganic nanoparticles and their toxicity

Of all the nanoparticles, inorganic nanoparticles (quantum dots, metallic NPs, etc.) are among those that are most abundantly produced and used commercially.[7] They are being used as therapeutic agents because of their anticancer and antimicrobial activities.[8] They can be used to create antimicrobial nanocomposite films. TYiO2-NPs were incorporated into chitosan to produce a biocomposite membrane that reduced the oxidative stress levels and apoptosis in mouse fibroblast cells due to the superior porosity, crystallinity, mechanical strength and structural flexibility.[9]

However, their increased exposure may cause inflammation, genotoxicity, and oxidative stress, leading to cancer and metabolic diseases.[10] Different types of inorganic nanoparticles and their associated toxicities are mentioned in Table 1.

Table 1. Inorganic nanoparticles and their toxicity

Sr No.	Type of inorganic nanoparticle	Source reducing Agent	Particle size	Mechanism	References
1.	TMAT-AuNP	Gold	1.3 nm	Progression of eye pigmentation	11
2.	Ag-NP	Silver	10 nm	Oxidative stress	12
3.	Multi-walled carbon nanotubes	Carbon nanotubes	15 – 50 nm	Inflammation	13
4.	TiO_2	Titanium	5-90 nm	Apoptosis	14
5.	ZnS CdS Quantum dots (QD)	cores: Zinc Cadmium shells: Sulphide	10 ±2 nm 8±2nm	Increased lipid peroxidation & catalase activity	15

Gold and silver nanoparticles toxicity

"Nanogold" is a suspension of sub-micrometer-sized gold particles in a fluid, usually water.[16] Because of chemical stability and good optical properties, gold nanoparticles (AuNPs) are being used in chemotherapy and drug delivery. They have shown the cytotoxicity in vitro on Balb/3T3 mouse fibroblasts.[17]

Silver nanoparticles (AgNPs), because of their antimicrobial activity, are used in medicine and drug delivery.[18] Oxidation of silver nanoparticles results in the release of silver ions that accounts for cytotoxicity related to the AgNPs.[19] A study showed that reactive oxygen species (ROS) generation was more by AgNPs than bulk silver, due to which AgNPs are more toxic than bulk silver.[20] Actually, the toxicity of AgNPs is related to surface area; as the concentration of AgNPs per unit volume of reaction mixture increases, the surface area increases as well. It causes an increase in reactive oxygen species (ROS) production, which ultimately contributes to cell toxicity.[21] Moreover, oxidative damage and subacute toxicity of AgNP-PVP and AgNP-20 on the kidneys, lungs and liver of mice have also been reported.[22]

Carbon nanotubes toxicity

Carbon nanotubes (CNTs) are the allotropes of carbon and possess fiber-shaped nanostructures.[23] In cell lines, CNTs can activate ROS-associated intracellular signalling pathways.[24] They have also been reported to trigger the release of cytokines including TNF-α, IL-1β, IL-8 and IL-6 from macrophages and mesothelial cells.[25] A study showed that Nanocomposites of chitosan CNTs not only improved antimicrobial activity but also caused DNA damage in hepatic cells of *Oreochromis Niloticus.*[26]

Titanium dioxide nanoparticles toxicity

Titanium dioxide nanoparticles (TiO_2 NPs) are used in cosmetics, food additives and pharmaceutical products because of their chemical stability and photocatalytic properties. TiO_2 NPs can induce cytotoxicity, genotoxicity and oxidative stress.[27] They have induced indirect genotoxicity in two lung cell lines, i.e., A549 and BEAS-2B due to impaired DNA repair processes.[28]

Quantum dots nanoparticles toxicity

Quantum dots (QDs), the semiconductor nanoparticles, are fluorescent and possess unique optical properties.[29] Just like other NPs, QDs cytotoxicity depends on their shape, size, concentration, redox activity, mechanical stability, surface coatings and charge.[30] Nitrogen and Sulphur co-doped graphene quantum dots are less toxic and used as fluorescent nano-sensors in living cells.[31]

Factors affecting the toxicity of NPs

Major factors associated with nanoparticles toxicity are given below:

Dose and time of exposure

The toxicity of nanoparticles is associated with their number. Cells with more particles have more toxic effects than cells with fewer particles**.** Both dose and time play a crucial role in determining the toxicity of nanoparticles.[32] However, NP penetration in the cells depends on their exposure time.

Concentration and aggregation

Increased concentration of NPs favours their aggregation as their size is in micrometers, they do not penetrate the cells and their toxicity is lost.[33] On the other hand, another study suggested that aggregation of NPs affects their stability, making them more toxic.[34]

Particle size and shape

The toxicity of nanoparticles also depends on their size.[35] Small-sized NPs are toxic than large-sized NPs, e.g., AgNPs of 10 nm have more significant toxicity than the larger AgNPs (20-100 nm).[36] The shape is another factor that helps to determine the toxicity of nanoparticles, i.e., different aspect ratios possess different toxicity levels.[37]

Long asbestos fibers (10 µm) can cause lung cancer, while short fibers (5-10µm) can cause mesothelioma or asbestosis (2 µm).[38] Multi-walled CNTs embedded in pleural membrane activated macrophages that secreted IL-1-beta, which amplify inflammation in mesothelial cells.[39]

Crystal structure and route of exposure

Different crystalline structures of nanoparticles can exhibit toxicity differently. Nanoparticles can show different oxidative mechanisms, cellular uptake and subcellular localization based upon their crystalline structure.[33] Route of exposure regulates the initial interaction of nanoparticles and cells.[40] Dermal exposure of nanoparticles activate the immune system while their systemic distribution causes spleen and liver toxicities.[36]

Pre-exposure and surface functionalization

Pre-exposure to low nanoparticle concentrations can stimulate phagocytic activity and adapt the human body to these nanoparticles.[41] Whereas their surface properties have drastic effects on oxidation processes. Nanoparticles with cationic surface are more cytotoxic than nanoparticles with anionic surface.[42]

Mechanisms of Nanoparticles Cytotoxicity

Nanoparticle toxicity mechanisms include DNA damage, oxidative stress, ROS production (Figure 1), and alteration of protein structures. Different mechanisms associated with nanoparticle cytotoxicity are mentioned in Table 2.

Table 2. Inorganic nanoparticles and their cytotoxicity

Sr.#	Type of inorganic nanoparticle	Source reducing agent	Particle size	Mechanism of cytotoxicity	References
1.	Au-NP	Gold	25 – 30 nm	Oxidative stress	43
2.	Ag20Pep	Silver	20 nm	ROS formation & calcium dysregulation	44
3.	Long and short multi-walled carbon nanotubes	Carbon	7 – 26 nm	Lipid peroxidation and oxidative stress	45
4.	TiO_2	Titanium	~26.4 ± 1.2 nm	ROS production and apoptosis.	46
5.	CdTe quantum dots	Cadmium	2.3 nm	ROS generation and apoptosis.	47

Two important cytotoxicity mechanisms are discussed below:

Inflammatory response and Oxidative stress

Inflammation is the defense mechanism of the body that involves many cytokines.[48] Macrophages in the macrophage-rich organs, including spleen and liver, usually take up the nanoparticles and release cytokines.[49] Reactive oxygen species induction is the leading cause of nanotoxicity.[50] A large number of nanomaterials have induced toxicity in human erythrocytes and skin fibroblasts through the production of ROS.[51] Moreover, an imbalance in redox state of the cell causes the oxidative stress.[52] Though ROS production is considered normal, but its excessive production is harmful to the cells. ZnO-NPs increase ROS inside cells and activate apoptosis via the caspase cascades in human gingival squamous cell carcinoma.[53]

Epigenetic modifications

Epigenetic modifications refer to the heritable changes that are not due to alterations in the nucleotide sequence of DNA. Instead, they are due to the alterations in chromatin structure and DNA accessibility, e.g., histone modification and DNA methylation.[54] Transcriptional machinery of the cell depends upon how tightly DNA is enfolded around histones, while DNA packaging depends upon histone post-translational modifications.[55]

Nanoparticle exposure can lead to epigenetic changes. iNPs can change the gene and chromatin packaging, e.g., Ag-NPs can cross the nuclear membrane and interfere with chromatin remolding enzymes that affect condensation of chromatin and accessibility of DNA, thus altering the expression of genes.[56]

Strategies to avoid toxicity caused by inorganic nanoparticles

The main cause of iNPs toxicity is oxidative stress, so their toxicity can be overcome by preventing oxidative stress. Interestingly, it is reported that vitamin C can decrease ROS production in acute myeloid leukaemia cells treated with silver nanoparticles.[57] Another strategy that can be used to avoid oxidative stress is to use methods that slow down the release of metal ions since metal ions play a role in the induction of oxidative stress, e.g., slowing down the release of silver ions produced by AgNPs can reduce AgNP-induced toxicity.[58,59] Nanoparticles can be coated with antioxidants or a polymer like polyethylene glycol (PEG) to reduce ROS formation. PEG-coated iron oxide nanoparticles reduce cytotoxicity by blocking the interaction of ROS with Fe_2O_3-NPs.[60] PVP-Bi2Se3 nanoparticles showed better radiotherapy efficacy in cancer treatment. As selenium can improve immune function by reducing the harmful effects of radiation on normal cells.[61] Nanoparticles toxicity can also be minimized by creating metal oxide nanoparticles that are toxic to cancer cells but not to normal cells, e.g., ZnO nanoparticles selectively target cancerous cells leaving normal cells.[62]

Interaction between phytochemicals and inorganic nanoparticles

Secondary metabolites derived from harmless microbes and plants are called phytochemicals.[63] These phytochemicals due to their therapeutic effects are used to prepare metal nanoparticles by green synthesis approach. Green synthesis is a biological method for synthesizing nanoparticles based upon oxidation-reduction reaction to reduce metal ions into stable nanoparticles using an organism's components or its extract.[64] Previous studies showed that phytochemicals with antioxidant properties could possess the ability to protect cells from nanoparticles' exposure. However, the interaction between phytochemicals, NPs, and their associated toxicities are yet to be understood.

Conclusion

Nanoparticles are being used in almost all fields of life today, but nanotoxicity has become a major issue. Oxidative stress is particularly associated with the toxicity of inorganic nanoparticles and reducing this stress may increase the biocompatibility of nanoparticles. Due to low toxicity and high bioactivity, phytochemicals can be coated on nanoparticles to reduce their cytotoxicity efficiently. Research on the toxicity of iNPs is highly dispersed and no definitive conclusions can be drawn from the available literature. So, there is a need for further research to understand the toxicity mechanisms, the interaction between various phytochemicals and inorganic nanoparticles and investigate strategies for synthesizing nanoparticles with optimal properties while minimizing adverse effects on living cells.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Consent to publish

Not applicable.

Authors' contribution

Dr. Rashid Bhatti substantially contributed to the conception and design of the article and interpreting the relevant literature. Dr. Rashid Bhatti, Hadia Shakeel, and Shajia Jabeen drafted the article. Dr. Rashid Bhatti and Dr. Mohsin Ahmad Khan revised it critically for important intellectual content. Dr. Kausar Malik helped to edit the manuscript. Dr. Muhammad Qasim and Dr. Nadeem Ahmed updated the manuscript according to the reviewers' suggestions and wrote the rebuttal letter.

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Legend for figures

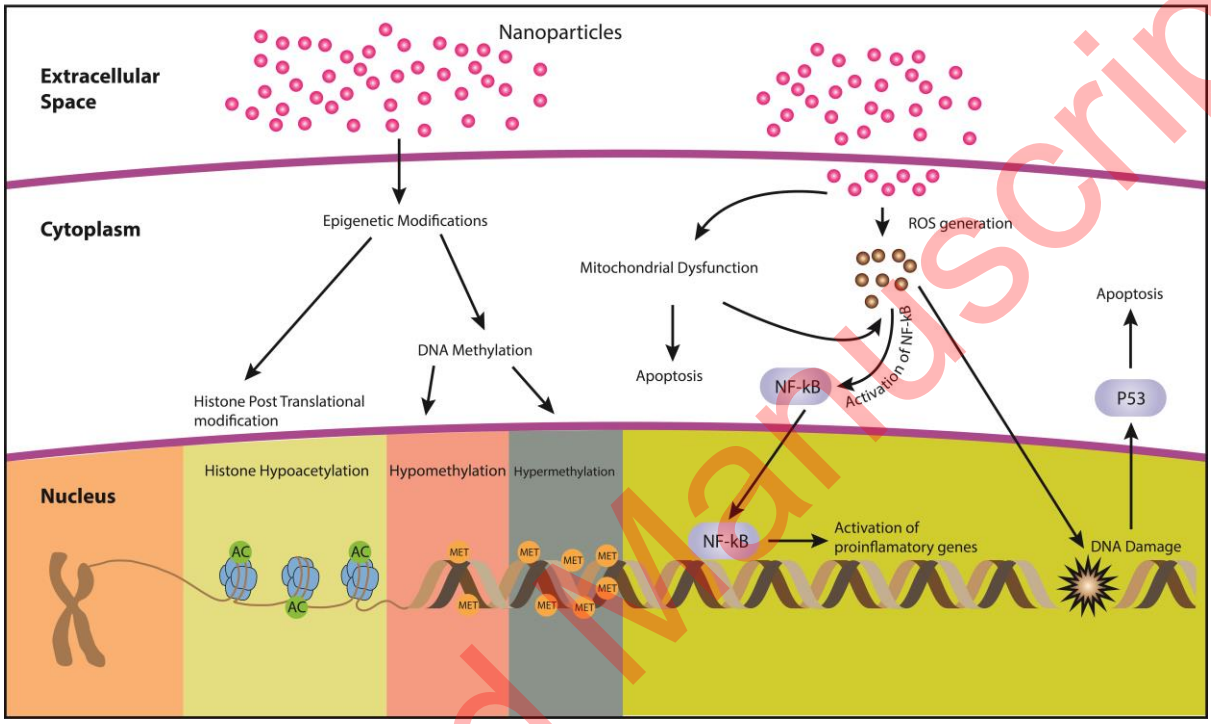

Fig. 1 Different mechanisms associated with nanoparticle toxicity