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REVIEW ARTICLE

Interactions of silver nanoparticles with biomolecules and resultant reactivated oxygen species generated immune responses

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Abstract

Nanotechnology is a key technology of the 21st century due to development of nano scale items and nanoparticles are important nowadays because of their small size and large surface area. Silver nanoparticles are one of the most essential and captivating nanomaterial among a few nanoparticles that are used in a wide range of biomedical and consumer products. The interaction of silver nanoparticles with biological systems leads to cause immunotoxic effects of AgNPs. They usually generate reactive oxygen species (ROS) which further lead to increase the release of pro-inflammatory reactions and oxidative stress destruction of the mitochondria due to depletion of antioxidant cause apoptosis or necrosis due to activation of intracellular signaling cascades. The mitochondrial GSH provides protection against the potential toxic effects of oxidative stress. This review article presents that the physiochemical properties of AgNPs like size, shape, surface area, solubility induce different cellular responses such as cellular uptake and intracellular biodistribution lead to the different immune responses in species due to exposure of AgNPs via several routes. Mostly the toxicity is based on the size and dose of AgNPs. It is concluded that small sized silver nanoparticles of 20nm produce biological responses and lead to cause organ toxicity and inflammatory responses. This article reveals the in-vitro biological responses induced by AgNPs which further lead to immunotoxicity.

Keywords

Nanoparticles
Silver nanoparticles
ROS
Oxidative stress
Glutathione
Immunotoxicty

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Introduction

Nanotechnology is a developing technology (Zahin et al., 2019) that is defined as science and engineering of material building in a scale of under 100nm (Alkahtani, 2018)**.** Nanotechnology is handling and control of matter on the nanoscale dimension by utilizing scientific information of different mechanical and biomedical applications. (Jeevanadam et al., 2018). It improved the clinical fields by improving mechanical and physical properties of materials, help presented new diagnostic modalities and nano-delivery system. (Alkahtani, 2018). Nanotechnology is quickly developing technology by creating nano items and nanoparticles that have novel and size related physiochemical properties contrasting notably from large matter (Tran et al., 2013).

Nanoparticles are particles that exist on a nanometer scale below 100 nm in at least one dimension (Kononenko et al., 2015). Nanoparticles demonstrate novel properties which relay upon their size, shape, and morphology which empower them to interconnect with plants, animals, and organisms. (Siddiqi et al., 2018)

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These properties are being quickly utilized in various territories such as bioimaging, biotechnology, energy sector, medicine, material science. (Beyene et al., 2017).Nanoparticles can produce from various substances for example polysaccharides, synthetic polymers, and proteins. Matrix material determination depends upon numerous components such as degree of biodegradability, toxicity, biocompatibility, size and surface characteristics of nanoparticle (Mohanraj and chan, 2006). Nanoparticles are classified as Carbon based nanoparticles, Metal NPs, Semiconductor NPs; Lipid based NPs, Ceramics NPs, polymeric NPs and dendrimer NPs (Khan et al., 2019; Subramani et al., 2009 ; Yih and Al-Fandi, 2006).

Silver Nanoparticles and their biomedical applications: Silver nanoparticles have been explored widely because of their high level physical, chemical, and biological characteristics and their predominance comes from size, shape, crystallinity, composition, and structure of Ag NPs compared to other nanomaterials (Lee and Jun, 2019). Silver nanoparticles are one of the most essential and captivating nanomaterial among a few nanoparticles that are associated with biomedical applications (Zhang et al., 2016). Silver nanoparticles are synthesized by physical, chemical/phytochemical methods, biological synthesis and Green chemistry synthesis approaches (Lee and Jun, 2019 ; Beyene et al., 2017 ; Zhang et al., 2016 ; Haider and Kang, 2014).

Silver nanoparticles, have favorable position over the other metallic nanoparticles due to their catalytic effect and chemical stability properties (Beyene et al., 2017). Silver nanoparticles have solid antimicrobial and novel physical and chemical properties; therefore, these are widely utilized in many fields including Medical, textiles, cosmetics, health care, industrial purpose, and food. Silver nanoparticles are considered as a rising contaminant in the sea (Li et al., 2018). Physical and chemical properties of silver nanoparticles include electrical, thermal, high electrical conductivity, optical and biological properties. Because of their exceptional properties they have been used for many applications such as antimicrobial agents, antibacterial agents, in household, health, industrial related products, biomedical device coatings, in the pharmaceutical industry, drug delivery carriers, optoelctronic platforms, water disinfection, in diagnostic, orthopedic, the food industry and consumer products, as anticancer agents furthermore, have at last improved tumor killing effects of anticancer medications(Lee and Jun, 2019 ; Zhang et al., 2016)dental applications, wound healing, bone healing, antifungal, antiviral, anti-inflammatory, anti-angiogenesis, antiplatelet activities (Burdusel et al., 2018). Synthesis of silver nanoparticles by utilizing Abutilon indicum leaf distillate showed profoundly strong antibacterial action upon Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, and Escherichia coli. In situ Synthesis of Silver nanoparticles inside the system of peptide strands utilizing UV illumination restrained the bacterial development of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. (Firdhouse et al., 2015).

A brief overview to Immune system: Host defense is the most primary functions of living organisms which consists of many biological structures and mechanisms within an organism that protects against disease (Alam, 1998). Immune system is a most sensitive part of human body (Galbiati et al., 2018). It has further two subtypes: innate immunity and adaptive immunity, both are two important aspects of the immune system (Luo et al., 2015). The innate immune system is the first line of defense against foreign invaders (Cheng et al., 2018). Innate immune system consists of cells that defend the host organism from the infection of other organisms by a non-specific manner (Castellheim et al., 2009). It relies on pattern recognition receptors (PRPs) which recognize wide and preserved molecular patterns that is found on pathogens named as pathogen-associated molecular patterns, PAMPs (Mogensen, 2009) belonging to toll-like receptors family (Takeda and Akira., 2004). The adaptive immune system is antigen-specific which requires some time to achieve its maximum effect, and typically generates an immunological memory and recognize pathogens more efficiently (Kononenko et al., 2015).

Reactive Oxygen Species and Effect on Immune System: Reactive oxygen species are defined as intracellular chemicals that contain oxygen which are chemically highly reactive, short lived and partially reduces or excited oxygen molecules. (Ahmad et al., 2017; Dutordoir et al., 2016; Glasauer and Chandel, 2013; Chen at al., 2016; Yang et al., 2013; Held 2015). These species are natural byproducts of cellular oxidative metabolism. ROS include free radicals having one or more unpaired electrons in the outermost shell of electrons and non-radicals which do not have unpaired electrons (Dayem et al., 2017). Three primary species superoxide anion, hydrogen peroxide and hydroxyl radicals are include in free radicals while hypochlorous acid, ozone, singlet oxygen, peroxynitrite and hydrogen peroxide are non-radicals. These non-radicals are either oxidizing agents or easily changed into radicals (Collin, 2019; He et al., 2011; Ray et al., 2012; Sharma et al., 2012; Ozcan et al., 2015). ROS are chemically more reactive than oxygen and are able to activate many biological events (Dayem et al., 2017). ROS are necessary in redox homeostasis maintenance, cellular communication and various cellular signaling pathways in different organisms ranging from bacteria to mammals (Gorlach et al., 2015).

Generation of ROS: ROS are produced both endogenously and exogenously. The endogenous sources of ROS include mitochondria, endoplasmic

reticulum, peroxisomes and NOX complexes in cell membrane and by inflammatory cell activation, while exogenous sources of ROS generation include ROS inducing agents, such as radiation, pollutants and nanomaterials exposure (Dayem et al., 2017; Bhattacharya, 2015; Gorlach et al., 2015).

Mitochondria and the family of NADPH oxidases (NOXs) are two vital sources of ROS generation. ROS generation through mitochondrial electron transport system consider main intrinsic source of production (Dayem et al., 2017). Over 90% of ROS in eukaryotic cells are produced by mitochondria (Lushchak, 2014). There are eight sites in mitochondria that produce ROS, in which three best sites are complex I, II and III within the mitochondrial respiratory chain. In cytoplasm increased accumulation of calcium result in activation of mitochondrial electron transport chain and ROS generation. Small concentration of oxygen is produced during mitochondrial production of ATP and water, resulting in the early stages of ROS production. Superoxide anion, the 1st ROS element generated by mitochondria, is produced due to complex I (NADH ubiquinone oxidoreductase) and complex III (co-enzyme Q, bc1 complex, and uniquinone/cytochrome c reductase) activity in the mitochondrial matrix and intermembrane space respectively, where superoxide anion rapidly converts into hydrogen peroxide. NOX is a non-mitochondrial source of ROS generation and play significant role in superoxide formation through oxygen reduction mediated by the electron donor NADPH. NADPH donates electrons to the center of NOX catalytic subunit to generate reduced oxygen through the one electron reduction of O2. Superoxide dismutase in the cytosol converts NOX generated reduced oxygen to hydrogen peroxide (Dayem et al., 2017; Glasauer and Chanel, 2013). Endoplasmic Reticulum also plays a vital role in ROS production. Various cellular Enzymes such as cytochrome p450 monooxygenase, lipoxygenase, cyclooxygenase, Xanthine oxidoreductase, and nitric oxide synthase, are also involved in the process of ROS generation (Dayem et al., 2017).

ROS Potential damage on Immune System: ROS are crucial for redox homeostasis as well as proper function in cardiovascular and immune system. At moderate concentrations, ROS are considered to be necessary for normal physiological functions regulation including of growth factor signaling, cell cycle progression, multiplication, differentiation, migration and cell death, the hypoxic response, inflammation and immune response. Excess cellular level of ROS causes damage to DNA, proteins, lipids, membrane and organelles. ROS has an important role in the immune system. Body system requires balance in its ROS levels for homeostasis. If levels of ROS increase of such extent that body can't handle, then oxidative stress occurs. A lack of ROS level in the immune system results in disease states that affects an individual power to fight against foreign invasion. Macrophages, neutrophils and dendritic cells are part of immune system which phagocytes foreign material. The phagocytic process is made possible through the use of ROS. When ROS production is not appropriate many diseases can occur. Chronic granulomatous disease occurs due to defect in NADPH oxidase. Body can't fight the infection, so granulomas creates around infections. Reactive oxygen species are involved in disease states such as aging, cancer and atherosclerosis. Increased level of ROS production lead to mitochondrial and cell apoptosis and also inflammatory diseases such as rheumatoid arthritis, multiple sclerosis. Due to ROS inappropriate level muscle fatigue, DNA damage, pulmonary diseases, and neurodegenerative disorders such as parkinson's disease, Alzheimer's disease occur. (Duttordoir et al., 2016; Mittal et al., 2014; Bae et al., 2011; Zuo et al., 2015; Patel et al., 2017; Mittler 2017).

Role of glutathione in oxidative damage: Glutathione is a small molecular weight tripeptide thiol antioxidant that consists of L-cysteine, L-glutamic acid and glycine. It exists in two states: reduced GSH and oxidized GSSG. It participates in antioxidant defense systems and also in many metabolic processes and cell signaling. It is present in high concentration in cytoplasm and also distributed in intracellular organelles, including the endoplasmic reticulum (ER), nucleus, and mitochondria (Traverso et al., 2013). It is a major endogenous and exogenous antioxidant is produced by cells that helps in the neutralization of free radicals and reactive oxygen species and also reduce exogenous antioxidants such as vitamins C and E (Drigen, 2000). Many antioxidants are used to block oxidative stress that chemically converted into oxidation products and then react with glutathione to form glutathione adducts which provide protection against free radicals (Hou et al., 2015). Glutathione is quickly used by the cells so it is a most potent scavengers of ROS as GSH maintains the cellular redox state and protect the cells from oxidative damage (Habib et al., 2007).

The significance of GSH in intracellular ROS is that it involves protective defense against oxidative stress and facilitate in ROS cell signaling (E. Presnell et al., 2013; Gomez et al., 2015; Ghezzi,2011). Glutathione provides protection against reactive oxygen species (ROS) by the interacting with associated enzymes like glutathione peroxidase and glutathione reductase and also preserve all other antioxidants (Adeoye et al., 2018). GSH depletion due to reduced glutathione levels which depends on the glutathione enzyme pathway contributes to progression of many diseases (Deponte et al., 2017). Oxidative stress is initiated when there is decline in the antioxidative defense system or oxidative stress caused by other factors also lead to decrease the concentrations of antioxidants. Alterations of antioxidant defenses support the hypothesis that oxidative stress may play an

Table 1: Cytotoxicity of AgNPs on different cell lines

Nature of NPs	Cell line	Size and dose	Toxicity signs	Reference
AgNPs	BRL-3A	15,100nm 5-50µg/mL	Reduced mitochondrial function Increase of ROS Depletion of GSH level Leakage of LDH	Hussain et al., 2005
AgNPs	HepG2	5-10nm 0.1-10µg/mL	Increase of ROS Depletion of GSH level Reduced mitochondrial function	Kim et al., 2009
Starch coated AgNPs	U251 IMR-90	6-20nm 25-400µg/Ml	Increase production of ROS DNA damage Cell cycle arrest in G2/M phase Mitochondrial damage Reduction of ATP content	AshaRani et al., 2009
AgNPs	Alveolar macrophages	15nm 25-50µg/mL	Increase of ROS Reduced mitochondrial function Depletion of GSH level	Cralson et al., 2008

Table 2: Toxicity via oral exposure of AgNPs

Nature of NPs	Animal model	Size and dose	effect	Reference
AgNPs	Sprague Dawley rats	2onm 820mg/kg	Induce oxidative stress in liver and cardiac mild inflammation in liver	Elle et al., 2013
CT-capped AgNPs	Wistar rats	10±4nm 0.2mg/kg	Prolonged low dose induced oxidative stress in brain	Skalska et al., 2016
PVP-AgNPs	Male Sprague Dawley rats	20-30nm 50,100,200mg/kg	High dose increased ROS production enhanced autophagy depletion of insulin signaling pathway	Blanco et al., 2018
AgNPs	Mice	22nm,42nm,71nm 1mg/kg 14 days and 28 days	Increase release of pro-inflammatory cytokines Increase ALP, AST	Park et al., 2010

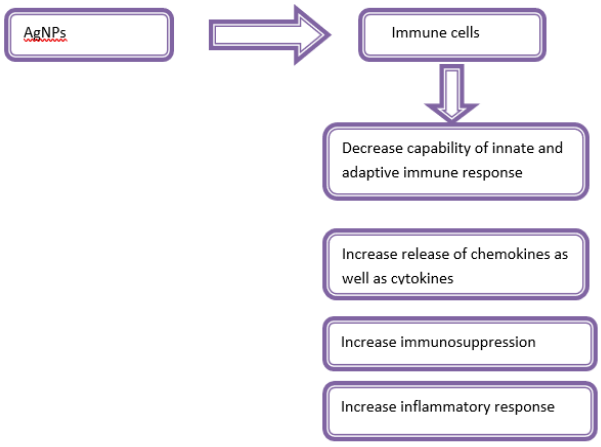

Figure 1: Schematic diagram of action of silver nanoparticles on immune system

important role in the pathophysiology of many diseases. The most robust and significant alteration in the antioxidant defense is a decrease in GSH concentration (Schulz et al., 2000). The mitochondrial GSH provides protection against the potential toxic effects of oxidative stress produced during coupled mitochondrial electron transport and oxidative phosphorylation (Armstrong et al., 2002).

Glutathione inhibitory role in immune system: GSH act as inhibitory factor for the inflammatory response but in fact, GSH also have some functions of the immune system for both innate and adaptive system. The first step in antigen degradation is the reduction of disulfide bonds that requires GSH (Arunachalam et al., 2000). As, GSH inhibits the production of most inflammatory cytokines, it is also required to maintain an adequate interferon gamma (IFN-gamma) production by dendritic cells that is vital for the host defense against intracellular pathogens (Ghezzi, 2011).

Interaction of NPs with biomolecules and consequent effects on immune system: The compatibility of an NP with the immune system is determined by the physiochemical properties of NPs include size, surface charge, hydrophobicity and hydrophilicity, as well as the steric effects of NP coatings (Buzea et al., 2007; Luo et al., 2015). Mostly, NPs interact with both innate and adaptive immune cells, and can either stimulate or inhibit the immune response (Gustafson, H. H et al., 2015).

Some nanoparticles don't exert immunomodulatory activity but they can suppress inflammatory responses (Engin and Hayes, 2018). Silver nanoparticles affect immune system by acting on different immune cells. Several studies have shown that NPs induce the release of inflammatory mediators from various cell types, like alveolar and bronchial epithelial cells, epidermal keratinocytes, and cultured monocyte–macrophage cells (Engin and Hayes, 2018).

NPs interact with variety of biomolecules and form "protein biocorona" (Neagu et al., 2017). "Protein corona" contains several proteins such as signaling and transport proteins, apolipoproteins, complement components, adhesion mediators or, coagulation factors, which give a unique "biological identity" to NP by opsonizing it (Shang et al., 2015; Walkey & Chan., 2012; Cedervall et al., 2007). The corona-coated NMs are then incorporate into the cells which further lead to the activation of the immune cells and lead to the secretion of proinflammatory cytokines such as IL-1b and IL-6. The complement system also becomes activated, which is associated to an inflammatory response, and functions to promote the elimination of NMs from the body (Corbo et al., 2016; Quach et al., 2018). The adaptive immune system recognizes these proteins on NMs and activates the signaling pathways that lead to secrete inflammatory cytokines (Corbo et al., 2016). The interaction of NPs with the immune system potentially leads to cause immunosuppression, hypersensitivity, immunogenicity, and autoimmunity (Engin and Hayes, 2018).

ROS mediated cytotoxicity by silver nanoparticles: Immunotoxicity is adverse immune modulation which shows undesirable effect on immune system (Frohlich, 2015). Human health risks increase due to the exposure of silver nanoparticle because of increasing number of nanoparticle having items and show adverse effects in different cell lines. (Kettler et al., 2016). Studies on toxicity of silver nanoparticles are frequently used to identify the biological response to AgNPs and these studies use to recognize risks linked with AgNPs exposure (Ferdous and Nemmar, 2020).

Silver NPs have been accounted to be cytotoxic to many cells, include human peripheral blood mononuclear cells, human alveolar epithelial cell line, human alveolar macrophage cell, neuroendocrine cell, normal human lung fibroblast cells, human glioblastoma cells, colon cells, hepatocytes, stem cells, skin keratinocytes, erythrocytes, human epidermal keratinocytes, embryo kidney cells, neuro blastoma cells, and procrine kidney cells, THP-1 derived macrophages, mast cells, neutrophils and natural killer cells ,rat liver cell line, mouse germinal cells and mouse fibroblast (Ge et al., 2014 ; Murphy et al., 2015 ; Ferdous and Nemmar, 2020 ; Kettler et al., 2016 ; Luo et al., 2015). Ag NPs upon interaction with cellular components lead to immunotoxicity which cause accumulation of oxidized glutathione due high levels of Reactive Oxygen Species induce by silver nanoparticle (Kettler et al., 2016). Diseases such as emphysema, asthma, lung cancer, bronchitis, neurodegenerative diseases, and autoimmune diseases such as scleroderma, erythematosus, systemic lupus and rheumatoid arthritis diseases also occur due to exposure of Ag NPs through inhalational route. (Singh et al., 2017) . The systemic toxicity produce due to immunotoxic effect of AgNPs can be reduced by the interaction of NPs and protein corona, by increasing the stability of particles and inhibiting the generation of reactive oxygen species (ROS) (Corbo et al., 2016). It is proposed that silver nanoparticles toxicity cause disruption of the mitochondrial respiratory chain by silver nanoparticles lead to production of ROS and interruption of ATP synthesis, which in turn lead to cause DNA damage. It is predicted that DNA damage is increased by deposition which is followed by interactions of silver nanoparticles to the DNA lead to cell cycle arrest in the G2/M phase (Asharani et al., 2009). Arora and his coworkers have also proposed that nanosilver is present in the mitochondria and triggers the antioxidant mechanisms (Arora et al., 2012).

It is also studied that silver nanoparticles lead to induce oxidative stress is due to cellular factors and cellular responses such as mitochondrial respiration, NP-cell interaction, and immune cell activation which are responsible for ROS-mediated damage. These oxidative stress responses cause further pathophysiological effects include inflammation, genotoxicity and fibrosis activation of related cell signaling pathways. As oxidative stress is a major element of NP-induced injury so it is compulsory to distinguish the ROS response resulting from NP. Due to physicochemical characterization and activation of the multiple signaling pathways by AgNP-induced ROS, a systemic toxicity occur with oxidative stress (Manke et al., 2013). Pandey and Prajapati proposed the mechanism of silver nanoparticles and effects on immune system which further lead to induction of oxidative stress by the activation of ROS (Pandey and Prajapati., 2018).

Glimpse of studies focusing on mechanisms of immunotoxicity by silver NPs: The physiochemical properties of silver nanoparticles such as size, shape, surface area, solubility produce toxicity that range from the beginning of inflammatory pathways through to cell death. When cell exposed to silver nanoparticles, it become abnormal sizes and shrink at high doses after 24 hour (Cralson et al., 2005; Hussain et al., 2005; Nel et al., 2006). Moreover, AgNPs also induced a variety of morphological malformations such as edema, spinal and heart malformations and eye defects (Wu et al., 2010).

Due to toxicity of silver, several studies are conducted with reference to the oxidative stress. Toxicity of nanoparticles is manifested by inflammation due to

activation of oxidative stress (Carlson et al., 2008; Folkmann et al., 2009; Park et al., 2009). It is widely approved from the previous published data that AgNPs cause cytotoxicity by oxidative stress that lead to induce apoptosis and damage to cellular components (Piao et al., 2011). In vitro cytotoxicity of silver nanoparticles on different cell lines at different sizes and doses are studies. These studies evaluated that at high dose of AgNPs after 24-hour exposure lead to mitochondrial damage, increased ROS levels, depletion of GSH levels and LDH leakage are mediated through oxidative stress (Kim et al., 2009; Cralson et al., 2005; Hussain et al., 2005). The later also study suggested that the cytotoxicity induced by Ag-nanoparticles against tissue cells are particle size-dependent, so the particle size should be according to biomedical uses (Kim et al., 2012). It is previously concluded that AgNPs extensively lead to increase cell death through oxidative stress-related mechanisms which cause DNA damage in mammalian cells (Hsin et al., 2008).

Mostly AgNPs lead to cause toxicity at low doses. One study reported that structure of retinal cells are disrupted at low concentrations of AgNPs with increased number of cells under oxidative stress (Söderstjerna et al., 2014). Asharani and his coworkers studied that induction of ROS cause DNA damage and chromosomal aberrations in human lung fibroblast cells (Asharani et al., 2009). In recent study it is suggested that small size AgNPs (10 nm) lead to cause toxicity to human blood mononuclear cells which is both time- and dose-dependent (Barkhordari et al., 2014). Silver nanoparticles are able to cross cell membrane and enter mitochondria which lead to oxidative stress, inflammation and thereby lead to apoptosis when incubated with human gingival fibroblast cells (Inkielewicz-Stepniak et al., 2014). In one study it is suggested that when an adult zebrafish are exposed to AgNPs, it lead to induced oxidative stress and cellular apoptosis is also observed in the liver cells (Choi et al., 2010).

Further studies revealed that small sized nanoparticles are more toxic as compared to their large sized AgNPs. Liu and his coworkers evaluated the cytotoxicities of three silver nanoparticles SNP-5, SNP-20 and SNP-50 with different sizes (5 nm, 20 nm and 50 nm) by using four human cell models (A549, SGC-7901, HepG2 and MCF-7) which shows that cell morphology, cell viability, cellular membrane integrity, oxidative stress and cell cycle progression are induced by SNP-5 and SNP-20 (Liu et al., 2010). The study related to toxicity on the embryo of zebrafish clarified that the smaller sized nanoparticles (20 nm) are more toxic as compared to 100 nm AgNPs (Kim and Tanguay, 2014).

Continuous oral administration may also cause toxicity in the organs and inflammatory responses (Park et al., 2010). For the exposure of AgNPs, oral route is important in many consumer products such as toothpaste, reusable bottles, nursing nipples, kitchen utensils, and toys (Chen and Schluesener, 2008; Edwards-Jones, 2009). One of the study of short term of high dose of AgNPs in rats (25-100mg/kg) via oral administration shows elevate ROS, alanine amino transferase (AAT), lipid hydroperoxide which lead to cause DNA damage. Immunoglobulin level decrease at high dose compared to short dose. Kim and his coworkers revealed that small-sized (10 nm size) AgNPs have a more ability to stimulate apoptosis in MC3T3-E1 cells than large-sized (50 and 100 nm) AgNPs (Kim et al., 2012).

The toxicity due to repeated-dose of AgNPs was also investigated in mice by oral administration for 14 days in control and treated groups which shows raised level of TGF-β in serum and increased distribution of NK cells and B cells and increased IgE production at small sized AgNPs (22nm , 42nm and 71nm) and for 28 days in which results show that small sized AgNPs are more active to produce biological responses and lead to cause organ toxicity and inflammatory responses in mice (Park et al., 2010).

Parenteral administration is another potential route of AgNPs entry in the cell. Several studies have done for the determination of potential systemic toxicity of silver nanoparticles (Ag-NP) via intravenous administration. These studies investigated that the most sensitive system which is largely affected by AgNPs is immune system which lead to affect many parameters include reduction in thymus weight, increase spleen weight and spleen cell number, strongly reduced NK cell activity, and reduction of interferon gamma production (De Jong et al., 2013; Vandebriel et al., 2014). In previous study the biokinetics and tissue distribution of silver nanoparticles are evaluated by intravenous administration of a dose of 120 mg/kg in both male and female mice in which post injection parameters are checked at 7 and 14 days shows that only inflammatory reactions in lungs and liver are induced in mice at high dose of 120 mg/kg (Xue et al., 2012).

The latest research has concluded that the wide use of nanoparticles as medical application has lead to raise potential toxicity. In one research it is investigated that the small dose of silver nanoparticles 5 mg/kg injected in rats indicate the immunotoxicity of AgNPs and which cause mild irritation in thymus and spleen and increased chromosome breakage and polyploidy cell rates (Wen et al., 2017).

It has been recently reported that in the initial stage of exposure silver nanoparticles, it affect cellular stress on the endothelial cells by triggering a pro-inflammatory pathways. In one research data it is evaluated that several proteins which are involved in neurodisorders and neurodegeneration are considerably upregulated in endothelial cells like 7-dehydrocholesterol reductase, zinc transporters 1 and 6, while proteins which are responsible for maintaining brain homeostasis are extensively

downregulated like anti-oxidative proteins glutathione peroxidase 1 and glutathione peroxidase 4. Many inflammatory pathways (C9 pathway), are appreciably upregulated at 24h post-AgNPs exposure while at 48h proteins which are involved in BBB damage and anti-inflammatory responses are upregulated like quinoneoxidoreductase1 and glutamate cysteine ligase catalytic subunit. This study suggested that soon after, cellular protection pathways have been activated to save the cells from AgNPs-induced toxicity (Khan et al., 2019).

Conclusion: The science and applications of nanotechnology are constantly evolving in presenting new diagnostic modalities. Ag NPs are one of the most attractive nanomaterials. They have been widely used for antimicrobial, electronic, and biomedical applications. In this review article, we provide a critical overview of the current state of knowledge of silver NPs, from synthesis methods, its applications, and special emphasis on toxicity related to immune system and its mechanism of interaction with immune system. Interaction of silver NPs with immune system involve production of ROS and interruption of ATP synthesis, which in turn lead to cause DNA damage. The physiochemical properties of NPs influence the immunological effects of NPs. The cytotoxic effects of Ag NPs are governed by factors such as size, shape, and dose and cell type. We studied cytotoxicity of silver NPs on different cell lines at different sizes, different doses and by different routes like intravenous, oral and parenteral route. Cytotoxic actions by oxidative stress lead to cell apoptosis and ultimately cell death. Several immune parameters that are affected because of Ag NPs toxicity include increase spleen weight and spleen cell number, reduction in thymus weight, NK cell activity reduction, reduce Interferon gamma production. Diseases such as neurodegenerative diseases and autoimmune diseases occur due to exposure of Ag NPs through inhalational route. Immunotoxicity due to silver NPs effect cell morphology, cell viability, and cellular membrane integrity. our study reveal that small size Ag NPs are more toxic as compare to large size Ag NPs. Conclusion is that though NPs are useful for many applications, but still there are some health hazard concerns.

Future Perspectives: More attention should be given to determine the mechanism of interaction between nanoparticles and immune system. Comprehensive studies to explore the effects of physiochemical properties of nanoparticles on immune system are still needed. Mechanistic studies investigating potential adverse effects of nanoparticles on immune system, immunomodulatory effects or inflammatory reactions of NPs are required to improve knowledge of the physiochemical properties which affect immune system. With new findings about interaction between nanoparticles and immune system, we will get potential to develop safe and better nanotechnology products.

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