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# Biochemical Toxicology: Heavy Metals and Nanomaterials

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## Abstract

The synthesis and application of nanoparticles have been actively studied in the modern era as it holds promises for effective and targeted strategies to deliver drugs inside the human body. Nanoparticles (NPs) play a big role in cancer diagnosis and have various advantages over other conventional chemotherapeutic drug delivery systems. But, the application of emerging engineered NPs to heavy toxic metals such as zinc, cobalt, and iron has resulted in a major source of toxicity. The toxicity of nanomaterials is majorly determined by their physical and chemical properties such as size, charge, and surface area. Also, the mechanism of nanotoxicity is majorly via the production of reactive oxygen species that create oxidative stress, thereby activating inflammatory cytokines and the mechanism of DNA damage that ultimately results in the cell death. So, mechanistic study needs to be done on nanomaterials to elucidate the mechanism involved in nanotoxicity and to generate less toxic and efficient nanomaterials.

**Keywords:** nanoparticle, heavy metals, nanotoxicity, ROS, inflammation

## 1. Introduction

Nanotechnology is one of the rapidly emerging fields in the twenty-first century with extensive increase of nanoparticle application for the treatment of a wide variety of chronic diseases such as cancer. P. Ehrlich's visionary concept of "magic bullet" based on the use of targeted medicines to effectively attack cancer cells has provided a promising field for cancer therapy [1]. Targeted delivery to solid cancers provides more bioavailability and effective approach for cancer treatment. The characteristics of nanocarriers such as their nanoscale, high surface-to-volume ratio, favorable drug release profiles, and targeting modifications allow them to target tumor tissue in an effective manner and release drugs in a stable and controlled manner [2]. NPs can accumulate in the leaky vasculatures of tumor tissue in an enhanced permeability and retention effect (EPR). The potential of nanomedicine can be explored in the field of early detection of cancer as well as in combination therapies for treating tumor earlier and effectively. NPs effectively solve the physiological barriers such as renal, hepatic, and immune related for effective drug delivery of conventional chemotherapeutic drugs [3]. NPs may be modified to utilize passive and active targeting mechanism to reach the tumor tissue. The nanodelivery-based carriers range from natural polymeric materials to nonbiodegradable gold NP, and magnetic mesoporous silica-based, metal-based NP. The surface of the NP can be suitably modified with ligands or drugs to offer multimodular treatment options [4]. The nanoparticle shape also plays an important role

in specific and effective nanodrug delivery. Nano-based drug delivery system has enhanced pharmacokinetic parameters, such as clearance value, volume distribution, and bioavailability to cancer cells through EPR. Unfortunately, these novel drug delivery systems still face barriers when delivered into the body, which can reduce the targeting efficiency as well as have increased toxic side effects. NPs have shown distinct toxicity patterns as compared with their larger counterparts [5]. As the size of NPs gets reduced for effective targeting, the number of surface molecules and surface area increase exponentially, which leads to complex bio-physiochemical interactions at the bio-nano interfaces when exposed to physiological environments. The potential paradigms of nanotoxicity can be understood possibly by understanding these bio-nano interactions. Since nanomaterials and therapeutic drug in combination work against cancer, the unfavorable toxicity of nanomaterials causes side effects and dysfunctions. Since the nanomedicines and therapeutic drugs share the same fate in the body, understanding the interconnections between nanotoxicity and drug delivery can widen our knowledge to improve the possibilities for cancer therapy. The effect of NPs can be divided into two categories, that is, primary and secondary depending upon the exposure time period [6]. The direct contact of NPs with cells results in primary effect, which involves toxicity, oxidative stress, DNA damage, and inflammation. Due to their nano-based size, the nanoparticles can translocate into the blood through tissue barriers where they can circulate and eventually accumulate in other organs, thereby, generating a secondary response of the NP. The secondary toxic effect of NPs might occur at the site of nanoparticle accumulation in organs such as the liver, spleen, or kidneys, and can stimulate systemic inflammation or can alter their systemic function [7]. The toxicity of NPs has been studied in different biological systems involving the cell lines as well as different organisms, which involve humans, rodents, zebra fish, catfish, algae, and macrophages. Carbon and metallic NPs are the most widely studied and used engineered nanomaterials. Nanometals, such as nanogold (nano-Au), nanosilver (nano-Ag), nanocopper, nanoaluminum, nanonickel, nanocobalt, and other NPs, have also been extensively studied. Toxic effect of metal oxide NPs such as nano-TiO<sub>2</sub>, nano-ZnO, nano-CuO, nano-CuZn, nano-Fe<sub>3</sub>O<sub>4</sub>, and nano-Fe<sub>2</sub>O<sub>3</sub>, with nano-TiO<sub>2</sub> and nano-ZnO in particular, has been reported [8]. As expected, different nanomaterials exhibit different toxic potency. For example, Zhu et al. compared the toxicity of three nanometal oxides, nano-CuO, nano-CdO, and nano-TiO<sub>2</sub>. Nano-CuO was determined to be the most potent in cytotoxicity and DNA damage, leading to 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation, while nano-TiO<sub>2</sub> was the least, without inducing a significant level of 8-OHdG [9]. The production of carbon nanotubes (CNTs) and graphene oxide is becoming commercially important. Under some experimental conditions, investigators have found that CNTs and graphene oxide are toxic. So, understanding the matter of safety and toxicity of nanomaterials has become an issue of interest to the public. Therefore, understanding the interactions of nanomaterials with biological systems is a particularly important scientific issue.

## **2. Physical and chemical properties of NPs in nanotoxicity**

Toxic effect of NPs can proceed through a variety of mechanisms. Toxicity from a nanoparticle depends on its physical and chemical properties as well as the testing systems such as different cell types. The fundamental physical and chemical properties, which include molecular shape, size, oxidation status, surface area, bonded surface species, surface coating, solubility, and degree of aggregation and agglomeration of nanomaterials, majorly lead to the generation of reactive oxygen

species and toxicity [10]. These intrinsic properties of nanomaterials can stimulate and generate toxic effects inside the biological system. Also, interaction with environmental factors such as light also determines how nanomaterials interact with the biological factors and lead to the mechanism of toxicity.

## 2.1 Size and shape

Their nanosize and large surface area are the unique physiochemical properties of nanomaterials that determine their toxicity. Due to their very small size, they have the ability to penetrate into cell membrane and other biological barriers into living organisms and can inhibit cellular functions [11]. The increased nanoparticle size decreases its ability for cellular uptake. Majorly due to their nanosize, nanomaterials can even target the lungs and give rise to several toxic effects. Yoshida et al. had reported that particle size plays a major role in intracellular disruption of amorphous silica and its induced reactive oxygen species (ROS) formation, leading to DNA damage in human skin HaCaT cells [12]. Moreover, as the size of nanoparticle decreases, the toxic effects increase. Alpha-MnO<sub>2</sub> nanowire, which is a wire-shaped nanomaterial, induces cytotoxicity, DNA oxidative damage, and apoptosis in HeLa cells [13]. In support of this statement, it was shown that long nanowires in cultured fibroblasts inhibited cell division, DNA damage, and increased ROS. Similarly, WISH cells when exposed to TiO<sub>2</sub> induced cytotoxicity alterations in morphology, production of ROS, and DNA damage. Sohaebuddin et al. determined the effects of the chemical composition of nano-TiO<sub>2</sub>, nano-SiO<sub>2</sub>, and multiwall CNTs on their toxicity in 3T3 fibroblasts, RAW 264.7 macrophages, and telomerase-immortalized bronchiolar epithelial cells [14]. The results indicated that the composition, molecular size, and target cell type are all critical determinants of intracellular responses, degree of cytotoxicity, and potential mechanisms of toxicity. Moreover, these nanomaterials induced cell-specific responses, resulting in variable toxicity and subsequent cell damage. A study by Yin et al. showed that the smaller the particle size, the greater the cellular damage induced. He studied the photocytotoxicity of four different sized (<25, 31, <100, and 325 nm) nano-TiO<sub>2</sub> and two different crystal forms anatase and rutile in human skin keratinocytes. Upon exposure to UVA radiation, all nano-TiO<sub>2</sub> particles induced cytotoxicity and cell membrane damage in a light- and dose-dependent manner. Similarly, in a study with different sizes of silica-titania hollow particle with uniform diameters of 25, 50, 75, 100, and 125 nm, the 50-nm silica-titania hollow NP showed the largest toxicity effect in macrophages [15].

The shape of the nanoparticle is one of the major determinants of nanomaterial-induced cytotoxicity. This was supported by the study done by Ray and his coworkers where they determined that a set of gold NPs with different shapes had similar cytotoxicity [16]. The shape of the nanoparticle is considered as a major determinant in the process of engineering and application. The characteristic shapes of NP are mainly spherical, ellipsoidal, sheet-like, cubic, and rod-like. Spherical NPs have shown to be more prone to endocytosis than nanotubes and nanofibers. Similarly, a study with different shapes (needle-like, plate-like, rod-like, and spherical) of hydroxyapatite NPs on cultured BEAS-2B cells showed that plate-like and needle-like NPs showed higher cell death than spherical and rod-like NPs [17]. This might be due to the fact that needle-like NPs have the capacity of damaging cells upon direct contact to the cell surface. An interesting study with graphene oxide nanosheets showed that the toxicity of these NPs was determined by their shape allowing them to physically damage the cell membrane. However, the toxicity of these NPs was reduced with increasing concentration of the fetal calf serum in the cell culture media. This phenomenon was explained on the basis

that graphene oxide NPs had the capacity to adsorb the protein molecules, which covered the nanoparticle surface which changed the shape of the nanoparticle and partly prevented cell damage.

## 2.2 Surface charge

The surface charge of NPs plays an important role in determining the nanotoxicity as it largely determines the interactions of the NP with biological systems. Positively charged NPs have been reported to have high toxicity due to their easy penetration into cells rather than the negatively charged nanoparticles [18]. This is due to the electrostatic attraction between the negatively charged cell membrane and positively charged NP. A comparative study of the toxic effects of negatively and positively charged polystyrene NPs on HeLa and H1H/3T3 cells has shown that the positively charged NPs were relatively more toxic. This is due to the ability of positively charged cells to easily penetrate through the cell membrane; also, they strongly bind to the negatively charged DNA, causing its damage, and prolong the G0/G1 phase of the cells. Negatively charged NPs have not been reported to have any effect on cell cycle. Similar observations have been reported with gold NPs where positively charged NPs were highly adsorbed and showed toxic effects rather than the negatively charged gold nanoparticle. Positively charged NPs have increased capacity of opsonization, which involves the process of adsorption of proteins facilitating phagocytosis, including antibodies and complement components from blood and biological fluids [19]. The adsorbed protein to the surface of nanoparticle which is normally referred to as protein crown may affect the surface properties of the NP. The protein crown contains serum proteins such as albumin, fibrinogens, and immunoglobulin G and several other functional molecules. *In vitro* experiments with quantum dots coated with a hydrophilic polymer enhance the fibril formation of human  $\beta_2$  microglobulin, which is arranged into multilayered structures on the surface of nanoparticle resulting in local increase in the protein concentration on the nanoparticle surface, precipitation, and formation of oligomers [20]. The charge of the nanoparticle can be modified from negative to positive via various modifications of the surface. So, Xu et al. had developed a method of changing the charge in polymer NP with the help of a pH-sensitive polymer that helps the negatively charged particles in a neutral medium acquire a positive charge in an acidic medium of pH 5–6 [21]. The cytotoxic effect estimated from surface-modified cerium oxide NP in H9C2, HEK293, A549, and MCF-7 cells showed that different polymers enable the nanoparticle charge modification, thereby showing different biological and toxic effects. Specifically, positive and neutral charged NPs are absorbed by all cell types at the same rate, whereas negatively charged NPs have the tendency to accumulate inside the biological tissues. So, modifying the charge of NPs allows to control their localization and toxicity, which can help in improving effective systems for targeted chemotherapeutic drug delivery to the tumor site.

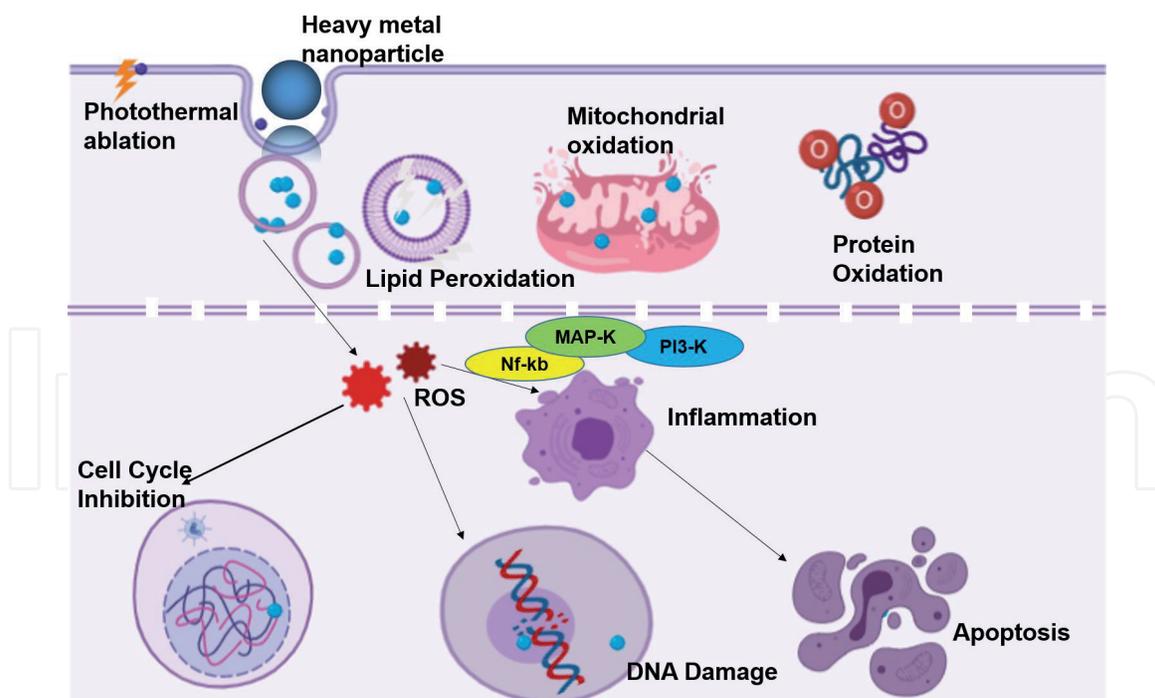
## 3. Nanoparticle shell

Improving the optical, magnetic, and electrical properties of nanomaterials application of a shell onto the surface of NP is quite important as it also improves the biocompatibility and solubility of NPs in water and other biological fluids by decreasing their capacity to aggregate and increasing their stability. Therefore, the shell reduces the toxic effect of NPs and provides them the capacity to selectively

interact with different types of cells and biological molecules [22]. In addition, the shell influences the pharmacokinetics of NP, which considerably changes the pattern of nanoparticle distribution and accumulation inside the body. Most of the nanoparticle toxicity has been reported due to the formation of free radicals inside the cells [23]. However, the shell has the capability to: reduce or eliminate these negative side effects as well as stabilize the NP, increase the resistance of NPs toward environmental factors, and enable them to acquire the capacity to selectively interact with the biological molecules. In regard to this point, Cho et al. demonstrated that polymer NPs could be modified with lectins and were able to selectively bind to the tumor cells presenting sialic acid on their surface, which made the nanoparticle suitable for specifically labeling cancer cells [24]. The surface of the NP can be modified using both organic and inorganic compounds such as polyethylene glycol, polyglycolic acid, lipids, proteins, low-molecular weight compounds and silicon. These modifiers make complex nanoparticle surface and change the nanoparticle properties for their specific transport and accumulation. The toxicity of quantum dots is significantly reduced using shells as the core of quantum dots is mostly hydrophobic and mainly consists of toxic heavy metals such as cadmium, tellurium, and mercury [25]. The shell enhances the stability of the core of quantum dots, thereby preventing its desalinization and oxidative or photolytic degradation. This ultimately prevents the leakage of heavy metal ions from the quantum core, thereby preventing nanotoxicity [26].

#### **4. Mechanism of nanotoxicity**

Nanotechnology has been an emerging field to determine the set standards or to formulate a set of designed rules for designing safe nanomaterials. The ability of nanomaterials to accumulate in different organs has resulted in some severe side effects and has hindered their use in the field of nanomedicine. So, understanding the mechanism that underlies the toxicity of nanomaterials may provide clues for overcoming the toxic effects of NPs. A major mechanism of nanotoxicity is by the generation of reactive oxygen species (ROS), which results in the subsequent formation of oxidative stress in tissues [27]. The induction of oxidative stress simultaneously activates the pro-inflammatory mediators via the principle cascades such as the nuclear factor- $\kappa$ B (NF- $\kappa$ B), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) pathways [28]. The most widely used nanomaterials are mostly the carbon nanotubes and metallic nanomaterials. Radomski et al. reported that engineered carbon NPs and nanotubes induced the aggregation of platelets in vitro, and enhanced vascular thrombosis in rat carotid artery [29]. Similarly, the single-walled carbon nanotubes showed enhanced cell apoptosis and decreased cell adhesion by upregulating genes involved in cell death or downregulating genes involved in cell proliferation and survival in cellular models of human kidney and bronchi. With the application of skin lotion and creams that majorly contain nano-TiO<sub>2</sub> and nano-ZnO, the skin is in continuous exposure to the toxic nanometals that can accumulate in the brain and can cause auxiliary toxicity resulting in the disruption of normal metabolism of neurotransmitters and ultimately leading to the cause of brain damage. While comparing the toxicity of three nanometal oxides, nano-CuO, nano-CdO, and nano-TiO<sub>2</sub>, nano-CuO was determined to be the most potent in regard to cytotoxicity and DNA damage, leading to 8-hydroxy-20-deoxyguanosine (8-OHdG) formation, while nano-TiO<sub>2</sub> was the least potent, without inducing a significant level of 8-OHdG [9] (**Figure 1**).



**Figure 1.** Mechanism of nanotoxicity. The major mechanism of nanotoxicity is by the generation of reactive oxygen species (ROS), which results in the subsequent formation of oxidative stress in tissues. The induction of oxidative stress simultaneously activates the pro-inflammatory mediators via the principle cascades such as the nuclear factor- $\kappa$ B (NF- $\kappa$ B), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) pathways. The other major effects are protein oxidation and DNA damage, which leads to apoptosis or cell cycle inhibition.

#### 4.1 Nanotoxicity via ROS production

The ROS generation and the subsequent production of oxidative stress are major causes of nanotoxicity, which involves DNA damage, unregulated cell signaling, changes in cell motility, cytotoxicity, apoptosis, and cancer initiation and progression. The amount and effect of ROS generation are completely dependent on the chemical nature of the nanomaterials [30]. Engineered nanomaterials have relatively small size, high specific volume-to-area ratio, and high surface reactivity, which results in higher production of ROS, simultaneously resulting in cytotoxicity and genotoxicity [31]. A variety of nanomaterials has been reported to induce nanotoxicity, that is, mediated by ROS in many biological systems such as human erythrocytes and fibroblasts. Quantum dots have been reported to have toxic effects produced by ROS-mediated oxidative stress and cell death. Akhtar et al. reported that silica NPs induced cellular stress and cytotoxicity in a dose-dependent manner, which is mediated by the induction of ROS and lipid peroxidation in cell membranes [32]. Nano-CuO induced cytotoxicity in mouse embryonic fibroblasts (BALB 3T3) by releasing lactate dehydrogenase, causing oxidative stress in a dose-dependent manner mediated by the induction of ROS and lipid peroxidation. Nano-ZnO has been reported to induce cytotoxicity that is mostly mediated by the induction of ROS, causing oxidative injury simultaneously releasing inflammatory mediators resulting in cell death in phagocytic RAW 264.7 cells, and transformation in human bronchial epithelial BEAS-2B cells [17]. Nano-Ag has been reported to induce apoptosis in NIH3T3 cells, which is mainly mediated via ROS and C-Jun terminal kinase-dependent mechanism involving the mitochondrial pathway. Also, nano-Ag-induced mutation and oxidative stress in mouse lymphoma cells. Shvedova et al. reported that keratinocytes incubated with high doses of single-walled CNTs resulted in ROS production, thereby leading to cellular and mitochondrial

dysfunction. Comparison of cytotoxicity of the four nanometal oxides nano-ZnO, nano-TiO<sub>2</sub>, nano-Co<sub>3</sub>O<sub>4</sub>, and nano-CuO in catfish hepatocytes and human HepG2 cells induced toxicity in the order of TiO<sub>2</sub> < Co<sub>3</sub>O<sub>4</sub> < ZnO < CuO and the major cause was the ROS generation leading to cell and mitochondrial damage [15, 33].

#### 4.2 DNA damage

DNA is one of the major targets of ROS. Toxicity of NPs is often specified for ROS production that ultimately damages the genetic material, thereby causing cell death. NPs are responsible for a wide variety of DNA damage such as chromosomal fragmentation, DNA strand breakages, and the induction of mutation in genes [34]. Gold NPs 20 nm in size at concentration of 1 nM have been reported to exhibit DNA damage in the form of 8-hydroxydeoxyguanosine (8OHdG), adduct formation in the embryonic lung fibroblasts, having a very low expression for DNA repair and cell cycle check point genes [35]. Several reports have also confirmed that metal oxide NPs induce DNA fragmentation and formation of oxidation-induced DNA adducts. The main functional molecule that comes into play in response to DNA damage is p53. Metal oxide NPs including TiO<sub>2</sub>, ZnO, Fe<sub>3</sub>O<sub>4</sub>, Al<sub>2</sub>O<sub>3</sub>, and CrO<sub>3</sub> of particle sizes ranging from 30 to 45 nm have been reported to induce apoptosis [36]. Cadmium telluride quantum dots were found to significantly increase p53 levels and upregulate the p53-downstream effectors Bax, Puma, and Noxa in human breast carcinoma cells [37].

#### 4.3 Inflammation

Oxidative stress induction is relatively linked to inflammation through the release of pro-inflammatory mediators through the cascade such as the NF- $\kappa$ B (nuclear factor- $\kappa$ B), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) pathways [38]. Inflammation is majorly a type of defense mechanism of the body that involves several immune regulatory molecules followed by the infiltration of phagocytic cells. The induction of inflammation in several cell types such as the alveolar and bronchial epithelial cells, epidermal keratinocytes, and cultured monocyte-macrophage cells has been reported with single and multi-walled carbon nanotubes and fullerene derivatives. A recent study has been able to provide a mechanistic explanation for immune and inflammatory responses initiated upon exposure to carbon NPs [39]. This observation reported that the immune system receptors like toll-like receptors recognize carbon nanotubes and C60 fullerenes as pathogens and thereby trigger the inflammatory responses by secreting inflammatory protein mediators such as interleukins and chemokines. Similarly, exposure of liposomes and other lipid-based NPs trigger the activation of the complementary cascade leading to hypersensitivity reactions and anaphylaxis [40]. However, the exact mechanism through which these complement proteins mediate nanotoxicity has not been elucidated. In the absence of a stimulus, NF- $\kappa$ B is degraded in the cytoplasm by the Inhibitor of  $\kappa$ B (I $\kappa$ B) family of inhibitors. The reactive oxygen species play a major role in the induction of the NF- $\kappa$ B, resulting in the inflammatory responses. Both *in vitro* and *in vivo* studies showed that nanoparticle-induced lung injury and pulmonary fibrosis lead to the ROS-mediated activation of NF- $\kappa$ B and production of pro-inflammatory mediators such as TNF- $\alpha$ , IL-8, IL-2, and IL-6 [41]. Metal oxide NPs such as zinc, cadmium, silica, and iron have also been reported to show toxic effects via the induction of inflammatory-related cytokine release induced by NF- $\kappa$ B. The single-walled and multiple-walled carbon nanotubes were also shown to promote inflammatory responses in mice by generating the TNF- $\alpha$  and monocyte chemoattractant protein-1 (MCP-1) [42].

The MAP-kinase pathway regulates critical cellular processes such as cell proliferation, differentiation, mitosis, cell survival, and apoptosis. Treatment of human bronchial epithelial cell lines with titanium dioxide NPs showed interleukin (IL)-8 production via p38 MAPK and/or ERK pathway and mediated toxicity in the cell lines [43]. The model organism *C. elegans* used for in vivo toxicity assay studies of silver NPs with a size range of 20–30 nm showed that the toxicity mediated was due to the production of ROS, which consequently increased the expression of PMK-1 p38 MAPK and hypoxia-inducible factor (HIF-1) [44]. The toxicity of silica NPs, which hinders their application as drug delivery systems, has been attributed to the activation of JNK, p53, and NF- $\kappa$ B pathways and an elevated expression of pro-inflammatory factors IL-6, IL-8, and MCP-1 [45]. Also, single-walled nanocarbon of size range 0.8–2 nm was reported to have potential adverse cytotoxic effects in mesothelial cells via the activation of signaling molecules, including PARP, AP-1, NF- $\kappa$ B, p38, and Akt, in a dose-dependent manner [46].

## 5. Organ-/tissue-specific nanotoxicity

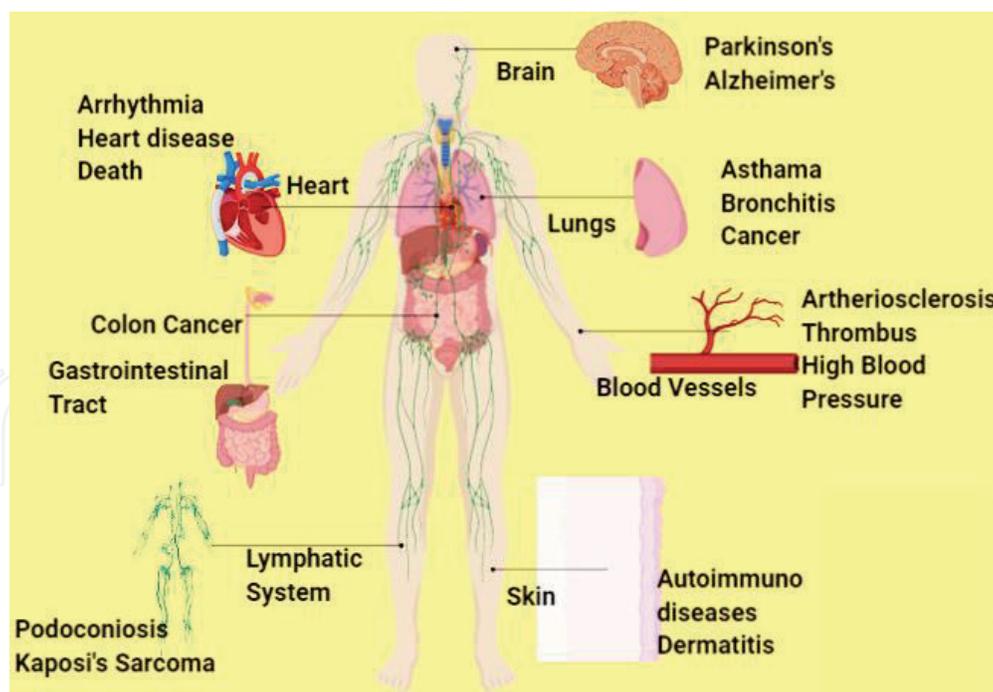
Nanoparticles can easily penetrate the tissue system and damage body organs because of their smaller size and high specificity to the tissue system. It has been observed that nanoparticles can move fast in the blood stream and easily cross the blood-brain barrier, this may induce toxicity, which can be harmful for the human organ system (e.g., pulmonary system, reticuloendothelial systems, cardiovascular systems, central nervous system, skin, and embryonic cells) (**Figure 2**).

### 5.1 Toxicity in pulmonary system

The small-sized NPs have the ability to penetrate easily through the lungs and can cause lung injuries and generate ROS [47]. The pulmonary toxicity studies in rats with ultrafine and fine NPs such as carbon black, nickel, and TiO<sub>2</sub> particles have shown enhanced pulmonary inflammation by the ultrafine NPs [48]. It is being reported that the toxic effects of NPs on lungs show characteristics such as development of exaggerated lung responses, high rate of pulmonary inflammation, failed clearance, cellular proliferation, fibroproliferative effects, and inflammatory-derived mutagenesis, ultimately leading to chronic effects like tumor development in lungs. Factors that mainly influence nanotoxicity in lungs are the particulate characteristics of NPs, such as particle size, number, surface area, surface dose, surface modifications, degree of aggregation, and method of particle synthesis [49, 50].

### 5.2 Toxicity in reticuloendothelial systems

The reticuloendothelial system in the liver is the main source of biological system where all the NPs get absorbed from the gastrointestinal tract into the cardiovascular systems, as all blood exiting from the gastrointestinal tract transport from the hepatic portal vein that directly diffuses to the liver. Carbon black and polystyrene NPs being less toxic NPs stimulate macrophages by the generation of ROS and activation of calcium signaling to release pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  [51]. Pro-inflammatory cytokines are also associated with pathology of liver disease where the generation of ROS molecule inhibits the hepatocyte function and bile formation.



**Figure 2.**

*Tissue- and organ-specific nanotoxicity. The toxic accumulation of nanoparticles can affect any of the tissue types in the body. The small-sized nanoparticles have the ability to penetrate easily through the lungs and can cause lung injuries and have an ability to generate ROS that lead to toxic effects in lungs. The reticuloendothelial system in the liver is the main source of biological system where all the nanoparticles get absorbed from the gastrointestinal tract into the cardiovascular systems, as all blood exiting from the gastrointestinal tract transport from the hepatic portal vein that directly diffuses to the liver. Increased exposure to nanoparticles majorly happens through the process of inhalation, which results in altered heart rate. The inhalation of nanoparticles acquires the ability to reach the brain system mainly through the route of olfactory epithelium by the mechanism of transsynaptic transport or through their uptake via the blood-brain barrier. Nanoparticles acquire the ability to penetrate inside the skin and cause toxic effects such as dermatitis and auto immune disorders.*

### 5.3 Toxicity in cardiovascular systems

The positively charged NPs such as gold and polystyrene have been reported to cause hemolysis and clotting of blood, while the negatively charged NPs are reported to be nontoxic in nature. Increased exposure to diesel-exposed particles (DEP) in hypertensive rats through the process of inhalation resulted in altered heart rate in rats as interpreted through the pacemaker that determines the activity of the heart [52]. Exposure to single-walled NPs also showed altered cardiovascular effects [53]. The injection of ultrafine carbon black NPs into the blood of normal rats caused platelet accumulation in the hepatic microvasculature of the rats and also caused prothrombotic changes on the endothelial surface of the hepatic microvessels [54].

### 5.4 Toxicity in central nervous system

NPs on inhalation of acquire the ability to reach the brain system mainly through the route of olfactory epithelium by the mechanism of transsynaptic transport or through their uptake via the blood-brain barrier [55]. Enhanced permeability of NPs through the blood-brain barrier has been reported to have increased the number of pathologies including hypertension and allergic encephalomyelitis. The surface charge of the nanoparticle has also been shown to have toxic effects on the brain leading to brain toxicity altering the blood-brain integrity [56]. NPs have

also been associated with the production of reactive oxidative species and oxidative stress, which are also associated with brain diseases such as Parkinson's and Alzheimer's [57].

### **5.5 Toxicity in skin**

The widely used cosmetic products for application in the skin contains mostly 3% NPs of size range approximately 50–500 nm [58]. These NPs behold the scattering properties that enhance the entering of UV photons from the optical source into the skin layer although the dermatological effects of NPs able to penetrate the skin are still under investigation. In vitro study with multi-walled carbon nanotubes reported that the carbon NPs have the ability to localize within and initiate an irritation response in human keratinocytes, which are the primary route of occupational exposure [59].

### **5.6 Toxicity in embryonic cells**

Fluorescence correlation spectroscopy played a major role in identifying the toxicity of nanomaterials in embryonic cells. The observation through this microscopy revealed that the accumulation of NPs especially NPs with carboxylate group on their surface takes place more in smaller blood vessels rather than larger blood vessels [60]. These findings are majorly important for finding the aggregation state that can likely influence nanoparticle accumulation in angiogenic tissue. The fluorescence correlation spectroscopy helps to measure the loss of NPs from the blood streams of live embryo [61]. This kinetic loss of NPs can be correlated to surface characteristics of NPs such as surface charge and size. Also, it has been reported that in a mature organism, the renal clearance of nanoparticles occurs only for NPs with size less than 5 nm in lateral dimension. NPs are being reported to act as effective targeted delivery agents in angiogenic tissues of adults as well as embryonic tissues. Larson et al. reported that quantum dots could be used to image vasculature (using two-photon excitation) in the dermis of mice [61]. Semiconductor quantum dots are NPs with intense, stable fluorescence and are a very good source to detect ten to hundreds of cancer biomarkers in blood assays, on cancer tissue biopsies, or as contrast agents for medical imaging. Smith and coworkers have developed some functionalized quantum dots for tumor targeting in mice; however, no study has been made to measure directly the concentration of the quantum dots in the blood or whether or not they were aggregated; hence, the toxicity level of these quantum dots has not been checked [62].

## **6. Conclusion**

The use of nanomaterials in biomedical sciences and health sciences has increased in recent years due to their size and surface characteristics appropriate for targeted and site-specific delivery of drugs to the affected areas. In cancer research, nanomedicine holds the massive potential for cancer therapy. The surface and tiny size and shape of NPs have been used as unique properties of NPs to play a key role for an efficient treatment and specific targeting. Nano-based therapeutic and diagnostic strategies pose as highly promising tools for easy and cost-effective diagnosis of cancer. But, the public interest's in accurate, relevant, and predictive nanotoxicological assessments also has been growing. Due to the complication of ROS formation and disruption to the normal biological events, the use of nanomaterials has created complicated situation. The usage of nanomaterials has been highly reported

to cause toxic events such as DNA damage, oxidative stress damage, and inflammatory responses. Major organs such as heart, brain, skin, etc. have been reported to have toxic responses related to nanoparticle applications. So, the development of a set of rules is needed for developing safe engineered nanomaterials, which can be determined by *in vitro* toxicity studies.

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