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Review Article

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Nanoformulation Safety versus Toxicity; What do the Recent Studies Tell Us?

Abdulkader Shaikh Omar[1,2]*

[1]Department of Biological Sciences, Faculty of Sciences, Kng Abdulaziz University, 21589, Jeddah, Saudi Arabia.
[2]Nagla Bint Saud Al Saud Center for Distinguished Research in Biotechnology, Jeddah, Saudi Arabia.

***Email:** akmshaikh90@gmail.com*

ABSTRACT

Nanoparticles (NPs) are a group of substances with characteristics that differ from their bulk and molecular counterparts. Different NPs have various size averages from 1 to 100 nanometers in two or three dimensions. NPs have been used for numerous industrial and domestic purposes, progressively increasing their production. Because of using NPs in different and multiple products, their presence in the environment around us has increased, raising the risk of potentially adverse effects of NPs in natural systems. NPs can enter the human body through different routes such as inhalation, ingestion, and skin contact. Applications of NPs in the medical field are variable; they are being utilized in diagnostic and therapeutic methods. The physico-chemical characteristics of NPs, such as size, shape, surface area, and dispersity, are considered crucial factors that significantly impact their safe or toxicological behaviors. Before clinical use, it is important to understand the characteristics of NPs, their effect on the body, and their toxicity mechanisms. This work aimed to summarize the studies that have described the great progression of nanotechnology and formulation of NPs in the last few years and its effects and toxicity on different human body systems.

Key words: *Nanoformulation, Safety, Toxicity, Studies, Mechanism*

INTRODUCTION

Nanoparticles (NPs) are structures with dimensions ranging from 1 to 100 nanometer (nm) at least one nm. They have different physicochemical properties, including lower melting points than their derivative metals and catalytic properties. Other characteristics include thermal and electrical conductivity, magnetic, wettability, light scattering and absorption [1].

According to the European (EU) Commission, there is another definition of NPs, and it is described as "natural or an industrial substance which is unbound, aggregated or even agglomerated elements and their dimensions between 1–100 nm" [2]. Because of the properties mentioned above of NPs and their small size and large surface-to-mass ratios, nanoparticles are widely used in industries, including electronics and cosmetics. They are used in the medical field, both diagnostic and therapeutic. The growing attention to the technology of nanoparticles has been improved due to advancement in NPs imaging methods that has atomic resolution abilities tandem electron microscopy, scanning transmission electron microscopy and scanning tunneling microscopy [2].

There are definitions scientific terms that have been used according to the British Standards Institution, first: nanoscale is approximately 1 to 1000 nm size range, second: nanomaterial is a material that has a nanoscale dimension in internal structures or external one while nano-object was known as material which has only one peripheral nanoscale dimensions that may be more than one. A nanoparticle is a nano-object having three exterior nanoscale dimensions. When a nano-longest object's and shortest axes have varying lengths, the terms nanorod or nanoplate are used in place of NPs [3].

The definition of nanoscience differs from the definition of nanotechnology. The definition of nanoscience is the science that studies and searches the material that has dimensions on the nanoscale. It provides information on their dimensions, structure, and characteristics, compares specific atoms, molecules, or bulk materials, and contrasts other materials. In comparison, nanotechnology is defined as manipulating and controlling matter on the nanoscale dimension using scientific knowledge of numerous biomedical and industrial applications [4].

Research in nanotechnology has increased in numerous fields of medicine, including delivery drugs and gene therapy. Also, there is a great progression in its use in the imaging field, the diagnosis of different medical diseases, and the medical devices industry. NPs are used as carriers for pharmaceutical drugs. Different NPs such as solid NPs, polymeric NPs, nanoemulsions, and liposomes possess possible clinical applications. Their using in the clinical field depends on variable parameters. It depended on their physical and chemical characters, drug release and loading efficiency. Applications of NPs are controlled by their efficacy/toxicity ratio [5]. There are 51 nanomedicines, according to the Food and Drug Administration (FDA) and European Medicine Agency (EMA), while there are 48 imaging agents based on nanoparticles. Liposomal and polymeric systems as examples of organic NPs. It has been known that most nanomaterials that the FDA permits use in the different medical fields are organic NPs. However, inorganic NPs have been used in medical applications, and inorganic NPs that have approval from the FDA are fewer [6]. On another side, different studies have proposed that NPs may be toxic. Our knowledge about the long-term effects of NPs is limited [7].

The time to produce nanomaterials and put them on the market for use is shorter than when it takes to evaluate their danger to the surrounding environment and the human body. For this aim, a lot of struggle and research is being made to study the impact of NPs on environmental and human health with a crucial requirement for designing safe NPs and decreasing their adverse effects [7]. In the last few years, the approval rate of the FDA for inorganic nanomaterials has gone down. This is mainly because researchers have tested all characteristics of nanomaterials and their effects on biological systems and analyzed their toxicities. Silver nanoparticles (AgNP) can penetrate the bacterial cell, resulting in cell death through the alteration of their cell membranes. In addition, releasing the ions of silver increases the permeability cell membrane, produces reactive oxygen species and disturbs the replication of DNA. Gold nanoparticles (AuNP) propagate corrosion, and depending on their size and shape, they might have some optical and electronic properties [8].

There are variable fields for using NPs and NMs that can be used in diagnosing, preventing, and even treating numerous diseases. NPs can be used as parts of drug delivery systems, diagnostic tools, and antibacterial agents. Also, NPs and NMs commonly used as components in skincare and cosmetics products. NPs present in household products that be used every day. These products can easily be conveyed into waterways and humans when disposed of in sewage [9].

Before clinical use, it is important to understand the characteristics of NPs, their effect on the body, and their toxicity mechanisms. Therefore, this work aims to summarize the studies that have described the great progression of nanotechnology of NPs in the last few years and its effects and toxicity on different human body systems.

Types and classification of nanomaterials

There are four categories of NPs depending on material based. They are, carbon-based nanomaterials, inorganic-based nanomaterials, organic-based, and composite-based nanomaterials [10]. Inorganic-based nanomaterials involve metal and metal oxide NPs. The metals are used as inorganic nanoparticles in a wide pattern, including gold (Au), silver (Ag), iron (Fe), cobalt (Co), zinc (Zn), aluminum (Al), lead (Pb), copper (Cu), and cadmium (Cd). While aluminum oxide (Al2O3), iron oxide (Fe2O3), zinc oxide (ZnO), cerium oxide (CeO2), titanium oxide (TiO2), magnetite (Fe3O4) and silicon dioxide (SiO2) are the common examples of the metal oxide-based NPs, have been used. Nanomaterials (NMs) have differed according to their physicochemical properties, chemical composition, effects on human health and the environment, and safety [10].

While NPs, which are mostly produced from organic matter and do not include any carbon, are known as organic-based nanomaterials. They consist of liposome NPs, micelles, and dendrimers polymer. Carbon is a component of carbon-based nanomaterials, which can be organized into various shapes such as ellipses, spheres, or hollow tubes. The category of carbon-based nanomaterials includes fullerenes (C60), carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr), and carbon onions. Important methods for production and fabrication of carbon-based materials including arc discharge, laser ablation and chemical vapor deposition (CVD) [11].

According to shape, NPs can be categorized into different shapes. Simple spherical, rod, triangle, circular, and more compound shapes octagonal or polyhedral. Gold Nanoparticles (AuNPs) have various shapes, including nanorod, nano-sphere, nano-cube, nano-star, nano-shell, sub-octahedral, icosahedral tetrahedral, nanocluster, decahedral, and octahedral. Also, AgNPs have variable shapes such as nano-platelet, spherical, nanorod, nano-

bar, triangles, cubic, five or six diagonals, nanowire and pyramid. NPs can be classified according to their origin, natural or synthetic. Mechanical grinding, engine exhaust, and smoke, as well as physical, chemical, biological, and hybrid approaches, are used to create manmade (designed) NPs. Currently, engineered NPs are created using various sources related to applications. The major challenge among synthetic NPs is if current knowledge is enough to guess their behavior and how they differ from natural NPs [12].

The crystalline shapes and chemical composition of the NPs were initially used to classify them. However, this classification was incomplete because it did not consider the NPs' dimensionality. The composites, such as 0D, 1D, 2D, and 3D NPs, were incorporated in a new classification scheme for NPs introduced in 2007. It depends on how the electrons flow within the NPs' dimensions [13]. In 0D NPs, electrons are imprisoned in a space with no dimensions, whereas in 1D NMs, electrons can move along the x-axis, which is less than 100 nm, for further explanation. Electrons move along the x-y-axis, as well as and the x, y, and z axes, in 2D and 3D NPs, respectively. The grain boundaries have a significantsignificantly impact on the characteristics of nanoparticles and nanomaterials (NMs). Therefore, grain boundary engineering will increase the traditional inner size effects, such as lowering the melting point lowering and diffusion augmentation [13].

Applications of NPs

Application of AuNPs

Variable fields used gold nanoparticles, such as immunology, medicine, and biotechnology. To increase the surface reactivity, AuNPs can be fabricated in different sizes to ensure a larger surface area to mass ratio. AuNPs have been used in medical fields for many years as AuNPs have unique tunable sizes and optical and thermal characters. It was discovered that AuNPs could inhibit the proliferation of pancreatic tumors and orthotopic when using the delivery vehicle with an anti-cancer drug (gemcitabine). Gold nanocage and nanorods have been examined in prostate cancer as photothermal components [14].

AgNPs applications

AgNPs have exclusive surface, optical, and electronic characteristics, leading to their application in the biomedical industry as they are easily adapted by cells. In heart disease detection or cancer biomarkers, they are utilized as tools in diagnostic research, molecular imaging agents, and even treatment as in drug delivery systems. Also, AgNPs are used in numerous health sectors and medical products; they can be used as disinfectants because of their antibacterial effect and wound dressings. They can help in wound healing, treating vascular diseases, parasitic infections and surgical nettings [15].

Applications of iron oxide and magnetic oxide nanoparticles

Iron oxide and magnetic oxide nanoparticles can have high magnetic susceptibility and superparamagnetic properties. However, the majority of NPs that have been studied are Fe2O3 nanoparticles. Different Fe2O3 nanoparticles have received FDA and EMA approval for the treatment of iron deficiency in people with chronic kidney disease and to be used in imaging liver lesions and lymph node metastases. These Fe2O3 nanoparticles include (Ferumoxtran-10, Ferrlecit®, Gastromark™, Venofer®, INFed®, Feraheme®, Resovit®, Dexferrum®, and Feridex I.V®. However, some of them were withdrawn from the market, for example (Feridex I.V®, Ferumoxtran-10, Resovit®, Gastromark™) [16].

Also, they can be used in applications in medical fields such as they can be used in imaging vessels, gene therapy, in the field of drug delivery and magnetic separation of molecules and cells. For glioblastoma treatment, in 2010 EU approved Nanotherm™; it is a superparamagnetic iron oxide nanoparticle (SPION) coated by aminosilane [16].

Applications of zinc oxide nanoparticles

They have been widely applied as antibacterial agents in the medical field, food preservation, textile coatings, and environmental applications. Research on their drug-delivery potential and their anti-cancer, antibacterial, antioxidant, antidiabetic, and anti-inflammatory effects has alo been done. They have also been utilized in various industrial products, including coatings, rubber, paint, and personal care items like sunscreen and cosmetics. In the textile industry, zinc oxide is added to the final fabrics; this encourages the development of antibacterial and deodorant qualities as well as resistance to UV and visible light [9].

Applications of silica nanoparticles (SNPs)

The structure of mesoporous silica NPs is distinctive. Its internal structure comprises several hollow tubes and appears like a porous honeycomb. High channel capacity, a sizable surface area, strong biological compatibility, easily functionalize surfaces, customizable size, and thermodynamic stability are among the traits of SNPs. The significant individual is not harmful. They can adsorb and release drug molecules. Hence they have been employed for drug delivery and release. Numerous release-controlling system types have been created using SNPs. Several mesoporous silica nanoparticles have been used in a system controlled releasing of antibiotics, and fluorescent SNPs have been used to deliver antisense nucleic acids, in addition to using some of them together with gene therapy and chemotherapy in cancer treatment [17].

Applications of titanium oxide, copper oxide, and other nanoparticles

Variable inorganic NPs have been used in drug delivery and therapy, such as barium-based nanoparticles, titanium oxide, copper oxide, and nickel oxide; however, not all NPs are used in this field. There are numerous applications for copper oxide nanoparticles and titanium-based nanoparticles in medical and non-medical fields. Copper oxide nanoparticles were used as antibacterial, biosensing, disinfectants for wastewater, and biolabeling [18]. While photodynamic therapy, photothermal therapy, antimicrobial agents, and even cosmetic industries are examples of applications in which titanium nanoparticles can be used. Hafnium oxide nanoparticles have been investigated to be used in treating soft tissue sarcoma. NBTXR3 is a compound investigated to treat rectal cancer, while PEP503 has been investigated to treat head and neck cancer [18].

Other NPs have great importance in anti-cancer photodynamic therapy, whereas these NPs are quantum dots. There are numerous applications in photovoltaic and light-emitting devices, solar cells, and catalysis. They are considered biosensors and imaging agents and are helpful in cancer detection playing an important role in diagnosis, disease staging, and event management [19].

Toxicity of nanoparticle

Nanoparticles' toxicity to different organs is related to their way of administration. There are various routes of exposure to NPs, such as inhalation that causes inflammatory reactions, fibrosis and necrosis in lung tissues, ingestion, skin contact, and injection. Also, NPs' origin affects how hazardous they are. While many of them appear to be harmless, others have negative impacts [20].

Toxicity can be classified according to different parameters. According to the time required for its manifestation, it is classified as immediate or delayed. If the effect is permanent or not, it is classified as reversible and non-reversible toxicity. At the same time, depending on whether the effects are limited to the site of drug administration and activity or spread throughout the entire body, it is categorized as local or systemic [21].

Acute nanoparticle toxicity

Acute toxicity occurs after exposure to a single dose within 24 hours or repeated exposures within 24 hours. The goal of different researches that study acute toxicity of NPs is to detect the maximum tolerated dose (MTD) of nanoparticles up to 14 days and the non-observable effect level (NOEL) [21]. There are different methods to evaluate the acute toxicity that has been detected. They are the fixed-dose procedure (FDP), 50% lethal dose (LD50), the up-and-down (UDP), and the acute toxic category (ATC) methods. Some of them are not approved tests [21].

Solid NPs result in oxidative stress, inflammation, and DNA damage following acute systemic exposure. The induction of oxidative stress by NPs exposure occurs in different organs, mainly the spleen, liver and kidneys. Also, NPs reduce the effects of antioxidants. In addition, the exposure causes activation of signaling pathways of cell stress, mitochondrial dysfunction, and DNA damage. The results are apoptosis and a stopped cell cycle [22]. Instead of focusing on the initial dose, it is important to normalize the hazardous responses of nanoparticles with their absorption and state of agglomeration. To assess the NPs toxicity, we should consider their crystallinity and composition, correlated to size distribution. Inorganic-based nanomaterials (metal oxide) solubility is key in assessing acute toxicity. After exposure, these NPs are thought to disintegrate and release their free ions, which causes toxicity. Soluble iron and zink oxide nanoparticles were in human mesothelioma cells at concentrations up to 30 ppm (μg/mL) for three days when used. There were signs of their toxicity, on the other hand, insoluble NPs of zirconia (zirconium dioxide), ceria (CeO_2) and titania (TiO_2) have no quantifiable toxic effects when used for six days at the same concentrations [22].

Following their cellular uptake, NPs go to lysosomes after cells uptake them. In lysosomes, the pH was acidic (5.5), which helped degrade NPs and release toxic heavy metals. These ions cause severe cell damage in various

ways, for example, the formation of reactive oxygen species and enzyme deactivation. Increasing free radicals by NPs is related to dose-dependent. The production of free radicals came from reduction or a catalytic process [23].

Subchronic and chronic nanoparticle toxicity

We cannot depend on the acute toxicity of NPs to detect their safety for numerous causes. Firstly, NPs exposure is a constant process example in employees in the industrial field or exposure due to daily use of cosmetics. The second cause is that the NP's degradation is a very long process that may take longer than the time taken in the NP's elimination process from the body. Additionally, the metabolites from NPs degradation may be toxic. Lastly, the accumulation of NPs and the biodistribution may be changed long run. This makes the need for additional research regarding the outcomes of NPs after chronic exposure to NPs. In other words, chronic exposure needs to be examined differently from acute exposure because they include various steps that not be the same as a single step of acute exposure [23].

The research on the sub-chronic and chronic toxicity of NPs should examine neurotoxicology, immunotoxicology, cardiovascular function, and ophthalmological evaluations after exposure to nanoparticles to detect long-term of them. Different systems' toxicity should be tested while studying the sub-chronic and chronic effects of NPs, such as genotoxicity, carcinogenesis, and embryotoxicity. A chronic study was examined to detect toxicity in reproduction systems one and two generations [16].

In sub-chronic studies to detect the doses of non-observable effect level (NOEL) in different routes of administration (ingestion, inhalation, and skin contact), the research period should be over 10% of the life span of animals. Furthermore, the number of animals in these studies should be 10 animals/sex/dose, no less than three doses of nanoparticles should be administrated, the exposure time for ingestion and skin contact routes should be seven days/week, and for inhalation route should be 6 hrs per day for seven days [24].

After NPs exposure, morbidity and even mortality may occur, and they should be assessed twice daily. It is very important to observe changes that occur in blood, urinalysis, clinical biochemistry, and necropsy analyses in organs of concern to evaluate organic NPs.

In chronic exposure studies, the number of animals in each study group should be at least 50 animals/dose/sex. Unfortunately, this number of animals can detect a side effect of examined NPs in less than 2% of the people. Chronic exposure to NPs usually associated with a different type of cancer, considered the main health problem. To know the carcinogenicity of NPs, studies should be done in vivo treatment in rodents for 24 months [25].

After prolonged exposure to zinc oxide and aluminum oxide, NPs to rats by oral route for 75 days, hepatic expression of mtTFA and PGC-1α proteins and toxicities in the kidney and liver [25]. In comparison, the morbidity of the parental planktonic crustacean female Daphnia Magna occurred after chronic exposure to gold NPs for 21 days due to diminished development. It declined fitness of reproduction that manifested by diminution of the aborted eggs and even total offspring [24]. When experimental rats were exposed to 12 weeks of inhalation of titanium dioxide NPs, lung toxicity manifested by the diminishing function of macrophages in the lung alveolus, inflammation and lung injury [25].

Silver nanoparticle (SNPs) toxicity

SNPs toxicities are linked with numerous physical and chemical properties, including size, surface area, chemical nature, reactivity and charge, aggregations and compositions. According to size-dependent toxicity in both vitro and vivo investigations, the lesser effects are realized from larger SNPs compared to the smaller ones [26]. On the other hand, the toxicity levels of the coated SNPs are higher than the uncoated ones according to the surface coating property, which is considered a vital character contributing to the NP's toxicity. Releasing silver ions is the reason for the acute toxicity of silver-related materials. When chloride ions (Cl^-) are highly concentrated in the media, the precipitation of silver ions can be helped, as shown in some studies on zebrafish and fish gill cell lines. When there are no chelators, the levels of free Ag^+ would be higher, which means more severe SNPs toxicity. Conversely, the ROS can be counteracted by chelators, thus improving the SNPs' toxicity [26].

Accumulation of SNPs within secondary organs such as the liver, spleen and brain. The toxicity of SNPs is influenced mainly by the distribution volume in the body. The different mice genders have different distribution and kinetic relations. After oral or intravenous administration, the SNPs concentration builds up in the brain and kidneys. Excretion is done in the form of stools and urine. SNPs may undergo biochemical transformation after interring different body systems, leading to the creation of secondary particles, leading to prolonged toxic

effects on health. The main sites of SNPs buildup and toxicity in the body after long-term exposure are the liver and spleen.

Gold nanoparticle toxicity

The key parameter that dominates the characteristics of gold nanoparticles is their size because the particle size controls different processes such as the effectiveness of endocytosis, cellular localization and accumulation sites in vivo. The cytotoxicity of gold materials depends on their size. Usually, cells can easily take up particles with 1nm to 200nm in diameter. So, the cellular uptake of AuNPs was seriously dependent on their size. AuNPs toxicity is linked with other properties and the size, including surface modification and particle shape [27].

Mechanism of NPs toxicity

The toxicity of nanoparticles may lead to increased pro-inflammatory cytokine expression and increased activation of pro-inflammatory cells (macrophages and neutrophils), leading to amplified excretion of reactive oxygen species (ROS). It is known that increased ROS damages DNA and activates several signaling pathways, i.e., serine/threonine-protein kinase B (AKT), p53 suppressor protein and mitogen-activated protein kinases (MAPK). While inorganic nanomaterial toxicities' primary mechanisms promote oxidative stress and inflammation, other NP types have specialized mechanisms [28]. Toxicity also leads to modification in functions and structures of protein and disruption in membrane integrity. Characters of NPs that simplify all mechanisms and assist in the molecular contact in the desired sites involve large surface areas. The human body tries to control the concentration of ROS in cells by different detoxifying enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Also, our body uses variable antioxidants for this purpose, including glutathione (GSH), ascorbic acids, flavonoids and vitamin E. NPs affect these protective mechanisms in different ways, reducing GSH to oxidized form, leading to induction of oxidative stress [28].

Additionally, protein denaturation, mitochondrial dysfunction, and disruption of phagocytic activities are all triggered by the acute toxic consequences of exposure to NPs. On the other hand, the reticuloendothelial system, nucleus, and neural tissue uptake of NPs, a familiar root of prolonged toxicity, results in the production of neoantigens, which ultimately causes organ malfunction and hypertrophy [29]. The easy dissemination of NPs across the environment and the resulting human exposure that results in health issues are caused by their free movement. On the other hand, when fixed NPs are handled properly, and the nanostructured elements are bonded to a huge object, no health issues arise [29].

Titanium dioxide encourages the nitric and glutamic acids to be realized in the brains of mice. Additionally, it increases the release of inflammatory cytokines from macrophages by activating signaling pathways of inflammation through NF-B pathways and c-Src, p38 MAP kinase, and following deposition of titanium dioxide in organs with high levels of macrophages (liver and spleen). Some NPs induced epigenetic modifications, for example, changes in histones post-translational, alterations in the DNA methylation status, remodeling in chromatin and RNA methylation [23].

Factors affecting nanoparticle toxicity

A variety of nanoparticle-specific variables influence engineered nanoparticles (ENPs) toxicity. These variables can be divided into three major categories: (i) physicochemical properties, (ii) interactions with co-pollutants present in aquatic environments, and (iii) functional behavior of ENPs. Examples of ENP functional activity influencing toxicity include ROS production and ENP ionization [30]. For instance, silver and copper (Cu) nanoparticles are ENPs that, in water, they dissolve. They produce free ions (Ag^{2+}, Cu^{2+}) respectively and peroxide radicals. The ionic form of metals is known to be more toxic than nanoparticles. ENP concentration in the aquatic environment is vital when examining the complete toxicity. Concentrations from 5 to 50 µg/L were small and may cause oxidative stress, physiological changes and chromosomal changes. While a concentration of 1 mg/L was considered a high concentration that caused mortality [31].

Ingressing ENPs into cells is a vital factor in their toxicity. The methodology can be summarized as follows: the adhesion to the pores of the cell membrane and then complete entry by ion transport systems or endocytosis. During ENPs entrance, ROS and interference with the electron transport process have extensive harmful effects, including damaging the cell membrane and nucleic acid and also changing the functions of organelles [31]. The research done on NPs toxicities was performed on multiple model organisms to show the various toxicity effects [30].

Furthermore, the duration, route and frequency of exposure, the dose of NPs, the age, sex and strain of experimental animals affect NPs toxicity. Biological interaction could modify NP's effect and behavior. We need

to know the changes in physicochemical characteristics of NPs which occurred in the body systems and how these alterations can impact their fate [32].

Tissues and organs affected by NP toxicity

Despite the variable applications of NPs, such as diagnosis and treatment of different diseases like malignant tumors and many other disorders and for targeted delivery of drugs to tissues and organs, NPs have high toxicity for living organisms which is considered a strong limiting factor to use them in. After inhalation, NPs reach the bloodstream and then to different organs in the body, including the liver, heart, or blood cells [33].

Developmental nanoparticle toxicity

NPs cause mortality and teratogenicity in zebrafish, as well as having negative impacts on the hatching rate and the developmental system. As an endpoint for toxicological analysis, embryonic development is a viable option. Delays in hatching and death were seen in embryos treated with AgNP. The developmental toxicity of AgNP therapies includes distorted notochords, aberrant body axis, sluggish blood flow, pericardial edoema, and even arrhythmia [33].

When zebrafish are exposed to copper oxide nanoparticles (CuONP), it may result in aberrant phenotypes such as smaller heads and eyes and delayed epiboly. When exposed to cetyltrimethylammonium bromide-coated gold nanorods, zebrafish embryos suffered death and delayed embryonic development. Examples of these changes include tail abnormalities, pericardial edema, decreased body length, deletion of the developing eye, and head and tail elongation [34].

ZnONPs have harmful consequences in zebrafish, such as delayed hatching, skin ulceration, and high mortality. The following list summarises the toxicity of TiO2NP to zebrafish embryos (premature hatching and a reduction in the normal hatching time) [34]. Most studies on the developmental toxicity of NPs were done on zebrafish for many reasons, for example, its transparency during embryo stages, its teratogenic test cycle is short (one week), it is suitable for image-based detection, zebrafish is suitable for large-scale gene mutant screening and analysis, it can record a variation of teratogenic indicators, such as cell movement during the intestinal phase, brain formation, heartbeat and blood circulation [35].

NPs can impact male fertility since spermatogenesis is extremely sensitive to these compounds. When male CD1 mice were exposed for 12 days by the intravenous route to small SNP concentrations, there was no meaningful reduction in testis weight and sperm numbers. Still, there was an alteration in Leydig cells' function, which increased testosterone levels in serum and even in testicular tissues. When pregnant CD-1 mice have been exposed to SNPs intravenously, the visceral yolk sac and endometrium are sites of accumulation of SNPs [36].

Nanoparticle immunotoxicity

There is an interaction between the immune system and the NPs. Variable factors contribute to these interactions, such as hydrophilicity, lipophilicity, catalytic activity, composition, electronic structure, solubility, dimension, and surface area. Some NPs accumulate in regional lymph nodes, where they are absorbed and processed by dendritic cells. Through their interactions with self-proteins, they are able to transform their antigenicity and amend immune responses that could lead to autoimmunity [37].

Allergic contact dermatitis or allergic sensitization can be generated by other NPs. NPs can also moderate the production of cytokine towards Th1 (Pd, Ni, Pl, and Co) or Th2 (Ti, mw and sw Carbon) production patterns. But NPs are unlikely can act as a hapten inducing a specific IgE production. Multiple allergic sensitizations to various allergens can be seen when they help induce particular patterns of cells, cytokines, and antibodies. Furthermore, NPs pro-inflammatory effects were demonstrated in the lungs of the affected test animals with higher manifestations of IL-1beta, MIP-1alpha, MCP-1, MIP-2, keratinocyte chemoattractant, TARC, GM-CSF, MIP-1alpha and activation of the stress-activated MAPKs p38 and JNK [37].

Nanoparticle hepatotoxicity

Chemical detoxification and toxicity are primarily focused on by the liver. Many nanomaterials have been described to accumulate and induce adverse side effects in the liver. Among these NPs are silica and several metallic nanoparticles. Hepatotoxicity has been reported after oral exposure and IV injection. Most toxic effects occurred in the hepatocytes, considered most liver cells. Also, other cells were affected badly, leading to liver damage (Kupfer cells) [38].

The endothelial cells were not observed as buildup zones of NPs. A common histological alteration has been detected in the form of sinusoidal dilation, considered a certain degree of endothelial cell dysfunction. The NP's size plays a crucial role in hepatotoxicity; nanosized NPs are more toxic than micro-sized ones. NPs below 100 nm tend to be more harmful than bigger ones. NPS can induce damage and apoptosis of human liver cells via several methods [38].

After inhalation, SNPs can be accumulated in the liver leading to vacuolization of cytoplasm and focal necrosis of liver cells. There is an elevation in liver biomarkers and histopathological parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-galactosyltransferase after SNPs administration. On another side is the inhibition of numerous cytochrome P450 (CYP) enzymes such as CYP3A, CYP2C, CYP1A, CYP2E1 and CYP2D after exposure [39].

Lung nanoparticle toxicity

Pulmonary surfactant (PS) is considered the early barrier of nano-bio interactions in the lungs. There was inhibition in PS caused by NPs. A low concentration of carbon nanotubes, graphene oxide, zinc oxide, and silver nanoparticles increased in vitro minimum surface tension of a modified natural PS. These led to the extensive alveolar collapse and inflammation observed in vivo in mice exposed to these NPs. In other words, there is a direct correlation between increasing surface tension resulting from PS inhibition due to NPs exposure that ends with lung toxicity.

Targets of SNPs that cause lung damage include connexin43 (Cx43) and gap junctional intercellular communication (GJIC). Both had increased expression in the cell line A549 of human lung cancer. The lung function of male and female rats exposed to SNPs was assessed, and it was found that female rats recovered gradually from the lung inflammation. The male rats in the high, however, displayed ongoing inflammation with a 12-week recovery time. Exposure to acute and subacute SNPs causes minor pulmonary tissue fibrosis and inflammation [40].

Renal nanoparticle toxicity

Kidneys are vital blood filtration and waste elimination organs, playing a key role in the transport and clearance of nanoparticles. The interactions of nanoparticles with different kidney compartments can be regulated by modulating their size, shape and surface chemistry. Understanding molecular nanoparticle–kidney interactions is significant in improving disease, controlling nanoparticle transport and clearance, and minimizing health hazards [41].

SNPs accumulate in the kidneys depending on doses. Exposure to SNPs caused inflammation, degeneration in the proximal convoluted tubule, thickening of the membrane and capsule, and adhesion to Bowman's capsule. There was a correlation between exposure to SNPs via skin and accumulation of it in the tissues of the kidney. After exposure to gold nanoparticles (AuNPs), there were alterations in the integrity of lysosomes, mitochondrial membrane potential, intracellular glutathione (total GSH) and ATP. AuNPs mainly targeted the mitochondria depending on their concentration. The integrity of the lysosomes was also partly affected [42].

Dermal nanoparticle toxicity

Some NPs have been used in medical applications and wound dressing to promote rapid healing. There is a strong association between NPs accumulation in different tissues and exposure through the skin. It is very important to improve public awareness concerning the environmental exposure to NPs, especially for the personnel in research and industry. The diminishing thickness of the dermis, papillary layers, and epidermis with the increasing number of Langerhans cells, inflammation markers, and levels of dermis collagen have occurred after exposure to SNPs. Gold nanoparticles with a size of 15 nm in aqueous solution aggregated on the skin surface (superficial stratum corneum) after 24 h exposure, while 6-nm nanoparticles in toluene penetrated through stratum corneum and into epidermal layers of human skin [43].

Central and peripheral nervous system nanoparticle toxicity

The brain and the spinal cord make up the central nervous system. They are both delicate body organs requiring protection from injury by xenobiotics. The distribution of various drugs into the CNS could lead to superfluous neurotoxicity. Nano-neurotoxicity of the NPs is a potential threat to the CNS via several possible mechanisms. Regarding the central nervous system (CNS), research data on nanoparticle interaction with neurons has provided evidence of both negative and positive effects [44].

Nanoparticles, such as polysorbate 80-coated PBCA NPs and pegylated PLA immunonanoparticles, can cross BBB through intravenous administration and follow the brain's accumulation. As these NPs can penetrate the BBB, resulting in some adverse effects on the BBB functions and brain physiology. The physical barrier can be permeated by various NPs of different particle sizes and materials, which enter the brain by the olfactory bulb's nerve endings. The initial pathway for NPs to reach the brain involves the uptake of nanoparticles by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to the structures of the CNS [45].

Activation of glutamatergic N-methyl-d-aspartate receptor (NMDA) is involved in the SNPs neurotoxicity. SNPs across the BBB lead to brain inflammation and neurotoxicity. After daily colloidal silver ingestion, epileptic seizures and coma occurred due to high levels of silver in different body tissues, including erythrocytes, plasma and even incerebro-spinal fluid. Exposure to SNPs can induce irreversible neurotoxicity that ends in death. Ag NPs could exacerbate aggravate the lack of sleep deprivation, resulting in brain damage and methamphetamine-induced brain damage. In a variety ofvarious circumstances, exposure to Ag NP could cause the BBB to break down in vivo in both cold and warm conditions [46].

Cardiovascular nanoparticle toxicity

There is a large interest in researching the harmful effects of NPs on the cardiovascular system. Metal-based NPs, including TiO2, ZnO and Ag NPs, are renownedly found in commercially accessible products. Additionally, they might find use in biomedicine, which would bring them into closer touch with cardiovascular systems. ZnO NPs administered orally to rats caused tissue damage to the hearts and increased inflammatory mediator levels in the blood. Contrarily, intratracheal administration of ZnO NPs to normal rats could cause dyslipidemia, increased inflammatory markers in the blood, and atherosclerotic changes. Human coronary artery endothelial cells overexpressed HO-1 and PECAM-1 after exposure to ZnO NPs in vitro, which may be the mechanism underlying atherosclerotic changes [47].

The introduction of Ag NPs to the embryos of zebrafish at the same doses was observed to cause pericardial effusion, decreased heart rate, aberrant cardiac shape, and circulatory abnormalities but not Au NPs. It was also demonstrated that early acute exposure of zebrafish embryos to Ag NPs caused a transitory gene expression associated with vascular endothelial growth factor (VEGF), which delayed vascular development at a later stage. Ag NPs were administered intratracheally to rats, which increased cardiac ischemia-reperfusion injury and decreased coronary vascular reactivity, probably due to an increase in circulating inflammatory mediators [26].

Nanoparticle genotoxicity

There is great importance to the study of the genotoxicity of metal NPs. Metal NPs caused chromosomal aberrations, DNA strand breaks, oxidative DNA damage, and mutations. SNPs interfere with the DNA replication causing mutations and genotoxic like in Drosophila; this happened after administration via a subcutaneous route, not an intramuscular injection. Chromosome damage and elevated micronucleus production are associated with toxic SNP concentrations. Twenty-eight days of in vivo trial administration of 60 nm SNP had no statistically significant genotoxic effects. Large DNA adducts and micronuclei forms were observed in human cell lines in an in-vitro research [48].

Challenges facing in NPs toxicity

There are variable challenges that need to be overcome. Several research studies have been conducted during the past decade on different NPs to explore the possible mechanisms of uptake, dosage, toxicity levels and how we can decrease their toxicity [6, 49]. Because numerous hazardous compounds are being included in goods with little chance of being released, there is currently a lack of a large-scale "nano" manufacturing industry. The particles are discharged into the environment, where they quickly combine and sequestered in big safer materials by encapsulation or another method [49].

Making reproducible data is further complicated by a lack of modeling tools, standardized processes, and theoretical understanding. Lessons from recent advancements in disease modeling and artificial intelligence can be applied to this scenario. There are few demonstrative functional assessments, few biomarkers, and scant information about the absorption process of nanoparticles, all of which the scientific community has to develop to advance its understanding of nanotoxicology. The absence of standardized materials consideration and dosimetry. Inadequate statistics, an absence of standardized dosimetry, and an absence of material consideration are also prevalent. By addressing these concerns, the potential for the faithful use of nanomaterials in modern

nanobiotechnology-based pharmaceutical design to treat uncommon diseases, battle infection, and address neurological issues, will be increased [49].

Further studies to avoid nanoparticles toxicity

Design more safe NPs by improving structured nanoemulsions and solid lipid nanoparticles formulated using food-grade ingredients that have been generally recognized as safe (GRAS) by the FDA. These NPs include proteins, lipids, surfactants and polysaccharides. The toxic effects of NPs are mostly because of metal-containing or solid NPs according to that, whole efforts have been awarded to limiting their use [50].

Defining the danger posed by NPs and methods for creating non-cytotoxic nanoparticles are other ways to make NPs that are safer. Classifying the potential dangers of various NPs is a practical way to create safer nanotechnology. This classification is to be founded on present toxicological assays that evaluate long-term and acute fatal effects. The present toxicological tests are used for chemicals and employed for degradable NPs, which have short residence durations in the body or the environment. This approach has this advantage. They assess the toxicity of NP and its breakdown products [23].

Another method of reducing the NP's hazard, particularly lipid-based NPs, is using "next-generation lipids." This class revealed rapid plasma removal and improved preclinical studies with high potency in vivo. Surface modification policies are another way to reduce the harm of NPs and create safer nanotechnology, and coating surface is the major of these strategies. Surface coating is any functionalization, alteration, or stabilization done to the NPs to change their characteristics [50].

CONCLUSION

There was great development in the field of nanotechnology and its applications. The effect of NPs on the surrounding environment and our bodies must take the main concern of societal awareness. Reducing NPs toxicity occurred by adjusting their characters by augmenting their efficacies. Approaches and guidelines to decline should be interested in studying acute and chronic exposures. When compared to insoluble NPs, the toxicity levels of the soluble NPs are higher. This shows that there is a great effect of nanoparticle dissolution on their toxicity. According to toxicity, the stabilized metal oxide NPs and non-modified NPs have lower toxic effects when compared with the stabilized metal oxide NPs. There is a great need for more and more studies on the fate of NPs in different body systems and how creatures respond to exposure to NPs for a long period. It is important to create approaches to produce safer NPs, till we complete our perception of the toxicological status of NPs.

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REFERENCES

1. Salih HH, El Badawy AM, Tolaymat TM, Patterson CL. Removal of stabilized silver nanoparticles from surface water by conventional treatment processes. Adv Nanopart. 2019;8(2):21.
2. Włodarczyk R, Kwarciak-Kozłowska A. Nanoparticles from the cosmetics and medical industries in legal and environmental aspects. Sustainability. 2021;13(11):5805.
3. Sudha PN, Sangeetha K, Vijayalakshmi K, Barhoum A. Nanomaterials history, classification, unique properties, production and market. InEmerging applications of nanoparticles and architecture nanostructures 2018 Jan 1 (pp. 341-384). Elsevier.
4. Dan DT. Nanotechnology, Nanoparticles and Nanoscience: A New Approach in Chemistry and Life Sciences. Soft Nanosci Lett. 2020;10(02):17.
5. Abdo NI. Comparative Study between Magnetite Nanoparticles and Magnetite/Silver as a Core/Shell Nanostructure. Adv Nanopart. 2021;10(4):115-22.
6. Aghebati-Maleki A, Dolati S, Ahmadi M, Baghbanzhadeh A, Asadi M, Fotouhi A, et al. Nanoparticles and cancer therapy: Perspectives for application of nanoparticles in the treatment of cancers. J Cell Physiol. 2020;235(3):1962-72.

7. Medici S, Peana M, Pelucelli A, Zoroddu MA. An updated overview on metal nanoparticles toxicity. InSeminars in Cancer Biology 2021 Nov 1 (Vol. 76, pp. 17-26). Academic Press.
8. Talarska P, Boruczkowski M, Żurawski J. Current knowledge of silver and gold nanoparticles in laboratory research—Application, toxicity, cellular uptake. Nanomaterials. 2021;11(9):2454.
9. Effiong DE, Uwah TO, Jumbo EU, Akpabio AE. Nanotechnology in cosmetics: basics, current trends and safety concerns—A review. Adv Nanopart. 2019;9(1):1-22.
10. Sannino D. Types and Classification of Nanomaterials. InNanotechnology 2021 (pp. 15-38). Springer, Singapore.
11. Kaya SI, Cetinkaya A, Ozkan SA. Carbon Nanomaterial-Based Drug Sensing Platforms Using State-of-the-Art Electroanalytical Techniques. Curr Anal Chem. 2022;18(1):79-101.
12. Barhoum A, García-Betancourt ML, Jeevanandam J, Hussien EA, Mekkawy SA, Mostafa M, et al. Review on natural, incidental, bioinspired, and engineered nanomaterials: history, definitions, classifications, synthesis, properties, market, toxicities, risks, and regulations. Nanomaterials. 2022;12(2):177.
13. Rizwan M, Shoukat A, Ayub A, Razzaq B, Tahir MB. Types and classification of nanomaterials. InNanomaterials: Synthesis, Characterization, Hazards and Safety 2021 Jan 1 (pp. 31-54). Elsevier.
14. Han X, Li Y, Xu Y, Zhao X, Zhang Y, Yang X, et al. Reversal of pancreatic desmoplasia by re-educating stellate cells with a tumour microenvironment-activated nanosystem. Nat Commun. 2018;9(1):1-8.
15. Jackson TC, Agboke AA, Udofa EJ, Ucheokoro AS, Udo BE, Ifekpolugo NL. Characterization and Release Kinetics of Metronidazole Loaded Silver Nanoparticles Prepared from Carica papaya Leaf Extract. Adv Nanopart. 2019;8(3):47-54.
16. Chrishtop VV, Mironov VA, Prilepskii AY, Nikonorova VG, Vinogradov VV. Organ-specific toxicity of magnetic iron oxide-based nanoparticles. Nanotoxicology. 2021;15(2):167-204.
17. Castillo RR, Lozano D, González B, Manzano M, Izquierdo-Barba I, Vallet-Regí M. Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery: An update. Expert Opin Drug Deliv. 2019;16(4):415-39.
18. Liu W, Chen B, Zheng H, Xing Y, Chen G, Zhou P, et al. Advances of Nanomedicine in Radiotherapy. Pharmaceutics. 2021;13(11):1757.
19. Sheng J, Zhang L, Deng L, Han Y, Wang L, He H, et al. Fabrication of dopamine enveloped WO3− x quantum dots as single-NIR laser activated photonic nanodrug for synergistic photothermal/photodynamic therapy against cancer. Chem Eng J. 2020;383:123071.
20. Badgar K, Prokisch J. Testing Toxicity and Antidote Effect of Selenium Nanoparticles with Paramecium caudatum. Open J Anim Sci. 2021;11(4):532-42.
21. Zhang H, Shi J, Su Y, Li W, Wilkinson KJ, Xie B. Acute toxicity evaluation of nanoparticles mixtures using luminescent bacteria. Environ Monit Assess. 2020;192(8):1-8.
22. Długosz O, Szostak K, Staroń A, Pulit-Prociak J, Banach M. Methods for reducing the toxicity of metal and metal oxide NPs as biomedicine. Materials. 2020;13(2):279.
23. Najahi-Missaoui W, Arnold RD, Cummings BS. Safe nanoparticles: Are we there yet?. Int J Mol Sci. 2020;22(1):385.
24. Luo Z, Li Z, Xie Z, Sokolova IM, Song L, Peijnenburg WJ, et al. Rethinking nano-TiO2 safety: overview of toxic effects in humans and aquatic animals. Small. 2020;16(36):2002019.
25. Mihailovic V, Katanic Stankovic JS, Selakovic D, Rosic G. An overview of the beneficial role of antioxidants in the treatment of nanoparticle-induced toxicities. Oxid Med Cell Longev. 2021;2021.
26. Tortella GR, Rubilar O, Durán N, Diez MC, Martínez M, Parada J, et al. Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment. J Hazard Mater. 2020;390:121974.
27. Gan N, Wakayama C, Inubushi S, Kunihisa T, Mizumoto S, Baba M, et al. Size Dependency of Selective Cellular Uptake of Epigallocatechin Gallate-modified Gold Nanoparticles for Effective Radiosensitization. ACS Appl Bio Mater. 2021;5(1):355-65.
28. Liu J, Wan M, Lyon CJ, Hu TY. Nanomedicine therapies modulating Macrophage Dysfunction: a potential strategy to attenuate Cytokine Storms in severe infections. Theranostics. 2020;10(21):9591.
29. Silva LF, Pinto D, Oliveira ML, Dotto GL. Dispersion of hazardous nanoparticles on beaches around phosphogypsum factories. Mar Pollut Bull. 2021;169:112493.
30. Wang F, Guan W, Xu L, Ding Z, Ma H, Ma A, et al. Effects of nanoparticles on algae: Adsorption, distribution, ecotoxicity and fate. Appl Sci. 2019;9(8):1534.

31. Chen F, Xiao Z, Yue L, Wang J, Feng Y, Zhu X, et al. Algae response to engineered nanoparticles: current understanding, mechanisms and implications. Environ Sci Nano. 2019;6(4):1026-42.
32. Alimohammadi S, Hosseini MS, Behbood L. Prenatal exposure to zinc oxide nanoparticles can induce depressive-like behaviors in mice offspring. Int J Pept Res Ther. 2019;25(1):401-9.
33. Raj A, Shah P, Agrawal N. Model organisms for in vivo assessment of nanoparticles. InToxicology of nanoparticles: Insights from Drosophila 2020 (pp. 29-57). Springer, Singapore.
34. Maheshwari RA, Sen DB, Zanwar AS, Sen AK. Evaluation of nanotoxicity using zebrafish: Preclinical model. InNanocarriers: Drug Delivery System 2021 (pp. 173-197). Springer, Singapore.
35. d'Amora M, Schmidt TJ, Konstantinidou S, Raffa V, De Angelis F, Tantussi F. Effects of Metal Oxide Nanoparticles in Zebrafish. Oxid Med Cell Longev. 2022;2022.
36. Singh S. Zinc oxide nanoparticles impacts: Cytotoxicity, genotoxicity, developmental toxicity, and neurotoxicity. Toxicol Mech Methods. 2019;29(4):300-11.
37. Petrarca C, Mangifesta R, Giampaolo LD. Immunotoxicity of Nanoparticles. InAllergy and Immunotoxicology in Occupational Health-The Next Step 2020 (pp. 75-94). Springer, Singapore.
38. Boey A, Ho HK. All roads lead to the liver: metal nanoparticles and their implications for liver health. Small. 2020;16(21):2000153.
39. Sepand MR, Aliomrani M, Hasani-Nourian Y, Khalhori MR, Farzaei MH, Sanadgol N. Mechanisms and pathogenesis underlying environmental chemical-induced necroptosis. Environ Sci Pollut Res. 2020;27(30):37488-501.
40. Qin Y, Han L, Yang D, Wei H, Liu Y, Xu J, et al. Silver nanoparticles increase connexin43-mediated gap junctional intercellular communication in HaCaT cells through activation of reactive oxygen species and mitogen-activated protein kinase signal pathway. J Appl Toxicol. 2018;38(4):564-74.
41. Oroojalian F, Charbgoo F, Hashemi M, Amani A, Yazdian-Robati R, Mokhtarzadeh A, et al. Recent advances in nanotechnology-based drug delivery systems for the kidney. J Control Release. 2020;321:442-62.
42. Li Y, Cummins E. Hazard characterization of silver nanoparticles for human exposure routes. J Environ Sci Health A. 2020;55(6):704-25.
43. Hashempour S, Ghanbarzadeh S, Maibach HI, Ghorbani M, Hamishehkar H. Skin toxicity of topically applied nanoparticles. Ther Deliv. 2019;10(6):383-96.
44. Engin AB, Engin A. Nanoparticles and neurotoxicity: Dual response of glutamatergic receptors. Prog Brain Res. 2019;245:281-303.
45. Furtado D, Björnmalm M, Ayton S, Bush AI, Kempe K, Caruso F. Overcoming the blood–brain barrier: the role of nanomaterials in treating neurological diseases. Adv Mater. 2018;30(46):1801362.
46. Sharma HS, Lafuente JV, Muresanu DF, Sahib S, Tian ZR, Menon PK, et al. Neuroprotective effects of insulin like growth factor-1 on engineered metal nanoparticles Ag, Cu and Al induced blood-brain barrier breakdown, edema formation, oxidative stress, upregulation of neuronal nitric oxide synthase and brain pathology. Prog Brain Res. 2021;266:97-121.
47. Cao Y, Gong Y, Liao W, Luo Y, Wu C, Wang M, et al. A review of cardiovascular toxicity of TiO2, ZnO and Ag nanoparticles (NPs). Biometals. 2018;31(4):457-76.
48. Narciso L, Coppola L, Lori G, Andreoli C, Zjino A, Bocca B, et al. Genotoxicity, biodistribution and toxic effects of silver nanoparticles after in vivo acute oral administration. NanoImpact. 2020;18:100221.
49. Paramo LA, Feregrino-Pérez AA, Guevara R, Mendoza S, Esquivel K. Nanoparticles in agroindustry: Applications, toxicity, challenges, and trends. Nanomaterials. 2020;10(9):1654.
50. Weng Y, Huang Q, Li C, Yang Y, Wang X, Yu J, et al. Improved nucleic acid therapy with advanced nanoscale biotechnology. Mol Ther Nucleic Acids. 2020;19:581-601.