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Potential Toxic Effects of Aluminum Nanoparticles: An overview

Basma Elkhadrawey, Badr El-Bialy*, Shimaa Abou-Zeid, Nermeen El-Borai, Hesham Elsabbagh

Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, University of Sadat City, 32897.

**corresponding author: badr_elsaid10@yahoo.com* *Received: 1/7/2021 Accepted: 21/7/2021*

ABSTRACT

Alumina (aluminum oxide) nanoparticles (AlNPs) have been categorized among the most important nano-metals, with wide applications in many fields as in food, agriculture, industry, engineering, pharmacy, medicine and others. The extensive use of AlNPs results in a massive release of them into the environment resulting in potential adverse impacts on animals and human health. Toxicokinetic studies demonstrated the rapid absorption and the systemic distribution of AlNPs to different organs. AlNPs mainly induced their toxic damage by induction of oxidative stress and mitochondrial dysfunction. Cellular accumulation of AlNPs resulted in interaction with cell components and binding with proteins and genetic materials (DNA and RNA) with producing other different chemical compounds resulting in cellular oxidative damage. Also, AlNPs induced cell death by disrupting the integrity of the mitochondrial membrane, depleting the mitochondrial thiols, activating apoptotic caspases (9 and 3) and generating more ROS. Previous in vivo and in vitro studies revealed hepato-, nephro-, myocardial, reproductive and neuro-toxic effects of AlNPs. Moreover, AlNPs exert inflammatory, apoptotic and geno-toxic effects. The aim here is to spot on previous studies demonstrating the toxic effects of AlNPs.

Keywords: *alumina nanoparticles, hepatotoxicity, nephrotoxicity, neurotoxicity, oxidative stress*

INTRODUCTION

Alumina (aluminum oxide) nanoparticles (AlNPs) have been categorized among the most important nano-metals because of their promising technological applications (Prabhakar *et al.*, 2012). They are listed among the most commonly manufactured nanoparticles (NPs), accounting for roughly 20% of the global nanoparticle market (Rittner *et al.*, 2002). AlNPs were produced at a global level of 18,500 tons per year in 2010. With their steady increase in output, it was expected that by 2020, the production of AlNPs would exceed 100,000 tons (Asztemborska, 2018).

AlNPs are employed in a variety of applications, including food, agriculture, engineering, industry, pharmacy, medicine and others (Alshatwi *et al.*, 2013; Canli *et al.*, 2019b; Yousef *et al.*, 2019b). AlNPs are used in heat transfer fluids, modification of polymers, and treatment of sewage. They also can be employed as a catalyst or a catalyst carrier in the field of catalysis (Kumar and Gill, 2014). Because of their highly effective catalytic action, they are used in solid rocket fuel. They also can be used in the ceramic sintering procedures as they exhibit large surface and superficial atom ratio (Prabhakar *et al.*, 2012). Moreover, aluminum and AlNPs are extensively used in ammunition, explosives, lithium batteries, artillery surface coatings, automotive finishing and flooring, and orthopedics (Tyner *et al.*, 2004; Monteiro-Riviere *et al.*, 2010). AlNPs have demonstrated great biological utility in biofiltration, drug delivery, and antigen delivery for vaccination (Kumar and Gill, 2014). Furthermore, they can be utilized as a carrier system in order to improve drug solubility (Tyner *et al.*, 2004). Also, it was documented that AlNPs can enhance the anticancer benefits of

immunotherapy using tumor cell vaccine (Sun *et al.*, 2010).

TOXICOKINETICS

It was reported that AlNPs were rapidly absorbed and systemically distributed to many organs such as liver, kidney, spleen, duodenum, and other organs in rats after oral administration (Krause *et al.*, 2020). Balasubramanyam et al. (2009b) studied the biodistribution of both aluminum oxide-bulk form and aluminum oxide-nanoparticles (30 and 40 nm). Results showed that AlNPs were absorbed from the GI tract and then passed through the lymph and lymph nodes and distributed to different tissues. AlNPs showed maximum accumulation in kidneys, whole blood, liver, and brain, while aluminum oxide-bulk biodistribution was significantly lower than AlNPs in all tissues and showed greater amounts in the feces than AlNPs. This indicated that size is a barrier to absorption of aluminum oxide in rats. Results also indicated that there was significant retention of AlNPs in kidneys, which might be attributed to the entrapment of these NPs in the reticular endothelial system and then excreted through the kidneys.

Park et al. (2015) mentioned that the kidneys, liver, and the immune system are the primary target organs for accumulation of AlNPs. They recorded that the no-observed adverse effect level (NOAEL) of AlNPs (rod-type) was lower than 6 mg/kg.

MECHANISM OF TOXIC ACTION

The mechanism of toxic action of NPs in general has been discussed by many researchers who reported that NPs can cross various cellular barriers and reach the most sensitive organs such as lung, liver, and kidney, thus resulting in mitochondrial damage, DNA mutations and eventually cell apoptosis/death. The production of reactive oxygen species (ROS), which could cause oxidative stress, inflammation and consequent damage to proteins, cell membranes and DNA, is a predominant mechanism leading to toxicity (Sengul and Asmatulu, 2020). Several studies reported that AlNPs mainly induced their toxic damage by induction of oxidative stress (Shah *et al.*, 2015; Yousef *et al.*, 2019b) and mitochondrial dysfunction (Alshatwi *et al.*, 2013; Yousef *et al.*, 2019b).

AlNPs can enhance free radical production resulting in oxidative stress in cells and tissues. Cellular accumulation of AlNPs resulted in direct interaction with cell components and binding with genetic materials, and proteins producing other different chemical compounds resulting in cellular oxidative damage (Morsy *et al.*, 2016a). Also, AlNPs induced cell death by enhancing ROS generation, disrupting the potential of the mitochondrial membrane, depleting the mitochondrial thiols, activating apoptotic caspases (Alshatwi *et al.*, 2013).

TOXIC EFFECT OF AlNPs

As a result of the extensive usage of AlNPs, a large amount of them is released into the environment (Dong *et al.*, 2019). Exposure to compounds containing AlNPs may cause toxic effects after certain thresholds. There are many factors affecting the AlNPS toxicity including: metal type, morphology, size and dose. For the same nanoparticle, smaller sized NPs have been shown to be more harmful than larger sized NPs (Wang *et al.*, 2009; Canli *et al.*, 2017, 2019b; Dong *et al.*, 2019).

The importance of the crystalline structure on the biological effects of alumina NPs was established in an in vitro research. The results of exposing six cell lines representing various target organs (liver, kidney, lung, brain, heart, and skin) to two particles of the same size but different phases (- AlNPs and -AlNPs) indicated that -AlNPs had better cell viability than -AlNPs. Park et al. (2016) demonstrated the importance of the crystalline structure on the biological effects of AlNPs, *in vitro*. The results revealed exposure of cell lines representing different target organs (kidney, lung, liver, brain, heart and skin), to two AlNPs of the same size but with different phases (α- and γ-AlNPs), resulted in higher cell viability after exposure to α-AlNPs, in comparison to γ-AlNPs.

1. Hepatotoxicity

I/P injection of rats with two forms of aluminum (nanoalumina and non-nanoalumina) at the same dose of 50 mg/kg, every two days for 60 days resulted in significant increase in plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) activities in nanoalumina group, in comparison to control and non-nanoalumina groups (Li *et al.*, 2012). Prabhakar et al. (2012) investigated the effects of acute oral treatment of rats with AlNPs at dose levels of 500, 1000 and 2000 mg/kg. Liver, kidney, brain and heart samples were taken on the 14th day. Only liver samples of the highest dose group showed significant

histopathological alterations, which include dilatation of central veins and expansion of portal tracts. These alterations were detected in 30% of the examined rats. It has been demonstrated that treatment of mice with AlNPs at doses of 1.5, 3, and 6 mg/kg, orally for 13 weeks, resulted in aluminum accumulation in tissues especially in the liver and kidneys. Serum activities of ALT, AST and lactate dehydrogenase (LDH) were decreased in the 1.5 and 3 mg/kg dose group, but these activities were significantly increased in the 6 mg/kg dose group. Hepatic histopathological alterations appeared only in the highest dose group in the form of chronic inflammation and moderate necrosis (Park *et al.*, 2015). Morsy et al. (2016c) investigated the effects of acute and subacute exposure to AlNPs in rats. Rats were injected I/P with a single-acute dose of AlNPs (3.9, 6.4 and 8.5 g/kg), or with a sublethal dose of 1.3 g/kg, day after day, for 28 days. Results revealed that the high acute and sublethal doses resulted in hepatic irregular disarray, necrosis of hepatocytes and Kupffer cells and congested blood sinusoids. The serum levels of total protein (TP) and total lipid were decreased, whereas the serum activities of ALT and AST were markedly elevated, in comparison to control groups. Oral treatment of rats with AlNPs at a dose of 70 mg/kg for a period of 75 days, markedly elevated the plasma activities of alkaline phosphatase (ALP), AST, ALT, gama glutamyl transferase (GGT) and LDH and the plasma levels of bilirubin, compared to control rats. Liver histopathology showed degenerative hydropic changes, cellular necrosis and congestion of sinusoidal blood vessels (Yousef *et al.,* 2019b). Treatment of male mice with AlNPs at the levels of 30 or 60 mg/kg, orally for 5 days resulted in significant elevation of serum ALT and AST activities. However, no changes were observed in the serum levels of TP, albumin and total bilirubin. Histopathological changes in the liver include dilated central vein and expanded portal tract (De *et al.*, 2020).

2. Nephrotoxicity

Morsy et al. (2016c) stated that I/P injection of rats with a single-acute dose of AlNPs (3.9, 6.4, and8.5 g/kg), or with a sublethal dose of 1.3 g/kg, day after day, for 28 days resulted in significant elevations of serum urea and creatinine levels in high dose acute and subacute groups. The renal histopathology revealed inter-tubular congestion, dilation of vascular glomeruli that that entirely filled Bowman's capsule and partial disappearance of the renal tubule's brush border. Treating rats with AlNPs orally at a dose level of 70 mg/kg for 75 days caused significant increase in plasma levels of creatinine, urea and blood urea nitrogen (BUN) and. Kidney histopathology of AlNP-treated rats showed hypertrophy, glomerular segmentation and hydropic degeneration of renal tubular epithelia (Yousef *et al.,* 2019b). Treatment of rats with AlNPs orally at the doses of 0.5, 5 and 50 mg/kg/day for 14 days caused AlNPs aggregates in all tissues including kidneys, at all dose levels (Canli *et al.,* 2019b).

3. Neurotoxicity

Many authors demonstrated that nano alumina can penetrate the blood brain barrier (BBB) easily regardless of the route of administration due to their small size and their influence on the BBB permeability through downregulation of expressions of tight junction proteins like claudin-5 and occludin (Chen *et al.,* 2008; Shah *et al.,* 2015). Several studies have documented that the neurotoxic effect of AlNPs was attributed to their ability to increase the brain aluminum content and to induce inflammation, ROS production, oxidative stress and mitochondrial dysfunction (Chen *et al.,* 2008; Shah *et al.,* 2015; Liu *et al.,* 2020). It was reported that I/P treatment of female mice with nano alumina at 50 mg/kg, twice weekly for three weeks, resulted in neurotoxicity in the form of increased brain aluminum content and ROS generation, disturbed brain energy homeostasis, and impaired the hippocampus-dependent memory. AlNPs induced AD neuropathology by enhancing the amyloid beta (Aβ) production, aggregation and implied the progression of neurodegeneration in the hippocampus and cortex of these animals (Shah *et al.,* 2015). I/V injection of male rats with AlNPs (20 mg/kg/day) for four consecutive days resulted in aluminum accumulation, disruption of mineral element homoeostasis, induction of oxidative stress in the hippocampus and disruption of spatial learning and memory performance in treated rats. In addition, decreased AChE activity in the hippocampus was recorded (M'rad *et al.,* 2018). Zhang et al. (2018) exposed female mice to AlNPs by nasal drip, at the concentration of 50 mg/kg, 3 times/day 2 weeks before matting and continued during pregnancy period until birth of offspring. Results demonstrated that

aluminum content in the hippocampus of newborns was significantly increased, in comparison to control group. Also, these newborns displayed stunted neurodevelopmental behaviors, increased anxiety-like behavior and impairment of learning and memory performance at one month of age owing to increased oxidative stress and alterations in neurotransmitter enzyme levels in brain cerebral cortex. These included reduction in choline acetyltransferase and increase in total cholinesterase activities. Treating female rats orally with different doses (0.5, 5 and 50 mg/kg/day) of AlNPs (40 nm) for 14 days caused significant reduction of brain AChE activity at all dose levels. ATPase activity was markedly inhibited in the kidney and brain but no discernible change in ATPase activity in the intestine was recorded (Canli *et al.*, 2019b). Huang et al. (2021) treated female mice, through a nasal drip three times per day, with 50 nm AlNPs at doses of 25, 50, and 75 mg/kg for 30 days. The results revealed that AlNPs impaired the spatial learning and memory in mice. Oxidative stress was observed together with marked pathological changes in the ultrastructure of mitochondria including mitochondrial swelling, sparsely arranged cristae, and vacuolation around the nucleus. Moreover, mitochondrial dysfunction was evidenced by diminished ATP activity and the content of mitochondrial respiratory chain complex IV.

4. Oxidative stress

Morsy et al. (2016a) injected rats I/P with single acute dose of AlNPs (3.9 or 6.4 and 8.5 g/kg) and with a sublethal dose of 1.3 g/kg, day after day for 28 days. Results revealed that AlNPs caused significant suppression of the liver, kidney and brain catalase (CAT), super oxide dismutase (SOD), and glutathione peroxidase (GPx) activities, and significant reduction of the reduced glutathione (GSH) concentrations associated with significant elevation of malondialdehyde (MDA) levels, in both acute and sublethal experiments. El-Hussainy et al. (2016) stated that I/P injection of rats with AlNPs at 30 mg/kg for 14 days resulted in significant elevation of serum NO and myocardial NO and MDA levels. Moreover, myocardial GSH concentration, SOD and CAT activities were markedly reduced in AlNP-treated rats. Mrad et al. (2017) studied the effects of four daily I/V injections of AlNPs (20 mg/kg) on male rat brain. They recorded that AlNPs elevated the levels of MDA and thiol group and inhibited the activity of CAT in the frontal cortex. Furthermore, SOD activity was inhibited in the frontal cortex and the cerebellum, whereas GPx activity was inhibited only in the cerebellum. Zhang et al. (2017) investigated the effects of AlNPs both *in vivo* and *in vitro*. Administration of AlNPs to mice I/P at doses of 300, 600 and 1200 μg/kg daily for 5 days, significantly elevated testicular MDA level and decreased SOD activity and levels of GSH and total antioxidant capacity, in a dose-dependent manner. Similar results were obtained when Chinese hamster lung fibroblast cells were treated *in vitro* with different concentrations (15, 30 and 60 μg/ml) of AlNPs. Mirshafa et al. (2018) injected male rats I/P with different doses (2, 4, and 8 mg/kg) of alumina (nanoparticle, microparticle, and ionic) forms for 28 days. They demonstrated that all forms of alumina increased ROS generation, lipid peroxidation and protein oxidation and depleted glutathione in the brain in a dose-dependent manner, with alumina nanoparticles being the most toxic. Daily I/V injection of male rats with AlNPs at the dose of 20 mg/kg for four successive days caused significant elevation in MDA level and significant reduction in SOD activity in the hippocampus, indicating the induction of oxidative stress. However, no change was recorded in thiol group levels or GPx and CAT activities (M'rad *et al.*, 2018). Zhang et al. (2018) exposed female mice to AlNPs by nasal drip at the dose of 50 mg/kg, 3 times/day, 2 weeks before matting and continued during pregnancy period until birth of the offspring. The newborns showed abnormal neurodevelopmental behaviors and neurotransmitter levels in the cerebral cortex. In addition, oxidative damage was observed in the form of elevated MDA level and inhibited SOD activity in the cerebral cortex. Oral treatment of mature female rats with different doses of AlNPs (0.5, 5, and 50 mg/kg/day) for 14 days resulted in significant and dose-dependent inhibition of liver SOD activity. The activity of GR was increased at the lowest dose level, while that of GPx was elevated at the highest dose level. On the other hand, liver GST and CAT activities showed no alterations (Canli *et al.*, 2019a). Mice were treated with ovalbumin to create the allergic asthma model while intratracheally given AlNPs (0.5, 5, and 50 mg/kg/day) for 3 weeks. Exposure to AlNPs

exacerbated airway hyperresponsiveness, airway remodeling, and inflammation, resulting in lung function impairment. In addition, results indicated that AlNPs increased ROS levels and decreased GSH concentrations in lung tissue (Cui *et al.,* 2019). Treatment of mouse neuroblastoma (N2A) and human bronchial epithelial (BEAS-2B) cells with α- and η-AlNPs (2.2 μg/cm^2) for 24, 48 and 72 hours caused oxidative stress represented by increased production of intracellular ROS and decreased GSH concentration and SOD activity. Both α- and η-AlNPs also caused marked elevation in CAT activity, with η-AlNPs showing higher activity, after 48 and 72 hours of exposure, for both cell lines (Nogueira *et al.,* 2019). Oral treatment of rats with AlNPs (70 mg/kg) for 75 days caused significant decrease in GSH levels and CAT, SOD and GPx activities, with significant increase of TBARS and NO levels in plasma, testis (Yousef *et al.,* 2019a), liver and kidneys (Yousef *et al.,* 2019b). Administration of AlNPs to rats orally as a single dose at 500 and 1500 mg/kg or cutaneously at 1000 and 2000 mg/kg significantly elevated the serum and brain MDA levels and reduced the activities of brain SOD and GPx (Arslanbaş and Coşar, 2019). The exposure of *Oreochromis niloticus* fish to different concentrations (1, 5, and 25 mg/L) of AlNPs for two weeks reduced the liver activities of SOD and CAT and increased liver activities GST. However, liver GPx and GR showed no significant changes (Canli and Canli, 2020). Treatment of mice orally with AlNPs (30 or 60 mg/kg) for 5 days resulted in disturbed redox homeostasis in brain, liver, kidney and spleen, appeared in the form of significantly increased lipid peroxidation levels and decreased glutathione concentrations at all doses. Moreover, CAT and SOD activities were significantly altered (De *et al.,* 2020). Administration of AlNPs to rats orally at the dose of 70 mg/kg daily for 28 days resulted in elevated testicular MDA level and decreased GSH level and CAT activity (Hamdi, 2020). Exposure of male mice to AlNPs (50 and 13 nm) through a nasal drip three times a day at a dose of 50 mg/kg resulted in increased aluminum accumulation in the spleen, with the 13 nm AlNP group having the highest accumulations. Alumina NPs induced oxidative damage in spleen and thymus appeared in the form of decreased SOD activity and GSH level with increased MDA level. Alumina NPs (13 nm) induced more oxidative damages than alumina NPs (50 nm) (Li *et al.,* 2020). It was documented that rats intranasally instilled with AlNPs at a dose of 20 μg/g bw daily for 15–30 days showed marked elevation of MDA levels and significant reduction of GSH concentrations in the olfactory bulb, hippocampus, and striatum after both 15 and 30 day-exposure. Also, GSH level was evidently reduced in the cerebral cortex after 30 day-exposure. All the alterations occurred in a time-dependent manner (Liu *et al.,* 2020). Treatment of female mice with 50 nm AlNPs through a nasal drip three times daily at the doses of 25, 50, and 75 mg/kg for one month resulted in impairment of the spatial learning and memory in mice. AlNPs induced oxidative stress in the cerebral cortex as evidenced by marked reduction of SOD activity and significant elevation of MDA level. Significant pathological changes in the ultra-structure and function of mitochondria were also recorded (Huang *et al.,* 2021).

5. Genotoxicity

5.1. In-vitro

Treatment of Chinese hamster ovary (CHO-K1) cells with AlNPs at the concentration of 0.5–10 μg/mL for 24 hours resulted in significant elevation of micronuclei (MN) frequency indicating the genotoxic effect. Also, AlNPs induced formation of perinuclear vesicles that were revealed by transmission electronic microscopy, but there were no NPs detected in the nuclei (Di Virgilio *et al.,* 2010). The genotoxic effect of AlNPs on human hepatocarcinoma cells (HepG2 cells) was examined using the alkaline single-cell gel electrophoresis (comet assay). Results revealed that exposure of HepG2 cells to AlNPs at the concentrations of 50, 150, and 450 μg/mL for 24 and 48 hours induced dose- and time-dependent increase in DNA fragmentation (percentage of tail DNA), compared to control HepG2 cells (Alarifi *et al.,* 2015). Furthermore, exposure of human peripheral blood lymphocytes to AlNPs for 24 hours resulted in a concentration-dependent elevation of DNA single-strand breaks (Sliwinska *et al.,* 2015). Hashimoto and Imazato (2015) documented that treatment of cultured macrophages (RAW264) with AlNPs at the levels of 200 and 400μg/mL resulted in nuclei and DNA damage. The both concentrations induced significant elevation in the frequencies of deformed nuclei and the DNA damage, while the MN frequency

was significantly elevated at the high concentration only. Zhang et al. (2017) stated that exposure of Chinese hamster lung fibroblast cells to different concentrations (15, 30 and 60 μg/ml) of AlNPs resulted in dose-dependent DNA damage that was demonstrated using the comet assay. The observed genotoxic effects were correlated with changes in the oxidant/antioxidant parameters.

5.2. In-vivo

Morsy et al. (2016b) demonstrated that AlNPs accumulated in the brain causing genotoxic effects, detected by the comet assay. The results showed significant increase in the % of DNA damage (significant increase in the tail intensity (TI), tail moment (TM) and olive tail moment (OTM) in the brain cells. Treatment of mice with AlNPs I/P at three dose levels 300, 600 and 1200 μg/kg (once daily for 5 days) resulted in dose-dependent elevation of the MN frequency in the bone marrow cells and the percentage of sperm deformities. The possible mechanism for these genotoxic consequences was the induction of oxidative stress (Zhang *et al.*, 2017). Oral treatment of mice with AlNPs at the doses of 15, 30 and 60 mg/kg for 5 days resulted in significant DNA damage in brain, spleen, tests (at all doses), liver, and kidney (at 30 or 60 mg/kg) appearing in the form of increased % tail DNA using comet assay. The highest DNA damage was documented in the brain. Moreover, sperm head anomalies were significantly elevated at the two high doses (De *et al.*, 2020). Administration of AlNPs orally to rats at a dose of 70 mg/kg once per day for 28 days resulted in significant elevation of DNA damage in testicular cells, evidenced by elevation of all comet parameters including % DNA damage, tail length, and tail moment (Hamdi, 2020). Treating male rats orally with three successive doses of AlNPs (25 mg/kg), resulted in significant DNA damage (increase in tail intensity) in bone marrow cells (Jalili *et al.*, 2020).

6. Inflammatory effect

Li et al. (2017) found that treatment of mice with AlNPs (0.4 or 2 mg/m^3) through inhalation for seven days resulted in emphysema and small airway remodeling in lungs, accompanied with enhanced inflammation and apoptosis. Exposure to AlNPs led to inhibition of protein tyrosine phosphatase, non-receptor type 6 (PTPN6) and phosphorylation of Signal transducer and activator of transcription 3 (STAT3), involved in the development of pulmonary inflammatory disease. AlNP-exposed mice showed dose-dependent increase in IL-6 and IL-33 levels, in bronchoalveolar lavage fluid. In addition, inflammatory cells were infiltrated around small airways and in alveolar areas. In another study, exposure of male rats to AlNPs through inhalation using a nose only inhalation system at the doses 1 and 5 mg/m^3 for 28 days, 5 days/week, resulted in significant increase in the neutrophil count and the levels of TNF-α and IL-6 in bronchoalveolar lavage fluid. Lung histopathology showed alveolar macrophage accumulation in the highest dose group during exposure and recovery (Kim *et al.*, 2018). It has been demonstrated that AlNPs enhanced inflammation by activating the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome, as evidenced by increased secretion of IL-1β and activation of caspase-1 (Manshian *et al.*, 2018). Additionally, treatment of rats with AlNPs alone or in combination with ZnONPs, daily, for 75 days resulted in significant elevation of liver, kidney and testicular levels of IL-6 and TNF-α (Yousef *et al.*, 2019a,b). Exposure of primary culture of neonatal rat cortex astrocytes to AlNPs in the form of nanoflakes or nanorods at three sublethal concentrations (31.25, 62.5 and 125 μg/mL) for 72 hours resulted in significant and dose-dependent increase in the levels of inflammatory cytokines IL-1β, IL-2 and IL-6. The increases induced by the nanorods were higher, by about 1.5- to 2-fold, than those induced by nanoflakes. This indicated that the morphology of AlNPs is an important factor for determining their toxic potencies (Dong *et al.*, 2019). In another study, exposure of male mice to AlNPs (50 and 13 nm) through nasal drip three times a day at a dose of 50 mg/kg resulted in significant increase in the levels of TNF-α, IL-1β and IL-6 in serum, spleen and thymus (Li *et al.*, 2020). Rats intranasally instilled with AlNPs (20 μg/g bw) once per day for 15–30 days showed significant increase of IL-1β and TNF-α levels in the olfactory bulb, hippocampus, and striatum after 15 and 30 day-exposure. Also, there was significant increase in the level of TNF-α in the cerebral cortex after exposure for 30 days. AlNP-exposure caused CNS damage, notably to the hippocampus and striatum. Such injuries might be caused by the release of inflammatory mediators and the toxic effects associated with oxidative stress. Thus,

AlNP-exposure increases the risk of development of neurodegenerative diseases (Liu *et al.,* 2020). Oral treatment of rats with AlNPs (<50 nm) at 50 mg/kg, once daily for 90 days resulted in hippocampal oxidative stress associated with neuronal ferroptosis. Moreover, significant increases in the hippocampus content of IFN-γ, TNF-α, IL-1β, and IL-6 were recorded (Zhang *et al.,* 2021).

7. Apoptotic effect

It was reported that exposure of human hepatocarcinoma cells (HepG2 cells) to AlNPs (50, 150, and 450 μg/mL) for 24 and 48 hours induced significant cytotoxic effect in a dose- and time-dependent manner, detected by the MTT and NR uptake assays. Also, significant oxidative stress and increased caspase-3 level. Moreover, the cells exposed to the high concentration changed into rounded and detached from the surface. Chromatin condensation was demonstrated after 48 hours at all dose levels (Alarifi *et al.,* 2015). Exposure of mice to AlNPs for seven days caused pathological changes in lungs, accompanied with apoptosis. AlNP-exposure induced inhibition of PTPN6 and phosphorylation of STAT3, resulting in increased expression of the apoptotic marker programmed cell death protein 4 (PDCD4) (Li *et al.,* 2017). It was documented that, *in vitro* exposure of rat brain mitochondria to AlNPs at the concentrations of 100 and 200 μM caused marked collapse of mitochondrial membrane potential, mitochondrial swelling, and increased cytochrome c release (Arab-Nozari *et al.,* 2019). Nogueira et al. (2019) exposed human bronchial epithelial (BEAS-2B) and mouse neuroblastoma (N2A) cells to α- and η-AlNPs (0.02 - 2.2 μg/cm^2) for 24, 48 and 72 hours. Results revealed cellular apoptotic morphological changes which include early apoptotic cells with perinuclear chromatin condensation or fragments and late apoptotic cells with condensed chromatin and blebbing of the cell membrane. Marked elevation in caspase 3/7 activity (apoptotic marker) was recorded in both cells after exposure to α- and η-AlNPs (2.2 μg/cm^2) for 24 and 48 hours. After 72 hours of treatment with η-AlNPs (0.02 μg/cm^2), significant increase in caspase 3/7 activity in BEAS-2B cells was observed. Analysis of nuclear morphology patterns revealed increased percentage of irregular nuclei in BEAS-2B cells exposed to η-AlNPs (2.2 μg/cm2) for 72 hours. Also, η-AlNPs markedly increased the percentage of nuclei with condensation in a near spherical form (characteristic of the apoptotic process) in N2A After 48 and 72 hours. Yousef et al. (2019b) documented that oral treatment of rats with AlNPs (70 mg/kg) for 75 days increased DNA fragmentation in liver and kidney. In addition, significant increase of p53 level and significant decrease of hepatic expression of PGC-1α and mTFA was recorded. The marked decrease in the PGC-1α and mTFA genes expression indicated a decreased mitochondrial biogenesis and impaired mtDNA replication and transcription causing mitochondrial dysfunction and alterations in mitochondrial membrane permeability owing to mitochondrial membrane potential disruption, resulting in forced apoptosis. Oral treatment of rats with AlNPs (70 mg/kg), once per day for 28 consecutive days significantly increased the intensity of activated caspase-3 immunostaining in seminiferous tubules and Leydig cells of the AlNP-treated rats (Hamdi, 2020). Liu et al. (2020) exposed rat pheochromocytoma (PC12) cells to various doses of AlNPs (12.5, 25, 50, 100, and 200 μg/mL) *in vitro*, for 12, 24 and 48 hours. They documented that AlNPs caused reduction in cell viability and elevation of LDH activity (cytotoxicity) in a dose-dependent manner. Exposure of PC12 cells to different concentrations of AlNPs for 48 hours resulted in significant decrease in mitochondrial membrane potential and ATP levels. AlNPs exposure resulted in apoptosis as evidenced by increased percentage of apoptotic cells, elevated expressions of p53, Rb, p21, and Bax and decreased expressions of cyclin D1, phospho-Rb, Mdm2 and Bcl-2.

Table 1: Hepatotoxicity of aluminum nanoparticles in mammals *in vivo*.

References	Animal	Route	Dose	Duration of exposure	Results/conclusion
Li *et al.*, 2012	rats	I/P	50 mg/kg every 2 days	60 days	significant increase in plasma ALT and AST activities
Park *et al.*, 2015	mice	oral	1.5, 3, and 6 mg/kg	13 weeks	Al accumulation, decreased serum activities of ALT, AST and LDH in the 1.5 and 3 mg/kg dose group, elevated in the high dose group with hepatic histopathological alterations
Morsy *et al.*, 2016c	rats	I/P	Acute (3.9, 6.4 and 8.5 g/kg) or sublethal (1.3 g/kg) every 2 days	Acute exposure and for 28 days	Decreased serum TP and TL levels, increased serum ALT and AST activities, hepatic histopathological alterations
Yousef *et al.*, 2019b	rats	oral	70 mg/kg	75 days	increased plasma activities of ALP, AST, ALT, LDH and GGT and plasma level of bilirubin, histopathological alterations
De *et al.*, 2020	mice	oral	30 or 60 mg/kg	5 days	elevated serum ALT and AST activities, no changes in the serum levels of TP, albumin and total bilirubin, histopathological changes

Table 2: Nephrotoxicity of aluminum nanoparticles in rats and mice *in vivo*.

References	Animal	Route	Dose	Duration of exposure	Results/conclusion
Morsy *et al.*, 2016c	rats	I/P	Acute (3.9, 6.4 and 8.5 g/kg) or sublethal (1.3 g/kg) every 2 days	Acute exposure and for 28 days	elevated serum urea and creatinine levels in high dose acute and subacute groups, renal histopathological alterations
Yousef *et al.*, 2019b	rats	oral	70 mg/kg	75 days	increased plasma levels of urea, BUN and creatinine, kidney histopathological changes
Canli *et al.*, 2019b	rats	oral	0.5, 5 and 50 mg/kg/day	14 days	AlNPs formed aggregates in all tissues including kidneys

Table 3: Neurotoxicity of aluminum nanoparticles in rats and mice *in vivo*.

References	Animal	Route	Dose	Duration of exposure	Results/conclusion
Shah *et al.*, 2015	mice	I/P	50 mg/kg, 2 times/week	3 weeks	Al accumulation, increased ROS, disturbed brain energy homeostasis, and impaired hippocampus-dependent memory, induction of AD neuropathology
M'rad *et al.*, 2018	rats	I/V	20 mg/kg/day	4 days	Al accumulation, disruption of mineral element homoeostasis, induction of oxidative stress, disruption of spatial learning and memory, decreased AChE activity in the hippocampus
Zhang *et al.*, 2018	mice	nasal drip	50 mg/kg, 3 times/day	2 weeks before matting till birth of offspring.	increased Al content in the hippocampus of newborns, stunted neurodevelopmental behaviors and increased anxiety-like behavior, impaired learning and memory performance, oxidative stress, alterations in neurotransmitter enzyme activities in cerebral cortex.
Canli *et al.*, 2019b	rats	oral	0.5, 5, 50 mg/kg/day	14 days	decreased brain AChE activity, decreased ATPase activity in the kidney and brain but not decreased in the intestine
Huang *et al.*, 2021	mice	nasal drip	25, 50, and 75 mg/kg, 3 times/day	30 days	impaired spatial learning and memory, oxidative stress, pathological changes in the ultra-structure of mitochondria, mitochondrial dysfunction

Table 4: Oxidative damage effect of aluminum nanoparticles *in vivo* and *in vitro* studies.

References	Animal or cell line	Route	Dose or concentration	Duration of exposure	Results/conclusion
Mrad *et al.*, 2017	rats	I/V	20 mg/kg/day	4 days	increased MDA and thiol group levels, inhibited CAT activity in frontal cortex, inhibited SOD activity in frontal cortex and cerebellum, GPx activity was inhibited only in the cerebellum
Zhang *et al.*, 2017	mice or Chinese hamster lung fibroblasts	I/P or *in vitro*	300, 600 and 1200 g/kg/day or *in vitro* concentrations (15, 30 and 60 µg/ml)	5 days or 12, 24 and 48 hours *in vitro*	elevated testicular MDA level, reduced SOD activity and GSH levels and TAC results of *in vitro* study are similar to those of the *in vivo* study
Mirshafa *et al.*, 2018	rats	I/P	2, 4, and 8 mg/kg	28 days	dose-dependent increase in ROS, lipid peroxidation, protein oxidation and glutathione depletion in the brain
Zhang *et al.*, 2018	mice	nasal drip	50 mg/kg, 3 times/day	2 weeks before matting till birth of offspring.	abnormal neurodevelopmental behaviors and neurotransmitter levels, increased oxidative stress in the form of elevated MDA level and inhibited SOD activity in the cerebral cortex
Canli *et al.*, 2019a	rats	oral	0.5, 5, and 50 mg/kg/day	14 days	dose-dependent inhibition of liver SOD activity, increased GR activity at the low dose, and GPx activity at the high dose. Liver GST and CAT activities showed no alterations
Arslanbaş and Coşar, 2019	Rats	Oral or cutaneous	500 and 1500 mg/kg or 1000 and 2000 mg/kg	24 hours (single dose)	elevated the serum and brain MDA levels and reduced the activities of brain SOD and GPx
Canli and Canli, 2020	*Oreochromis niloticus* fish	NPs in water medium	1, 5, 25 mg/L	14 days	reduced SOD and CAT activities and increased GST activity in liver. GPx and GR activities showed no significant change
De *et al.*, 2020	mice	oral	30 or 60 mg/kg	5 days	disturbed redox homeostasis in brain, liver, kidney and spleen, appeared in the form of significantly increased LPO levels and decreased GSH concentrations. CAT and SOD activities were significantly altered
Hamdi, 2020	rats	oral	70 mg/kg/day	for 28 days	elevated testicular MDA level and decreased GSH level and CAT activity
Huang *et al.*, 2021	mice	nasal drip	25, 50, and 75 mg/kg, 3 times a day	30 days	impaired spatial learning and memory, oxidative stress (decreased SOD activity and increased MDA level) in cerebral cortex, pathological changes in the ultra-structure of mitochondria and mitochondrial dysfunction

Table 5: Genotoxicity of aluminum nanoparticles *in vivo* and *in vitro* studies.

References	Animal or cell line	Route	Dose or concentration	Duration of exposure	Results/conclusion
Di Virgilio *et al.*, 2010	CHO-K1) cells	*in vitro*	0.5–10 μg/mL	24 hours	elevation of MN and formation of perinuclear vesicles, but no NPs was detected in the nuclei
Alarifi *et al.*, 2015	HepG2 cells	*in vitro*	50, 150, and 450 μg/mL	24 and 48 hours	time- and dose-dependent increase in DNA fragmentation (percentage tail DNA)
Zhang *et al.*, 2017	mice	I/P	300, 600 and 1200 μg/kg/day	5 days	oxidative stress, elevation of MN frequency in the bone marrow and the % of sperm deformities in dose-dependent manner
Zhang *et al.*, 2017	Chinese hamster lung fibroblasts	*in vitro*	and 15, 30 and 60 μg/ml	12, 24 and 48 h	oxidative stress and dose-dependent DNA damage
De *et al.*, 2020	mice	oral	15, 30 and 60 mg/kg	5 days	DNA damage in brain, spleen, tests (at all doses), liver, and kidney (at 30 or 60 mg/kg) appearing in the form of increased % tail DNA. The abnormalities in sperm head morphology were elevated at the two high doses
Hamdi, 2020	rats	oral	70 mg/kg/day	for 28 days	elevation of DNA damage in testicular cells as evidenced by elevation of % DNA damage, tail length, and tail moment
Jalili *et al.*, 2020	rats	oral	6, 12.5 and 25 mg/kg	gavage at 0, 24 and 45 hours	significant DNA damage (increase in tail intensity) in bone marrow cells at the high dose

Table 6: Studies on inflammatory effect of aluminum nanoparticles.

References	Animal or cell line	Route	Dose or concentration	Duration of exposure	Results/conclusion
Kim *et al.*, 2018	rats	inhalation	1 or 5 mg/m^3, 5 days/week	28 days	increase in the neutrophil count and the levels of TNF-α and IL-6 in BAL fluid. Lung histopathology showed alveolar macrophage accumulation in the highest dose group
Yousef *et al.*, 2019a	Rats	oral	70 mg/kg	75 days	significant elevation of liver and kidney levels of IL-6 and TNF-α
Yousef *et al.*, 2019b	rats	oral	70 mg/kg	75 days	significant elevation of testicular level of IL-6 and TNF-α
Dong *et al.*, 2019	neonatal rat cortex astrocytes	*in vitro*	31.25, 62.5 and 125 μg/mL	72 hours	dose-dependent increase in the levels of IL-1β, IL-2 and IL-6. The increases induced by the nanorods were higher by about 1.5- to 2-fold than those induced by nanoflakes
Li *et al.*, 2020	mice	nasal drip	50 mg/kg, 3 times a day		increased levels of TNF-α, IL-1β, and IL-6 in serum, spleen and thymus
Zhang *et al.*, 2021	rats	oral	50 mg/kg/day	90 days	hippocampal oxidative stress, neuronal ferroptosis, significant increases in the hippocampus content of IFN-γ, TNF-α, IL-1β, and IL-6

Table 7: Apoptotic effect of aluminum nanoparticles

References	Animal or cell line	Route	Dose or concentration	Duration of exposure	Results/conclusion
Li *et al.*, 2017	mice	inhalation	0.4 or 2 mg/m3	7 days	Pathological changes in lungs, enhanced apoptosis. AlNPs induced inhibition of PTPN6 and phosphorylation of STAT3, resulting in increased expression of PDCD4
Nogueira *et al.*, 2019	BEAS-2B and N2A cells	*in vitro*	0.02 - 2.2 μg/cm^2	24, 48 and 72 hours	cellular apoptotic morphological changes. Caspase 3/7 activity was increased in both cells after exposure to α- and η-AlNPs (2.2 μg/cm^2) for 24 and 48 hours. After 72 hours of treatment with η-AlNPs (0.02 μg/cm^2), significant increase in caspase 3/7 activity in BEAS-2B cells was observed. Proportion of irregular nuclei was increased in BEAS-2B cells exposed to η-AlNPs (2.2 μg/cm2) for 72 hours. Also, η-AlNPs markedly increased the percentage of nuclei with condensation in a near spherical form in N2A After 48 and 72 hours
Yousef *et al.*, 2019b	rats	oral	70 mg/kg	75 days	increased DNA fragmentation in liver and kidney, increased p53 level and decreased expression of PGC-1α and mTFA in liver, mitochondrial dysfunction and forced apoptosis.
Hamdi, 2020	rats	oral	70 mg/kg/day	for 28 days	increased intensity of activated caspase-3 immunostaining in seminiferous tubules and Leydig cells
Liu *et al.*, 2020	PC12 cells	*in vitro*	12.5, 25, 50, 100, and 200 μg/mL	12, 24 and 48 hours	reduction in cell viability, mitochondrial membrane potential and ATP levels and elevation of LDH activity. AlNPs induced apoptosis as evidenced by increased percentage of apoptotic cells, elevated expressions of p53, Rb, p21, and Bax and decreased expressions of cyclin D1, phospho-Rb, Mdm2 and Bcl-2.

CONCLUSION

The extensive use of AlNPs in various applications, leads to adverse impacts on animals and human health. Exposure to AlNPs may induce hepatotoxicity, nephrotoxicity, neurotoxicity, oxidative stress, genotoxicity, inflammatory response and apoptotic effects.

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