An Insight about Toxicological effects of Titanium Dioxide Nanoparticles

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Conflict of interest: None declared.
Funding: No funding sources

Abstract

NMs, which is the umbrella term for other nano sized morphologies such as NPs, nanofibers, and nanotubes, are defined as very small materials having at least one dimension below 100 nm in size. They can be synthesized by two primary strategies: the top-down fabrication, which crushes bulk material into smaller particles, and the bottom-up method, that uses chemical reactions to originate NPs from atoms or molecules. Nanoparticles are commercially produced from metal and nonmetal, polymeric materials and bioceramics have widespread application in all aspects of modern life. These particles have unique features such as small size, high surface area, special physicochemical and electrical properties and high reactivity. Nanosized Titanium dioxide particles (TDN) or (TiO2) are one of the most commenly manufactured nanoparticles. Titanium dioxide (TiO2) is primarily used as a whitening agent due to its brightness and resistance to discoloration in consumer products and food. Titanium dioxide is approved as a white-colored food additive in Europe (E171). the toxic effects of test substances are usually measured in terms of acute, sub-acute, sub-chronic or chronic exposure conditions. Studies with a maximum of 2 weeks (14 days) study duration are normally referred to as acute toxicity studies. Sub-acute toxicity studies last for a maximum of 4 weeks (28 days), sub-chronic toxicity studies for a maximum of 13 weeks (90 days) and chronic toxicity studies last longer than 4 months. Despite several benefits of nanotechnology, unique properties of NPs can cause harmful effects on biological systems.
Keywords: Titanium Dioxide Nanoparticles

Tob Regul Sci.™ 2023 ;9(1): 7968 - 7984

DOI: doi.org/10.18001/TRS.9.1.563

Introduction

NMs, which is the umbrella term for other nano-sized morphologies such as NPs, nanofibers, and nanotubes, are defined as very small materials having at least one dimension below 100 nm in size. They can be synthesized by two primary strategies: the top-down fabrication, which crushes bulk material into smaller particles, and the bottom-up method, that uses chemical reactions to originate NPs from atoms or molecules [1].

Nanoparticle (NP) is an ultrafine unit with size measured in nanometres (nm; 1 nm = 10−9 metre). Nanoparticles may be present in the natural world and are also produced as a result of human activities [2].

Nanomaterials have always been released into the air by various natural phenomena, e.g. volcano ashes or wild fires, and this is how they unintentionally come into contact with humans, animals, and the environment. Besides, anthropogenic NMs set free by diesel engine exhaust, combustions, welding or cigarette fume are part of the possible exposure to nano-sized particles [3].

Nanoparticles are commercially produced from metal and nonmetal, polymeric materials, and bioceramics, having widespread application in all aspects of modern life. These particles have unique features such as small size, high surface area, special physicochemical and electrical properties, and high reactivity [4].

Nanotechnology is a growing research field, with many potential applications in pharmaceuticals, cosmetics, medicine, engineering, biology, biotechnology, agriculture, and industry [5].

Owing to their minute size, NPs can get an entrée to many biological structures, interacting with molecules such as lipids, proteins, and nucleic acids that may interfere with their normal function, damage the subcellular organelles and cause cell death [6].

Metal oxide nanoparticles such as (titanium dioxide, zinc oxide, copper oxide, molybdenum dioxide, and tungsten oxide) are belonged to a category of nanomaterials (NMs) which are synthesized widely for manufacturing and domestic requests [7].

Titanium (Ti) is the world's fourth most abundant metal and is the ninth most common element in the earth's crust [8]. But there is no proof of Titanium being a vital element for human or animals [9].

Nanosized Titanium dioxide particles (TDN) or (TiO2) are one of the most commonly manufactured nanoparticles [10] and the production volume of nano-TiO2, which is increasing annually, is expected to reach nearly 2.5 million metric tons per year in 2025 [11].

TiO2 is odorless, low-solubility crystal which has thermal stability and combustibility, excellent physical properties, corrosion resistance, biocompatibility and excellent electrical and optical performance [12].

The molecular weight of Titanium dioxide is 79.9 g/mol , boiling point of 2972°C, melting point of 1843°C, and relative density of 4.26 g/cm3 at 25°C [13].

TiO2 exists in three crystalline forms, anatase, rutile, and brookite [14, 15]. The anatase and rutile forms have natural and industrial importance, while the brookite is rarely used. Generally, anatase is more toxic than rutile and, unfortunately, being used abundantly [15, 16].

It was thought that TiO2-NPs were non-toxic minerals [17]. However, many studies recommended that TiO2-NPs could be more toxic than their original materials [18].

Uses of titanium dioxide

Titanium dioxide (TiO2) is primarily used as a whitening agent due to its brightness and resistance to discoloration in consumer products and food. Titanium dioxide is approved as a white-colored food additive in Europe (E171) [19].

Many popular consumer products such as candies, gum, and baked goods contain 0.01 to 1 mg Ti per serving. The products with the highest titanium contents are sweets or candies, for example, powdered donuts can contain up to 100 mg Ti per serving [20].

Also, it is used in sunblock creams to protect the skin from UV light and are under study as a treatment for acne vulgaris, recurrent condyloma accuminata, atopic dermatitis, hyperpigmented skin lesions, and other non-dermatologic diseases [21].

Also, TiO2 nanoparticles are potential photosensitizers, and in nanotherapeutics, they can be used for advanced imaging and photodynamic therapy [22].

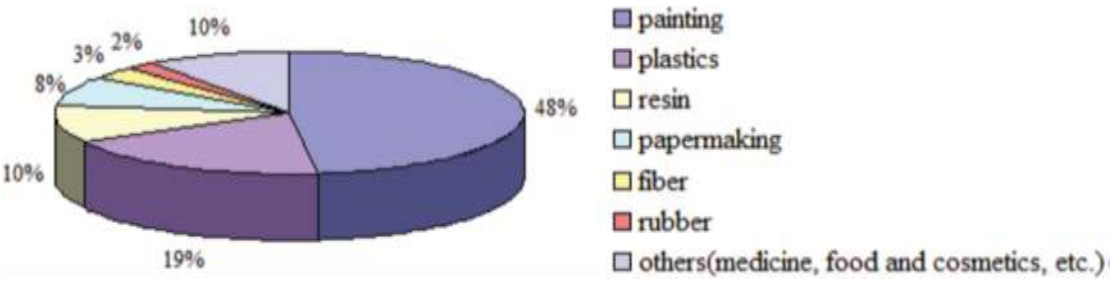
Intensive uses of titanium dioxide (TiO2) nanoparticles in many consumer products, e.g., toothpaste, cosmetics, paints, and in cancer therapy as a result of their high optical performance, high redox activity, and large surface area [23, 24, 25]. Definitely, nano-TiO2 was reported to kill viruses, e.g., Poliovirus 1, hepatitis B virus, Herpes simplex virus, and MS2 bacteriophage [26, 27].

In medicine, nano-TiO2 are under investigation as useful tools in medical instruments and supplies as advanced imaging techniques, operating rooms, catheters and nano therapeutics. For example, nano-TiO2 are being evaluated as potential photosensitizers for use in photodynamic therapy [22, 28].

Titanium dioxide nanoparticles absorb ultraviolet radiation from sunlight so they are used in sunscreens due to their effective block to long-wave ultraviolet light. Nano-TiO2 are also used under investigation as treatment for acne vulgaris, hyperpigmented skin lesions, recurrent condyloma accuminata, and atopic dermatitis [29, 30, 21].

Titanium is widely used for a wide range of implanted medical devices, such as dental implants, joint replacements especially for the hip and knee, cardiovascular stents, and spinal fixation devices [31].

Titanium dioxide nanoparticles also have antibacterial properties under UV light irradiation, so it is highly efficient in killing antibiotic-resistant bacteria by destroying bacterial spores [32, 28, 33].



The application areas of TiO2 NPs. [34]

Routes of exposure

The exposure to nanoparticles can be either by accident due to occupational exposure, or intentionally through different routes such as the nose by inhalation, mouth by intake, skin contact, or intravenous injection [35].

The major routes of TiO2 NPs that have toxicological relevance in the workplace are inhalation and dermal exposure [36].

Weir et al. [20] postulated that titanium dioxide nanoparticles are found in toothpaste, food colorants, candies, sweets, and chewing gums, so oral exposure may occur during usage of these products.

In nanomedicine, subcutaneous injection of TiO2 nano particulate carriers is a unique way to deliver TiO2 NPs into the human body [37]. Or injected directly intravenously into the blood [38].

TiO2 nanoparticles are found in a number of consumer products like sunscreens and cosmetics, so skin exposure to TiO2 nanoparticles may occur [39].

Pharmacokinetics:

It means the kinetics or rate of nano-TiO2 to migrate from the initial site of exposure to the final site in human organs and accordingly the concentration of nano-TiO2 in the human body depends on the rate of their absorption, distribution, metabolism, and excretion in that body. [40].

Absorption

Absorption of Titanium dioxide nanoparticles in the GIT was supposed to take place via the Peyer's patches, as they have been absorbed through the lymphoid tissues surrounding the GIT. They are present in high concentration in the lymphoid tissues [41].

Under normal conditions, Titanium dioxide nanoparticles cannot penetrate the intact skin but they can penetrate the slightly injured skin as that caused by physical force or sunburn [39].

Distribution

Whatever the route of exposure to TiO2 nanoparticles is, once they entered into the circulatory system, the nanoparticles are transported into various parts of the body [40].

After the initial absorption of nano-TiO2, the particles reach the systemic circulation and are distributed to all organs and tissues in the body. In the systemic circulation, nano-TiO2 potentially interact with plasma proteins, platelets, coagulation factors, and red or white blood cells. This binding to the components of the plasma may have an effect on the distribution, metabolism, and excretion of the NPs [42, 43].

In general, nanomaterials rapidly distribute from blood to tissues [44, 45, 46, 47]. Particularly, highly perfused reticulo-endothelial system (RES)-containing tissues such as the liver and spleen are target tissues for nanomaterials. Distribution across protecting membranes has also been observed as nanoparticles have been detected in the brain, fetus, and testis [48].

As regard to the inhalation route, intra-nasally instilled nano-TiO2 could reach the central nervous system via the sensory olfactory nerves [49].

Geiser and Kreyling [50] reported that NPs including nano-TiO2 in the size range of 5-100 nm could be absorbed through the air-blood barrier reaching the systemic circulation.

Several studies have shown that most of the nano-TiO2 after intravenous or intraperitoneal administration were translocated to the liver [51, 52, 53].

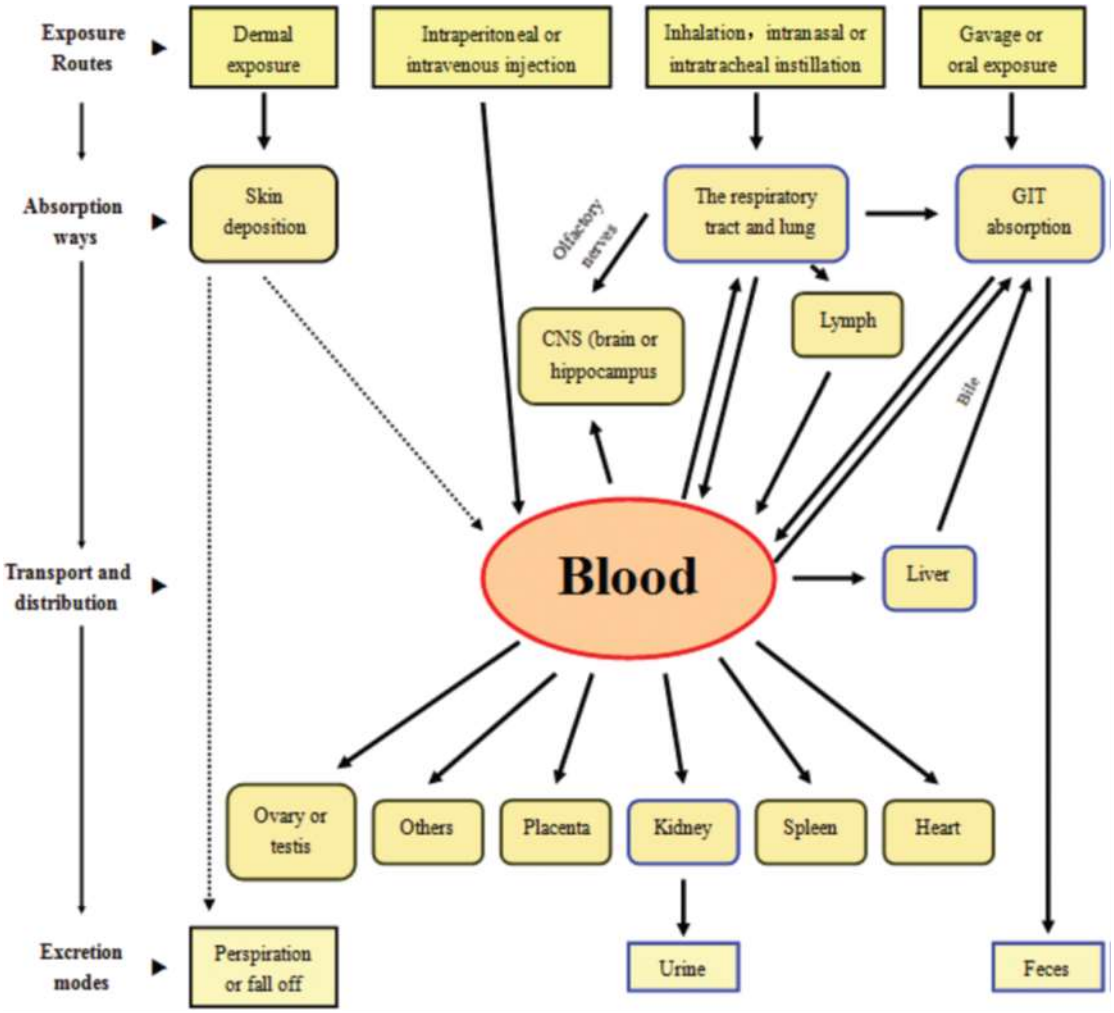
Execretion

Titanium dioxide nanoparticles in the systemic circulation have two potential pathways for clearance, i.e., kidneys/urine, and bile/feces. The International program on chemical safety for TiO2 shows that most ingested TiO2 is excreted with urine [40].

Inhaled TiO2NPs which are deposited in the airways of the respiratory tract and phagocytized by alveolar macrophages may be transported by mucociliary action to the larynx to be cleared via spitting of sputum or be swallowed entering the GIT [54, 17, 55].

Furthermore, elimination is generally quite slow, and for metal and metal oxide nanoparticles like titanium dioxide, metabolic degradation does not seem to occur. Elimination of these materials may rather be related to dissolution, which can be very slow. Limited elimination in

combination with a low fraction that becomes systemically available will eventually result in accumulation in tissues upon repeated exposure [56].



The toxicokinetics of TiO2 NPs in vivo (the arrows in dotted lines represent uncertainties). [40]

Mechanism of Toxicity of nanoparticles

Shi et al. [40] postulated that the toxic effects of test substances are usually measured in terms of acute, sub-acute, sub-chronic, or chronic exposure conditions. Studies with a maximum of 2 weeks (14 days) study duration are normally referred to as acute toxicity studies. Sub-acute toxicity studies last for a maximum of 4 weeks (28 days), sub-chronic toxicity studies for a maximum of 13 weeks (90 days), and chronic toxicity studies last longer than 4 months. Despite several benefits of nanotechnology, unique properties of NPs can cause harmful effects on biological systems [57].

The lethal effects of nanoparticles can be accredited to their small size and huge surface area which results in increased rates of chemical reaction and infiltration into the cells interfering with numerous subcellular physiological mechanisms [58].

Nanoparticles stimulate excessive production of reactive oxygen species (ROS) leading to a state of oxidative stress that is responsible for hazardous biological effects produced by NPs [59, 60, 61]. Cells constantly generate ROS under normal conditions as a consequence of aerobic metabolism. When cells are exposed to any insult (chemical or physical), the cell protects itself by the production of an excess amount of ROS leading to this state of oxidative stress [62].

Mates et al. [63] stated that the cell also protects itself by the production of a number of strong antioxidant enzymes against ROS. These antioxidants such as nitric oxide synthase (NOS), catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), thioredoxin reductase, vitamin C, vitamin E, vitamin A, melatonin, methionine, homocysteine, β-carotene, adenosine, cysteine, taurine, and selenium.

Reactive oxygen species (ROS) are constantly generated in cells under normal conditions as a consequence of aerobic metabolism. When cells are exposed to any insult (chemical/physical), it results in the production of an excess amount of ROS leading to a state of oxidative stress [62].

The term oxidative stress is generally applied when oxidants are more than antioxidants [64]. The imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as oxidative stress [65]. The main destructive aspects of oxidative stress are the production of ROS, which include free radicals and peroxides [66].

Additionally, the generation and accumulation of reactive oxygen species (ROS), which give rise to an inflammatory response [67], depletion of cellular antioxidants such as glutathione [68], and mitochondrial damage with prevention of ATP synthesis [69], and causing several adverse effects represented in DNA injury [70], induction of apoptosis, and genotoxicity [71].

The mechanisms contributing to the generation of ROS in cells exposed to nanoparticles are that some types of nanoparticles act as photosensitizers, facilitating the generation of 1O2 and O2 from ground state molecular oxygen under the influence of light [40]. In tissues that are not exposed to daylight, several other types of reactions may account for ROS generation, such as the release of organic matter from combustion-derived nanoparticles, including quinones prone to redox cycling. Also, transition metal ions may be released from nanomaterial preparations, partly derived from particle impurities, catalyzing Fenton-type reactions.

In addition to such chemical reactivities, the interaction of particles with subcellular structures involved in the catalysis of redox reactions may modulate the generation of ROS. Supposed targets include the plasma membrane with its enzyme complexes, including NADPH oxidases, whose activity and regulation may be affected by interaction with nanoparticles. Moreover, nanoparticle interactions with mitochondria or the endoplasmic reticulum are anticipated to contribute to ROS formation. For example, the physical interaction between nanoparticles and cell structures might directly affect electron flow and leakage from the inner mitochondrial

membrane. In fact, the enhanced generation of ROS, and damage to mitochondria, as well as the presence of ultrafine particulate matter inside mitochondria of exposed cells, was demonstrated [72, 73]. The interaction of nanomaterials with mitochondria and the endoplasmic reticulum, two major cellular Ca2+ stores, may cause the dysregulation of calcium ion levels. This, in turn, might activate calcium-dependent enzymes involved in the generation of ROS/RNS, such as NO synthases [74, 75, 76].

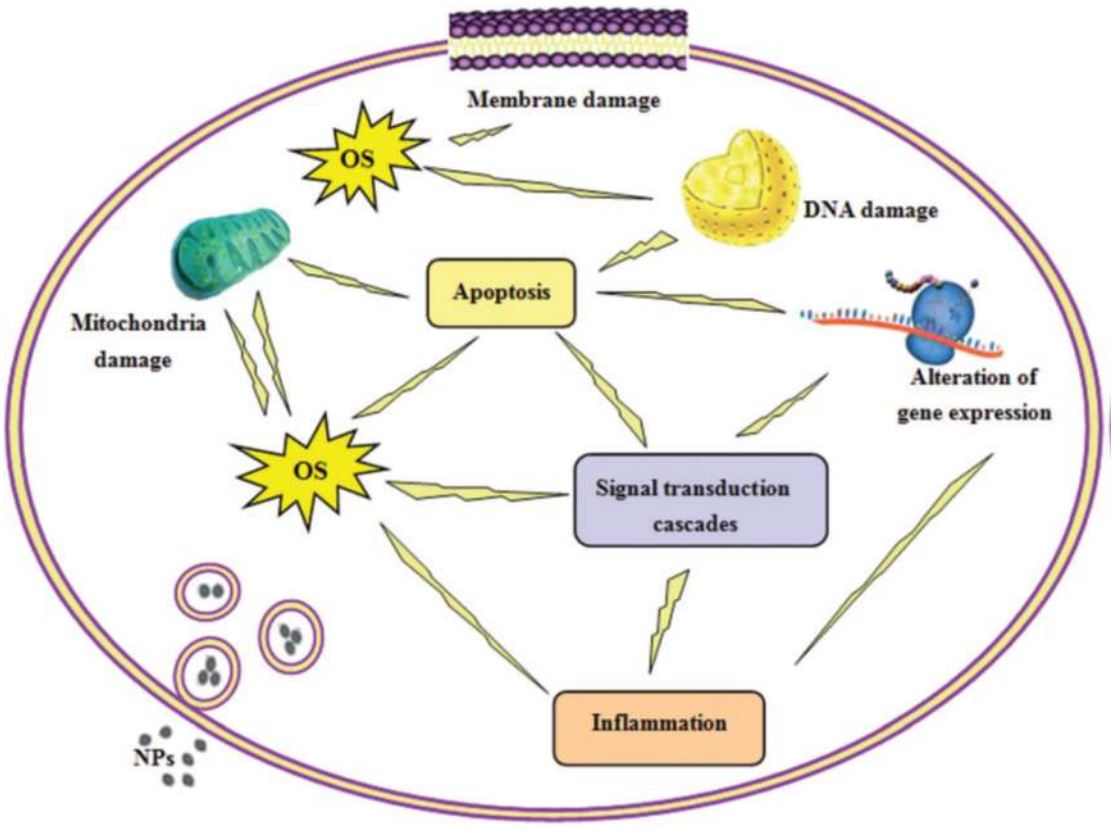
Apoptosis is a kind of programmed cell death that plays an important part in the early development and growth of tissue and metamorphosis. Apoptosis is mostly synonymous with the suicide of the cell, and it also refers to the controlled regulation of the cycle and removal of unnecessary cells at appropriate times without causing surrounding tissue damage [77].

Damage induced by any toxicant, including free radicals, can result in cell death by apoptosis or necrosis. Apoptosis is a structured form of cell death characterized by cell rounding, a decrease in cell volume, nuclear condensation, and plasma membrane leaking. The initiation and execution of apoptosis are mediated by a family of cysteine-aspartate proteases (caspases) [78].

Two principal pathways for apoptosis initiation exist. The ‘extrinsic’ pathway is mediated by death receptors, a subgroup of the TNF receptor superfamily. The second pathway, which is also referred to as the ‘intrinsic’, or possibly more accurate, ‘Bcl-2-controlled’ pathway, is consequently controlled by members of the Bcl-2 family [79, 80, 81].

Apoptosis is controlled by genes, revealing its clinical relevance. Apoptosis disrupted by mutations in these genes results in malignancies, autoimmune diseases, the spread of viral infection, and neurodegenerative disorders, while excessive apoptosis occurs in AIDS and ischemic diseases [82].

Necrosis, on the contrary of apoptosis, is considered a passive form of cell death. This is characterized by swelling of organelles (edema), energy breakdown, random DNA degradation, relatively early breakdown of the plasma membrane and eventually total cell disintegration that leads to inflammation around the dead cells, attributable to the release of cellular contents and proinflammatory molecules [83, 84].



The potential mechanisms of TiO2 NP-induced toxicity.

Toxic effects of titanium dioxide nanoparticles:

Braydich-Stolle et al. [85] demonstrated that anatase TiO2 NPs resulted in lactate dehydrogenase leakage and widespread necrosis; rutile NPs resulted in higher levels of ROS and consequently apoptosis. TiO2 NPs have been reported to cause toxicity both in vitro and in vivo, attributed to the generation of reactive oxygen species (ROS), resulting in apoptosis, but cell dysfunction has also been associated with micronuclei formation [86, 87], mitochondrial abnormalities [70], and other forms of cell toxicity [71].

It has been demonstrated that oxidative stress is one of the most important toxicity mechanisms of TiO2 NPs in the lung [88, 89], liver [90, 91, 92], spleen [93], kidney [94], and reproductive system [95, 96, 97].

1- Respiratory System Toxicity

Warheit et al. [12], Li et al. [98], and Chen et al. [52] reported that acute exposure to Nano-TiO2 induced pulmonary toxicity in the form of inflammatory cellular infiltration, structural damage of lung tissue, and increased bronchoalveolar lavage inflammatory parameters. Several studies have reported that chronic lung inhalation resulted in several pathologies in pulmonary tissues such as enhanced proliferation of pulmonary cells, squamous metaplasia, increased incidences

of pneumonia, defects in function of macrophage, alveolar epithelial metaplasia, progressive fibro-proliferative lesions and accumulation of macrophages in interalveolar septa [40].

Immune response to inhaled toxic gases and particles might lead to pulmonary emphysema and chronic obstructive pulmonary disease (COPD), which is a widespread illness with an increasing prevalence and mortality rate [99].

2-Nervous System Toxicity

Generally, most molecules cannot cross the BBB, as the BBB is a tight barrier to protect the brain from the penetration of xenobiotics. However, NPs made of certain materials and with varying particle sizes can overcome this physical barrier and enter into the brain, or enter into the brain by the nerve endings of the olfactory bulb [100].

Neurotoxicity of Nano-TiO2 is attributed to their ability to pass to the brain, regardless of the route of demonstration. Their accumulation in the brain triggers alterations in the production and metabolism of neurotransmitters beside numerical and structural changes in the neuronal architecture. [41, 49, 101, 102].

Nano-TiO2 induces some neurons to be transformed into filamentous shapes and others into inflammatory cells. Oxidative stress and injury of the brain are inducers of cascade reactions as lipid peroxidation, decreases of the total anti-oxidation capacity and activities of antioxidative enzymes, reduction of glutamic acid, excessive release of Nitric oxide and a decrease in acetyl cholinesterase activity [103].

3- Cardiovascular System Toxicity

Liu et al. [53], Wang et al. [104], and Faddah et al. [105] reported that the accumulated Nano-TiO2 in the heart tissue induced DNA damage and oxidative stress in mice and rat cardiac cells. Also, several studies reported that intraperitoneally injected Nano-TiO2 caused severe alteration in myocardial function indicated by a significant increase in the activities of aspartate aminotransferase, creatine kinase alpha-hydroxybutyrate dehydrogenase, and lactate dehydrogenase.

Previous studies demonstrated that TiO2 NP exposure resulted in titanium accumulation in the heart, myocardium dysfunction, oxidative stress, cardiac inflammation, and atherosclerosis in mice [106, 107], increased plaque progression in the aorta in mice [108], and induced an inflammatory response in primary vascular endothelial cells [109].

4-Renal System Toxicity

Several studies reported severe alterations of functional parameters of the kidney, in the form of increased and decreased blood urea nitrogen, increased and decreased creatinine, reduced uric acid levels beside multiple histopathological alterations [41, 110, 53, 104, 55, 111].

An in vitro study showed that high concentrations of Nano-TiO2 caused renal proximal cell death [112]. Also, Nano-TiO2 were supposed to impair nephric functions and cause nephric inflammation through reactive oxygen species (ROS) accumulation, revealing its toxicity [113]. A previous study on Nano-TiO2 by Gui et al. [94] also demonstrated an induced nephric inflammation and nephric cell necrosis. Jeon et al. [114] in a recent report found that proteins were differentially expressed in the mouse kidney by exposure to Nano-TiO2.

Mohamed and Sayed [115] reported histopathological alterations in almost all components of the renal cortex; numerous mesangial cells with dark nuclei, numerous secondary lysosomes; bizarre-shaped mitochondria with a marked decrease in basal enfolding, and excessive deposition of collagen bundles in the interstitium.

5-Liver Toxicity

Chen et al. [52] reported that intraperitoneally injected mice with different doses of Nano-TiO2 resulted in significant alteration in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), necrosis and apoptosis of liver cells, hepatic inflammation, and fibrosis (high deposition of extracellular matrix proteins, as glycoproteins, collagen, and proteoglycans).

Ma et al. [116] intraperitoneally injected mice with Nano-TiO2 and reported an accumulation in the liver, swollen blood vessels, and a series of diseases as diffuse and focal ischemic basophilic changes. Also, swelling of mitochondria and nuclear vacuoles were shown indicating hepatic cell apoptosis. There was a significant increase of both mRNA and protein expression levels of several inflammatory cytokines and mediators in the liver.

6-GIT Toxicity

The impact of TiO2 nanoparticles (TiO2-NPs) on gastro-intestinal cells is poorly documented, and there is relatively lower information on the possible toxic effects of NPs on this tract [117, 118, 119].

Most studies reporting the impact of TiO2-NPs on gastrointestinal tract models were performed in vitro on Caco-2 cells, either non-differentiated [120] or fully differentiated. TiO2-NPs<40 nm were shown to translocate through differentiated Caco-2 epithelia, triggering morphological changes in microvilli and disorganization of the brush border [120].

7-Spleen toxicity

TiO2 NPs induce histopathological changes of the spleen, including congestion and lymph nodule proliferation, and splenocyte mitochondria swelling, splenocyte nucleus exhibiting the classical morphology characteristics of apoptosis or necrosis as reduction in nuclear size, chromatin condensation, and nucleolus cap appearance. The mitochondria swelling suggested that TiO2 NPs enter the cell and bind to the mitochondria [10].

No Conflict of interest.

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