ABSTRACT

Background: Silver nanoparticles became one of the most manufactured nanomaterials introduced in the medical field, that’s why it was mandatory to evaluate their biological toxicity.

Aim: In this study, the aim was to evaluate the size-dependent toxicity of intraperitoneal administered characterized Ag NPs of different sizes on the submandibular salivary glands of adult albino rats.

Materials and methods: Fifty adult male albino rats were randomized into three groups: group 1 (control), group 2 received intraperitoneal injection of 13mg/kg body weight of Ag NPs (10 nm), and group 3 received intraperitoneal injection of 13mg/kg body weight of Ag NPs (60 nm). The rats were sacrificed after 3 and 6 hours after treatment. The submandibular salivary glands of the all groups were subjected to histopathological examination, histomorphometric analysis, real time PCR analysis and colorimetric assay.

Results: Histologically, the submandibular salivary glands of group 2 revealed signs of acinar degeneration, represented by loss of normal architecture (amalgamation), intracytoplasmic vacuolization. The nuclei of the acinar cells revealed pleomorphism and pyknosis. However, the seromucousacini relatively preserved their shape in group 3. RT-PCR results revealed significantly decreased GPx mRNA gene expression in all Ag NP-treated groups.

Conclusion: In a rat model, Ag NP induced cytotoxic effect. The cytotoxic effect was size- and time-dependence.

Keywords: Nanotechnology, Silver nanoparticles, Cytotoxicity, submandibular salivary glands, antioxidant.

I. INTRODUCTION

Nanotechnologies are defined as the manipulation of materials of controlled shape and size at the nanometer scale (1-100 nm). Sindhu and Ranjith (2016) & Khan et al. (2019). Owing to their size range, engineered NPs hold great interest for nanotechnologies as they have different or enhanced properties such as reactivity, strength, electrical and optical characteristics compared with the same materials at a larger size. The two main reasons for this behavior change are the increased relative surface area and the surface reactivity Nagarajan and Rajeswari (2017).

Silver nanoparticles (Ag NPs) exist in a huge variety of different forms; they can be of different shapes (spheres, rods, wires, and triangles), coatings (citrate, polymer, peptide, sugars), and of different sizes.
Owing to their tiny size, they could pass more easily through cell membranes and other biological barriers such as the blood-brain barrier as well as the placental barrier, and subsequently induce potential cytotoxicity Xu et al. (2020).

Silver nanoparticles (Ag NPs) display unique physical and chemical properties and represent useful materials in biological applications. Ag NPs are now being developed for various biological applications such as medicines, antimicrobial agents Nam et al. (2015), anti-bacterial, anti-viral, anti-fungal, anti-inflammatory as well as anti-cancerous abilities Ullah Khan et al. (2018), wound dressing Rath et al. (2015) drug targeting and deliveries Soumya and Hela (2013) transfection vectors Dzięgiel (2015), bio-imaging, and labeling agents Sotiriou and Pratsinis (2011).

Despite the promising advantages of NPs, safety concerns have been raised over the usage of nanoparticles because they pose potential hazards to human health and the environment Sheehan et al. (2018).

Reactive oxygen species (ROS) play essential roles in many biological processes. Sies et al. (2017). Indeed, excess ROS accumulation will lead to oxidative stress that causes an imbalance in that redox system resulting in cellular dysfunction. These free radicals trigger the oxidation of essential biomolecules like DNA, RNA, protein and, lipids, leading to multiple disease conditions Styskal et al. (2012).

Lipid peroxidation generates a variety of relatively stable decomposition end products, such as malondialdehyde (MDA). MDA is used as a marker of cell membrane injury as its production is increased with an increase in free radicals. Ferdous et al. (2018).

Anti-oxidants such as catalase, superoxide dismutase (SOD), and glutathione (GSH) dependent enzymes e.g. glutathione peroxidases (GPx) act as scavenger that slow damage to cells caused by free radicals in the body Kurutas (2016).

The present work was carried out to investigate the toxic effect of Ag NPs on the submandibular salivary glands as well as oxidative stress induction using histopathological examination, histomorphometric analysis, real time PCR analysis.

II. MATERIAL AND METHODS

Chemicals
All the chemicals were purchased from Sigma-Aldrich Chemical Co. St. Loutis, USA. The main chemicals used for the synthesis of the Ag NPs were silver nitrate, trisodium citrate, and deionized water.

Preparation of Ag NPs
Synthesis of Ag NPs was carried out in the Egypt Nanotechnology Center, Sheikh Zayed City, Cairo University. Two different sizes of 10 and 60 nm of Ag NPs were prepared using the chemical reduction method according to Youssef et al. (2020). Ag NPs were given intraperitoneal injection at dose 13 mg/kg body weight Cho et al. (2018).

Characterization of Ag NPs:
Transmission electron microscopy (TEM):
TEM (Jeol, JEM-2100 high-resolution, Japan) was used for determining the size of NPs. A small drop of Ag NPs was placed onto TEM grids, coated with carbon film and allowed to evaporate. Digital pictures of several locations on the grid were taken.

Zeta potential measurement
Samples of Ag NPs were diluted in distilled water. The zeta potential was measured using a Nano Zetasizer particle analyzer (Entrgris instruments model of Z3000, USA) using the software provided by the manufacturer.

Animal treatment:
The study was carried on 50 adult male albino rats weighing 150g-200g in the animal house - Faculty of medicine - Cairo University after submission for approval from the Institutional Animal Care and Use Committee (IACUC) - Cairo University. The animals were housed under standard conditions, with free access to water and standard food pellets and in a controlled 12/12hr light/dark cycle. Room temperature was kept at 22±2º C. Rats were randomized into 3 equal age- and weight-matched groups each containing 10 rats as follows:

**Group 1 (control group):** included 10 normal rats that received a single intraperitoneal injection of 1 ml/kg body weight of citrate buffer *Mohamed et al. (2020).* They were subdivided into G1A and G1B groups and were sacrificed after 3 and 6 hours, respectively.

**Group 2 (treated group, G2):** included 20 Ag NPs treated rats that received single intraperitoneal injection of Ag NPs of average size 10 nm at dose 13 mg/kg body weight *Cho et al. (2018).* They were subdivided into G2A and G2B groups and were sacrificed after 3 and 6 hours, respectively.

**Group 3 (treated group, G3):** included 20 Ag NPs treated rats that received single intraperitoneal injection of Ag NPs of average size 60 nm at dose 13 mg/kg body weight *Cho et al. (2018).* They were subdivided into G3A and G3B groups and were sacrificed after 3 and 6 hours, respectively.

All the animals in groups 1, 2, and 3 were sacrificed by intraperitoneal injection of ketamine 100 mg/kg body weight after 3 hours (for G2A & G3A) and 6 hours (for G2B & G3B) from the beginning of Ag NPs treatment *Lairez et al. (2013).*

The specimens of the dissected glands were processed for histopathological examination, histomorphometric analysis and real time PCR analysis.

**Light microscopic study:**

The left side were fixed in 10 % neutral buffered formalin for 24 hours, then dehydrated in ascending grades of ethyl alcohol, cleared in xylene, embedded in paraffin wax, sections of 3-4 µ were obtained, mounted on clean slides and then done using hematoxylin and eosin stain according to the convention method of *Bancroft et al. (1977)* then used for histological examination by ordinary light microscope.

**Histomorphometric analysis (evaluation of the degenerated seromucousacin number/µm²):**

H&E stained sections were assessed by ordinary light microscope using an image analyzer computer system for histomorphometric measuring of the degenerated seromucousacin number/µm². The equipment consists of a digital camera attached to a light microscope and a computer system equipped with the software Leica Quin 500 (Leica Microsystem Inc., Switzerland).

**Quantitative reverse transcription-polymerase chain reaction (q RT-PCR) examination:**

Quantitative PCR amplification and analysis were performed using an Applied Bio-system with software version 3.1 (Step One™, USA). The quantitative PCR assay with the primer sets was optimized at the annealing temperature to localize Glutathione peroxidase (GPx) mRNA genes expression *Farrell (2010).*

**Statistical analysis:**

The recorded values from histomorphometric analysis as well as the q RT-PCR results were statistically analyzed. Statistical analysis was then performed using a commercially available software program (SPSS 18; SPSS, Chicago, IL, USA).

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and the post-hoc test (Tukey) for pair wise comparison. The level of significance was set at P value ≤ 0.05.

III. RESULTS

NPs characteristics:
TEM results showed that all Ag NPs samples were homogenous spherical shape and dispersed without any extreme agglomeration with size range of 10- 36 nm and 60- 96 nm. The zeta potential of 10 nm and 60 nm Ag NPs were -31.5 mV and -18.1 mV respectively, thus they were considered stable.Figures (1A, 1B).

**Histological results:**

**Group 1 (control group)**
Both control groups (G1A and G1B) revealed normal architecture consisted mainly of seromucous acini. Different types of closely packed ducts and connective tissue stroma.

The seromucous acini appeared rounded, small in size with a very narrow lumen. They were lined by a single layer of pyramidal cells. The lining cells showed basally located spherical nuclei with granular basophilic cytoplasm.Figures(2A, 2B).

The duct system was composed of intercalated, granular convoluted tubules (GCT), striated ducts and excretory ducts.

The granular convoluted tubules were lined by tall columnar cells with rounded or oval basally located nuclei and discrete eosinophilic granules occupying its apical cytoplasm.Figures(2B, 2C). The striated ducts appeared rounded, lined by a single layer of columnar cells having large, rounded, central and darkly stained nuclei. The cells showed characteristic prominent basal striations with intensely eosinophilic cytoplasm.Figure(2A).

The excretory ducts were lined by pseudostratified columnar epithelium with rounded or oval deeply stained nuclei appearing at different levels.Figure(2B).

**Group 2 (10 nm Ag NP-treated group)**

**Group 2A**
The submandibular salivary glands showed loss of the normal architecture. Seromucous acini appeared shrunken, atrophied, darkly stained pyknotic nuclei and intracytoplasmic vacuolization Figure (3).

The GCT showed marked degree of destruction Figure (3C). Most of the ducts were shrunken due to atrophy with an ill-defined outline,

The striated ducts showed widening lumen and their epithelial lining cells showed signs of degeneration with loss of basal striation and perinuclear vacuolization. The excretory ducts epithelial lining showed areas of atrophy and appeared as cuboidal or flat cells Figure (3B).

**Group 2B**
The submandibular salivary glands showed marked histological changes as compared to group 2A with loss of the normal architecture (amalgamation). Seromucous acini appeared atrophied with ill-defined cell borders and massive intracytoplasmic vacuolization as well as pyknotic nuclei Figures (4A, 4B).

The GCT appeared atrophied with interrupted boundaries and pyknotic nuclei. The striated ducts showed wide lumen and pyknotic nuclei. Dilated blood vessels engorged with red blood cells were adjacent to the striated ducts Figures (4A, 4B).

Excretory ducts showed interrupted epithelial lining and stagnant secretion in their lumens. The epithelial lining cells had pleomorphic nuclei with different sizes and stainability Figure (4C).

**Group 3 (60 nm Ag NP-treated group)**

**Group 3A**
The submandibular salivary gland showed seromucous acini with intracytoplasmic vacuolization. The nuclei showed different sizes and shape (pleomorphism) as well as increased basophilia (hyperchromatism) Figures (5A, 5C).

The GCT appeared atrophied with interrupted epithelial lining in certain areas. The striated ducts showed separation of their lining epithelial cells away from the basement membrane with apparent loss of their basal
striations. The nuclei exhibited increase in basophilia (hyperchromatism) and perinuclear vacuolization Figures (5A, 5C).

The excretory ducts showed disturbed epithelial lining cell with different stainability of their nuclei and stagnant secretion. Dilated and congested blood vessels engorged with red blood were observed Figure (5B).

**Group 3B**

The seromucousacinar lining cells showed atrophy with intracytoplasmic vacuolization and pyknotic nuclei Figure (6).

The intercalated duct was atrophic Figure (6C). The GCT showed loss in their cytoplasmic granules, and ill-defined cell boundaries Figures (6A, 6C). The striated ducts showed ill-defined epithelial lining and loss of basal striation, pyknotic nuclei and perinuclear vacuolization.

The excretory ducts showed retained secretion in their lumens and pleomorphic nuclei. The connective tissue stroma was dissociated and edematous Figure (6B).

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**Figure (1):** An electron photomicrograph showing; (A) 10 nm Ag NPs. (B) 60 nm Ag NPs.
Figure (2): A photomicrograph of rat submandibular salivary gland of control group (G1A & G1B) showing 
(2A) Seromucous acini (red arrows) having granular basophilic cytoplasm and striated ducts (yellow arrows) with rounded deeply stained nuclei and apparent basal striation. (2B) showing excretory duct (asterisk) lined by pseudostratified columnar epithelium with the nuclei appearing at different levels, seromucous acini (red arrows), granular convoluted tubule (black arrows) and blood vessels (green arrow) embedded in the connective tissue stroma (H&E, Orig. Mag. X 400). (2C) A high magnification showing the GCT having multiple eosinophilic granules (H&E, Orig. Mag. X 1000).
Figure (3): A photomicrograph of rat submandibular salivary gland of control group (G2A) (10 nm Ag NP-treated group for 3 hours) showing (3A) loss of normal glandular architecture, degenerated granular convoluted tubule (black arrows) and striated duct (yellow arrows). Widely separated atrophic seromucous acini (red arrows) with marked intracytoplasmic vacuolization and darkly stained pyknotic nuclei. (3B) showing atrophied excretory duct (asterisk) lined with a single layer of cuboidal and flattened cells. The epithelial lining of the excretory duct was disrupted at certain areas. Striated ducts (yellow arrows), atrophied seromucous acini (red arrow), atrophied GCT (black arrow) were also seen. Note dilated and congested blood vessels (green arrow) (H&E, Orig. Mag. X 400). (3C) A high magnification of the granular convoluted tubules (G) with ill-defined outline, Intracytoplasmic vacuolization, and loss of their cytoplasmic content (asterisk). Atrophied intercalated ducts (red circle) were also observed (H&E, Orig. Mag. X 1000).
Figure (4): A photomicrograph of rat submandibular salivary gland of control group (G2B) (10 nm Ag NP-treated group for 6 hours) showing (4A) marked loss of normal glandular architecture. The gland appeared less impact with relative loss of connective tissue septa between the seromucousacini and the ducts. Atrophied GCT (black arrows) with interrupted boundaries, the epithelial lining cells exhibited intracytoplasmic vacuolization with loss of some nuclei and pyknosis of the remaining seromucousacinar (red arrows). Note atrophied intercalated ducts (red circle). (4B) showing striated ducts (yellow arrows) with wide lumen disrupted epithelial lining. The epithelial lining cells showed intracytoplasmic vacuolization and pyknotic nuclei. Atrophied GCT (black arrows) and seromucousacini (red arrows) with intracytoplasmic vacuolization. Dilated blood vessels (green arrows) engorged with red blood cells adjacent to the striated ducts (H&E, Orig. Mag. X 400). (4C) showing high magnification of excretory duct (asterisk) with interrupted epithelial lining and stagnant secretions in its lumen. Thick collagen fibers surrounding the excretory duct was noticed (H&E, Orig. Mag. X 1000).
Figure (5): A photomicrograph of rat submandibular salivary gland of control group (G3A) (60 nm Ag NP-treated group for 3 hours) showing:

(5A) seromucous acini (red arrows) with intracytoplasmic vacuolization and some mitotic nuclei. Atrophied GCT (black arrows) with interrupted epithelial lining in certain areas. The epithelial lining cells showed intracytoplasmic vacuolization and pyknosis of some nuclei.

(5B) showing excretory duct (asterisks) with different stainability in their nuclei and retained secretion in their lumen. Note dilated and congested blood vessels (green arrow) engorged with red blood cells. Atrophied GCT (black arrows) was also noticed (H&E, Orig. Mag. X 400).

(5C) A high magnification of the granular convoluted tubules with intracytoplasmic vacuolization and pyknotic nuclei of their epithelial lining cells (H&E, Orig. Mag. X 1000).
Statistical results:

Histomorphometric Analysis (Number of degenerated seromucousacini/ µm²)

Results of histomorphometric analysis are summarized in Table (1) and Figure (7A).

Comparison between groups

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After 3 hours from treatment
The highest mean value was recorded in Group 2 (10 nm Ag NP-treated group) (39±3.54), followed by Group 3 (60 nm Ag NP-treated group) (16±1.58), with the least value was recorded in group 1 (control group) (5±1.58). ANOVA test revealed that the difference between exposure times was statistically significant (p=0.00). Tukey’s post hoc test revealed a significant difference between each 2 groups.

After 6 hours from treatment
The highest mean value was recorded in Group 2 (10 nm Ag NP-treated group) (64±10), followed by Group 3 (60 nm Ag NP-treated group) (21±4), with the least value was recorded in group 1 (control group) (5±1.58). ANOVA test revealed that the difference between exposure times was statistically significant (p=0.00). Tukey’s post hoc test revealed a significant difference between each 2 groups.

Quantitative real time -PCR molecular assessment (GPx mRNA gene expression)
qRT-PCR molecular assessment (GPx mRNA gene expression) is summarized in Table(1) and Figure (7B).

Comparison between groups
After 3 hours from treatment
The highest mean value was recorded in group 1 (control group) (1.01±0.01), followed by Group 3 (60 nm Ag NP-treated group) (0.41±0.1), with the least value was recorded in Group 2 (10 nm Ag NP-treated group) (0.28±0.06). ANOVA test revealed that the difference between exposure times was statistically significant (p=0.00). Tukey’s post hoc test revealed a significant difference between each 2 groups.

After 6 hours from treatment
The highest mean value was recorded in group 1 (control group) (1.01±0.01), followed by Group 3 (60 nm Ag NP-treated group) (0.34±0.11), with the least value was recorded in Group 2 (10 nm Ag NP-treated group) (0.25±0.02). ANOVA test revealed that the difference between exposure times was statistically significant (p=0.00). Tukey’s post hoc test revealed no significant difference between group 2 and group.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control group)</th>
<th>Group 2 (10 nm Ag NPs-treated group)</th>
<th>Group 3 (60 nm Ag NPs-treated group)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Mean value of the seromucousacini number/μm²</td>
<td>5.00±1.58</td>
<td>5.00±1.58</td>
<td>39.00±3.54</td>
<td>64.00±10.00</td>
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<tr>
<td>Mean value of the GPx mRNA gene expression</td>
<td>1.01±0.01</td>
<td>1.01±0.01</td>
<td>0.28±0.06</td>
<td>0.25±0.02</td>
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Table (1): Descriptive statistics of both histomorphometric analysis (seromucousacini number/μm²) and qRT-PCR molecular assessment (GPx mRNA gene expression) between groups (ANOVA test)
IV. DISCUSSION

Nanotechnology has gained a great interest in the medical field as it offered exciting possibilities in the medical applications. Among the numerous engineered nanoparticles, Ag NPs have gained a high interest in many fields due to their superior properties.

The present study was conducted to investigate the possible toxic effect of different particle sizes of Ag NPs at different exposure times on the submandibular salivary glands of adult albino rats. The submandibular salivary gland was examined histologically, histomorphometrically and by qRT-PCR.

The results of the current investigation showed that intraperitoneal injection of Ag NPs of two different sizes (10 and 60 nm) for two different time of exposure (3 and 6 hours) resulted in histological alterations in the rat submandibular salivary gland. However, such alterations were more aggravated in the smaller particle size (10 nm)-treated group as compared to the larger particle size (60 nm) -treated group, as well as in the longer time of administration (Ag NP-treated groups for 6 hours as compared to those for 3 hours).

These histological findings of the ongoing study were in accordance with Taha and Said (2019) who found degenerative change in the parotid gland of 20 nm Ag NP -treated rat in the form of atrophied serous acini that lost their normal architecture, hyper chromatic and pleomorphic nuclei, inflammatory cell infiltration, dilated blood vessels, and thick connective tissue septa. They explained that Ag NPs could induce pro-inflammatory cytokines which resulted in massive amount of free radicals in cells through increase the expression of NADPH oxidase family, which might cause cytotoxicity, cellular dysfunction, and DNA damage.

The findings of the present study were in accordance with Zaki et al. (20 20) who reported the cytotoxic effect of Ag NPs on circumvallate and filiform papillae of albino rats. They found degeneration of some taste cells, while some taste buds appeared atrophied with irregular outline. The von Ebner salivary glands serous acini appeared amalgamated with ill-defined cell borders.

Bio et al. (2018) reported histopathological changes of albino mice ovaries after administration of Ag NPs for 21 days. They reported these changes in form of fatty degeneration, pyknosis, necrosis of the nuclei of the follicular cells, as well as congestion in the blood vessels.

The revealed histological findings of the present investigation showed variable degrees of histological alterations which were more pronounced in group 2 (10 nm Ag NP-treated group) as compared to group 3 (60 nm Ag NP-treated group). The possible explanation to these findings might be that the smaller sized Ag NPs (10 nm) could internalize faster into the cells due to their larger total surface area and increased particle number for the same mass and more surface energy than larger one (60 nm) and performed their cytotoxicity and genotoxicity on the glandular elements Zhang et al. (2018).

Wang et al. (2013) showed that PVP-coated 20nm Ag NPs induced more cellular toxicity than larger particles (110 nm) through generated acute neutrophilic inflammation in the lungs of Ag NP-exposed mice. They
explained that small size Ag NPs were able to cross the basement membrane and accumulated in lung tissues, causing their cytotoxic effects.

Cho et al. (2018) reported in their investigation that the different incidences of lesions in the liver of 60 and 100 nm Ag NP-treated mice might be due to lower uptake or ROS generation rates than those in the 10 nm Ag NP-treated mice. However, high reactivity and easy transportability of small Ag NPs (10 nm) into the cell might lead to such high toxicity.

Stensberg et al. (2011) postulated in their study the acute toxicity of Ag NPs to zebrafish embryos. They hypothesized that the toxicity of Ag NPs was due to the dissolution of Ag NPs and released silver ions. They reported that small sized Ag NPs have high dissolution rate as compared to the larger size, subsequently, more release of silver ions as well as more toxic effect.

The histological results of group 2 (10 nm Ag NP-treated group) of the present study appeared amalgamated and atrophied with ill-defined cell border. Of the present study, Zaki et al. (2020) also found in their study that the serous acini of rat’s von Ebner salivary gland after treatment with Ag NPs showed atrophy of some and degeneration in others. A finding that Ag NP’s toxicity could be due to the induction of ROS and the oxidative stress in the cells.

In the current study, examination of Ag NP-treated group revealed intracytoplasmic vacuolization of both seromucousacinar and ductal cells as compared to the control group. Fabrega et al. (2011) reported in their study zebrafish’s gills that the intracytoplasmic vacuolization might be due to disturbance of the epithelial lining cell membrane function owing to Ag NP’s toxicity that blocked the Na’ K’ ATPase and subsequently, affected the Na’Cl- ions regulation across the gills, influx of water and Na’ ions, causing cellular swelling.

Loghman et al. (2012) suggested that the cytotoxicity of Ag NPs on hepatic tissue of chickens was due to the reduction of mitochondrial function, and that the presence of intracytoplasmicvacuolations were due to mitochondrial damage (apoptosis). A finding that could be a possible cause for those revealed in the present study.

Almansour et al. (2016) observed intracytoplasmic vacuolization of rat’s hepatic cells and referred it as leakage of lysosomal hydrolytic enzymes due to Ag NP exposure that led to cytoplasmic degeneration.

The histological results of present study in Ag NP-treated groups revealed interrupted cell membrane of the seromucousacinar and ductal cells and degenerated in some areas. These findings were more apparent at group 2 than group 3. Wang et al. (2017) reported bacterial cell membrane disruption after Ag NP treatment. They attributed that the interaction of Ag NPs with the phospholipid molecules and the proteins in the cell membrane might cause cell membrane damage, this finding could be a possible cause for those reported in the present study.

Kim et al. (2008) reported that Ag NP-treated dermatophytes showed cell membrane damage. They suggested the chelating action of Ag ions with thiol group containing molecules in the cell membrane, could hinder the enzymatic function of the affected protein, and caused membrane dysfunction and cell membrane deformity, a finding that in accordance with those of the present study.

Zhu et al. (2016) also suggested that the Ag NP induced increase in ROS, and DNA damage that activated a signaling pathway and eventually led to cell death mainly by apoptosis, as finding that is in agreement with those of the present study.

The results of the ongoing research were in similarity with those done by Taha and Said (2019) on parotid gland of rats received orally Ag NPs of size 35 nm. Their histological findings revealed that the acinar cells of parotid gland showed deeply stained, large hyperchromatic nuclei and mitotic figure of the seromucousacinar cells in Ag NP-treated rats. Furthermore, Taghyan et al. (2020) also reported that Ag NPs exposure caused signs of degeneration in the rat parotid gland in the form of pleomorphism, binucleation, and pyknosis- and crescent-shaped nuclei. They suggested these results indicated DNA damage and potential genotoxic effects of Ag NPs.

A noticeable histological finding in the current study was the pyknotic nuclei of the seromucousacinar cells and the ductal epithelial lining cells in group 2 (10 nm Ag NP-treated group) and group 3B (60 nm Ag NP-treated group for 6 hours). A suggestion for these findings was reported by Panzarini et al. (2018) who found that the small-sized Ag NPs (10 nm) could disrupt the nuclear membrane or passed through the nuclear pores, causing...
severe oxidative stresses and DNA damage, while in group 3B, the nuclear damage might be due to the factor of time where more time of Ag NPs exposure on the cells might affect their nucleus causing its dramatic nuclear damage.

Obvious dilated and congested blood vessels were observed adjacent to the striated ducts and in the connective tissue stroma of all the Ag NP-treated groups of the current work. Taha and Said (2019) explained in their study the presence of vasodilatation of the blood vessels engorged with red blood cells in the connective tissue septa of parotid glands of rat after Ag NP treatment as Ag NPs might cause mitochondrial oxidative stress which subsequently caused mitochondrial membrane depolarization that activated endothelium-derived relaxing factors which promoted tissue perfusion and vascular dysfunctions.

The present investigation reported marked fibrosis and edema of the connective tissue septa surrounding the excretory ducts in all Ag NP-treated groups. These findings were in agreement with Lui et al. (2010) who suggested that the oxidative stress caused by Ag NPs might cause increase in the transforming growth factor β which was the most potent profibrogenic cytokine that promoted the fibrosis in most fibrotic diseases.

Ahmed et al. (2016) also reported in their study marked collagen fibers deposition within the alveolar tissue and thickening of interalveolar septa in rats that received Ag NPs as compared to the control group.

The histomorphometry analysis as seen in the present work revealed significant increase of the defected seromucous acini per unit area in the Ag NP-treated groups as compared to the treated group. These findings were in accordance with Taghyan et al. (2020) who revealed in the histomorphometry analysis a highly significant increase in the mean surface area of the defected serous acini of parotid gland in Ag NP-treated rats as compared to the controls.

Throughout the results of the ongoing study, group 2 (10 nm Ag NP-treated group) showed statistically significant increase in the defected seromucous acini per unit area as compared to that of group 3 (60 nm Ag NP-treated group). This finding was in agreement with other studies that considered the size of Ag NP a key factor of their toxicity. Such results indicated the progressive toxic effect of Ag NPs on the seromucous acini which is size- and exposure time-dependent McShan et al. (2014).

In the current study, qRT-PCR results demonstrated statistically significant decrease in the expression of GPx in Ag NP-treated groups (group 2 and group 3) as compared to the control group (group 1). These findings were in accordance with Fang et al. (2019) who speculated in their study a statistically significant decrease in the GPx activity of erythrocytes after Ag NPs exposure. They explained their findings as Ag NP-induced oxidative stress in erythrocytes though direct interaction of Ag NPs with GPx protein molecules where it changed the protein skeleton of enzyme molecules, thus loosening the skeleton structure, unfolding the polypeptide chain, and gradually it could alter the enzymatic structure and function.

Wu and Zhou (2013) concluded that dose dependent decrease in antioxidant activity after Ag NPs exposure suggested an excessive consumption of this antioxidant enzyme in the tissue to overcome the oxidative stresses induced by Ag NPs treatment.

In the present work, the seromucous acinar and some of the ductal cells showed sign of degeneration in form of pleomorphism, mitosis, and pyknosis. Katsnelson et al. (2013) referred to the DNA damage and potential genotoxic effects of Ag NPs. The authors also reported a statistically significant genotoxicity of Ag NPs and DNA damage in various tissues such as liver, spleen, and kidney. El Mahdy et al. (2014) also detected chromosomal aberrations and DNA damage after injecting Ag NPs at different doses intraperitoneal for 28 days in rats.

V. CONCLUSION

According to the results of the present study, the following conclusions could be reached:

- Intraperitoneal injection of Ag NPs induced cytotoxic effect in rat's submandibular salivary gland which was manifested histologically, histomorphometrically, and by qRT-PCR.
- The degree of cytotoxicity was size-and time-dependence.
The cytotoxicity effect of Ag NPs was attributed to oxidative stress through significant decrease in GPx mRNA gene expression.

REFERENCE


