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Silver Nanoparticles Induce Time- and Tissue-Specific Genotoxicity in *Oreochromis niloticus*: Utilizing the Adsorptive Capacities of Fruit Peels to Minimize Genotoxicity

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Abstract

Fish were exposed to a sub-lethal concentration of silver nanoparticles with and without orange and banana peels water treatment for 24, 48, and 96 h. The adsorption of AgNPs on both peels was recognized by scanning electron microscopic, energy dispersive X-ray spectroscopy, and laser ablation imaging. The % of DNA damage in liver and muscle tissues (comet assay) showed significant elevations in all studied groups with the maximum level in liver tissues after 24 h. DNA damage was markedly decreased after 48, and 96 h signifying the presence of an effective repairing mechanism. Micronucleus and nine nuclear anomalies were recorded in the peripheral blood cells. All anomalies were observed in all studied groups with a maximum induction rate after 96 h. of exposure. Based on the % of DNA damage and the frequencies of nuclear anomalies, water treatment with orange and banana peel succeeded to reduce AgNPs-induced genotoxic damage.

Keywords Silver nanoparticles · Genotoxic biomarkers · Comet assay · Micronucleus test · Bioremediation · Fruit peels

The exponential growth of metallic nanoparticles (NPs) and their massive discharge in aquatic bodies have increased the susceptibility of aquatic organisms to unknown threats. The unique features of NPs are the primary explanation of their entrance into many industries and their obvious existence in the aquatic ecosystem (Nnamdi et al. 2019; Abdel-Khalek et al. 2021). Silver nanoparticles (AgNPs) are the most widely utilized NPs due to their various and unique characteristics. The production and usage of AgNPs have increased during recent years, estimated as 50% of global products of nanomaterials, and are expecting to rise to 63% by 2024 (Inshakova and Inshakov 2017). AgNPs are among the very attractive groups of nanostructured metals that have promising roles in the medical and industrial sectors due to their distinctive physical, chemical, and biological properties (Hedayati et al. 2019). These NPs are frequently used in several industries like dyes, toothpaste, cosmetics, clothes, shampoos, food complements, and washing machines (Inshakova and Inshakov 2017; Hedayati et al. 2019). The excessive usage of AgNPs is a problematic issue as the nano-scale size, high surface area to volume ratio, positive surface charge, and high bioactive properties have contributed to high toxic potential compared to their micro-sized particles. Owing to their tiny sizes, discharged AgNPs may invade the vital tissues of aquatic organisms making several adverse effects at the cellular and molecular levels. Bressan et al. (2013) reported that AgNPs can bind with membrane proteins and enter the cell by diffusion or endocytosis causing mitochondrial damage, reactive oxygen species (ROS) production, and DNA damage. Moreover, Asharani et al. (2009) suggested that the disturbance in the respiratory chain induced by AgNPs may increase ROS level and interrupt the synthesis of ATP, leading to DNA damage. The comet assay is widely used because it is a very sensitive technique that can measure a variety of DNA lesions in each cell. Thus, this assay has been effectively applied to evaluate DNA damages in fish exposed to various genotoxic agents (Simoniello et al. 2009). Measuring the percentage of migrated DNA from immobilized nuclear DNA can help us to evaluate DNA single and double-strand breaks and alkali-labile sites. On the other hand, further genotoxic effects for chromosomal abnormalities, such as acentric and dicentric

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chromosomal fractures or combinations can be assessed using the micronucleus (MN) test. The induction of MN and other nuclear anomalies were considered a hallmark of clastogenic and aneugenic chromosomal changes. The MN test in the erythrocytes of the fish is generally favored as fish erythrocytes are normally nucleated (Arslan et al. 2015). Bacchetta et al. (2017) reported several genotoxic effects in fish after short-term exposure to sub-lethal concentrations of AgNPs. Therefore, AgNPs should be carefully treated to reach standard values before their release into aquatic environments. Several methods for purifying polluted water are available like ultra-filtration, coagulation, precipitation, membrane separation (Alyasi et al. 2020). All those techniques are complicated, time-consuming, and expensive. Biosorption "removal or recovery of organic and inorganic substances from solution by biological material such as living or dead microorganisms and their components, seaweeds, plant materials, industrial and agricultural wastes, and natural residues" is one of the most effective, low-cost, and useful processes for metal removal (Fomina and Gadd 2014). Some agricultural waste has a unique structure and chemical characteristics with high levels of cellulose and lignin therefore they can be used as potential adsorbents (Singh et al. 2018). In this context, orange peel (OP) and banana peel (BP) can be modified and converted to be useful biosorbents due to their cellulose and other polysaccharides enrichment, large adsorptive areas, swelling capacities, and excellent mechanical strengths (Kelly-Vargas et al. 2012). As a continuation of the study of Abdel-Khalek et al. (2021), the present study aims to (1) evaluate the genotoxic impacts of AgNPs on *O. niloticus*; (2) assess the effectiveness of orange and banana peels as biosorbents to reduce the genotoxicity of Ag NPs.

Materials and Methods

Silver nano-powders with 99.5% purity (Product No. 576832) were obtained from Sigma-Aldrich, St. Louis, MO, USA. The characterization of the used AgNPs was done in our previous work Abdel-Khalek et al. (2021) using transmission electron microscopy (FETEM, JEM-2100 F, JEOL Inc., Japan) for actual particle sizes determination (41.3 ± 5.2 nm); dynamic light scattering (DLS, Nano-zeta sizer-HT, Malvern Instrument, UK) to assess the aggregation behavior of AgNPs in water (84.23 nm); Malvern Zeta Sizer Nano ZS instrument to determine zeta potential of the studied NPs (− 13.8 mV). The selected sub-lethal concentration (4 mg/L) of the present work was according to Afifi et al. (2016) and Abdel-Khalek et al. (2021) who reported this concentration as a sub-lethal concentration for *O. niloticus* with eliciting biological effects. The actual concentration of the present work was obtained after dispersing silver NPs in dechlorinated water (pH: 7.4). The nominal concentration of AgNPs in water was 87% ± 2% of the actual concentration as confirmed by inductively coupled plasma (ICP-AES), Thermo Sci, model: iCAP6000 series. The limit of detection was 0.3 µg/L. The procedure blanks (all used reagents without samples) were absorbed along with the measurement process to rectify the background absorption to increase the accuracy of the measuring instrument. Recovery ranges for silver metal (using Lake Superior fish 1946 NIST, National Institute of Standards and Technology, USA) were between 94% and 106%. A standard sample of a specified Ag concentration was used every five samples to assess the correctness and accuracy of the test method. The prepared AgNPs solution was ultrasonicated (BioLogics, Inc., Manassas, VA, USA, 100 W, 40 kHz) for 60 min before use to increase NPs dispersion.

OP and BP were sliced into tiny fragments and washed with distilled water then left for 24 h. in the oven at 80°C. The obtained dry peels were ground to convert peels into powdered particles with 1–5 mm as suggested by Annadurai et al. (2003). The obtained dry particles (of both peels) were used in the bottom of fish aquaria and segregated by a porous mesh, to permit AgNPs movement through it but at the same time without the release of peel powders to water to avoid their intake by fish. The used concentration of the absorbents was 40 mg/L (i.e.: 10 times the used NPs concentration) as recommended by Akpomie and Conradie (2020) who observed that a higher dose of adsorbent materials can provide more active sites for potential interaction with the target contaminants.

From a private fish farm (Kafr El-Sheikh governorate, Egypt) adult male *O. niloticus* were collected as suitable bioindicator in many toxicological works (Abdel-Khalek et al. 2018) with 41.91–50.17 g body weight range and 10–15 cm body length range. The transportation process was done in the early morning in large plastic containers with good aeration conditions. Fish were maintained for 2 weeks in glass aquaria (40 × 70 × 26 cm) with 50 L of aerated, dechlorinated tap water with 7 fish/aquarium. Water parameters were tested regularly and maintained with a constant range during this period (25 ± 1°C for temperature, 7.2–7.4 for pH, and 7.1–7.8 mg/L for dissolved oxygen). Fish began to eat 1 day after the transportation process using commercial pellets (20% crude protein, 4% crude fat, 5% crude fiber, 12% crude ash, and 10% crude moisture). During this period, dead fish or fish with unusual behavior were excluded immediately. Water change was done every day (40% of the water) after the meal and any excess food or wastes were removed using suction pumps.

After the acclimatization period, the experiment was done using 84 healthy fish as shown in Fig. 1.

Both utilized biosorbents (OP and BP) were collected after each study period (24, 48, and 96 h) to confirm the

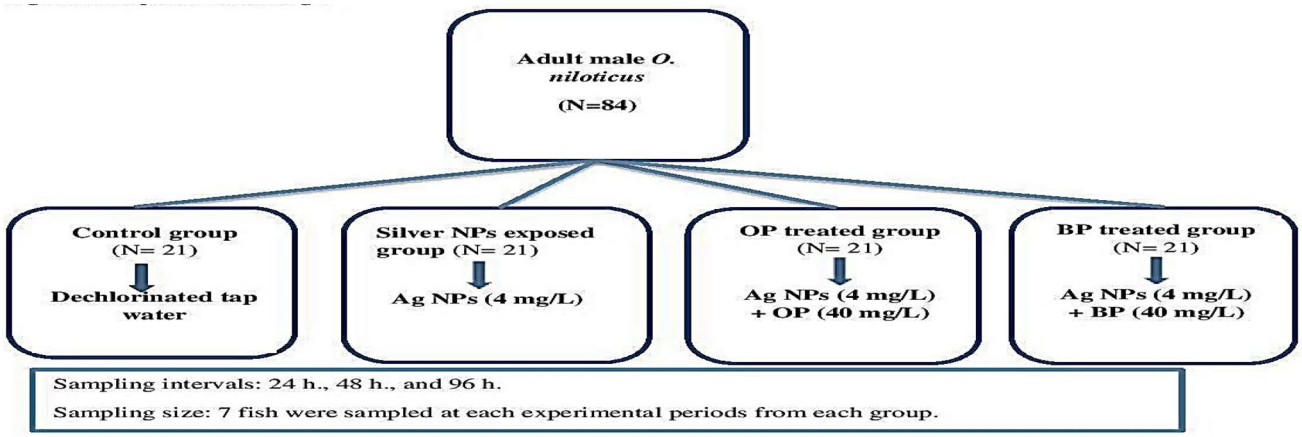

Fig. 1 The experimental design

adsorption of AgNPs on their surfaces using scanning electron microscopic models FEI Inspect S 50-Netherlands and energy dispersive X-ray spectroscopy (EDS) attachment with Bruker AXS-Flash Detector, 410-M-Germany, and laser ablation imaging.

Blood samples from 7 fish from each experimental period/group were collected from the caudal vein using heparinized syringes and clove oil (25 mg/L as an anesthetic agent). Fish were then dissected to isolate liver and muscle tissues for comet assay analysis.

The alkaline comet assay (pH > 13) was conducted as described by Tice et al. (2000) and Recio et al. (2010). In 1 mL cold Hank's balanced salt solution (containing 20 mM EDTA and 10% DMSO; free from Ca^{++} nd Mg^{++}), a tiny fragment of liver and muscle tissues (1 cm^2) was minced into fine pieces. Then 10 µL cell suspension (containing about 10,000 cells) was combined with 80 µL of 0.5% low melting point agarose and distributed on a fully frosted slide which is dipped before in normal melting agarose (1%). The slides (after solidification) were placed in cold lysis buffer (2.5 M NaCl, 100 mM EDTA, and 10 mM Tris, pH 10, with freshly added 10% DMSO and 1% Triton X-100) for 24 h at 4°C in dark conditions. Slides were incubated into a freshly prepared alkaline buffer (300 mM NaOH and 1 m MEDTA, pH > 13) for 25 min at 300 mA and 25 V (0.90 V/cm) to unwind DNA. In an excess amount of 0.4 M Trizma base (pH 7.5), the neutralization of slides was done. Then the slides were fixed using absolute, cold ethanol and kept at room temperature. All slides were stained with ethidium bromide (2 µg/mL) and the extent of migration among cells was analyzed and photographed at ×400 magnification power using Komet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK). Based on the intensity of all tail pixels/the total intensity of all pixels in the comet (% tail DNA), the DNA damage was compared among different groups using the mean of 100 cells/time interval/group.

The induction of MN and other nine deformations were recorded in the peripheral erythrocytes as previously described in Abdel-Khalek (2015). On a clean slide, a blood drop was smeared and fixed using absolute methanol for 10 min. The staining process was done using Giemsa stain (10%) for 10–15 min. then all slides were immersed in distilled water to remove excess stain. For each group, 7 fish were used at each experimental period and 7000 cells (1000 cells/fish) were examined under a light microscope (1000× magnification) for the scoring process. A blind evaluation was done using randomized and encoded slides to reduce the technical variance. The observed anomalies in the present study were: micro-nucleated erythrocyte; bi-nucleated erythrocyte; blebbed nucleus; fragmented nucleus; karyolysis; notched nucleus; erythrocyte with more than one MN; nuclear thread; microcytic cell; vacuolated cytoplasm. The observed anomalies were examined and photographed by ZEISS Primo Star light microscopy with Tucsen IS 1000, 10.0 MP Camera.

The data were represented as mean ± standard error. All data were statistically analyzed using analyses of variance (ANOVA) to estimate the significant differences ($p < 0.05$) among different groups and time intervals. Duncan's multiple ranges were used to examine the homogeneity among different groups as indicated by case letters in the descending order of A, B, C, and D. The statistical analysis was done using Statistical Processor Systems Support, SPSS software, version 20, IBM, Chicago, IL, USA.

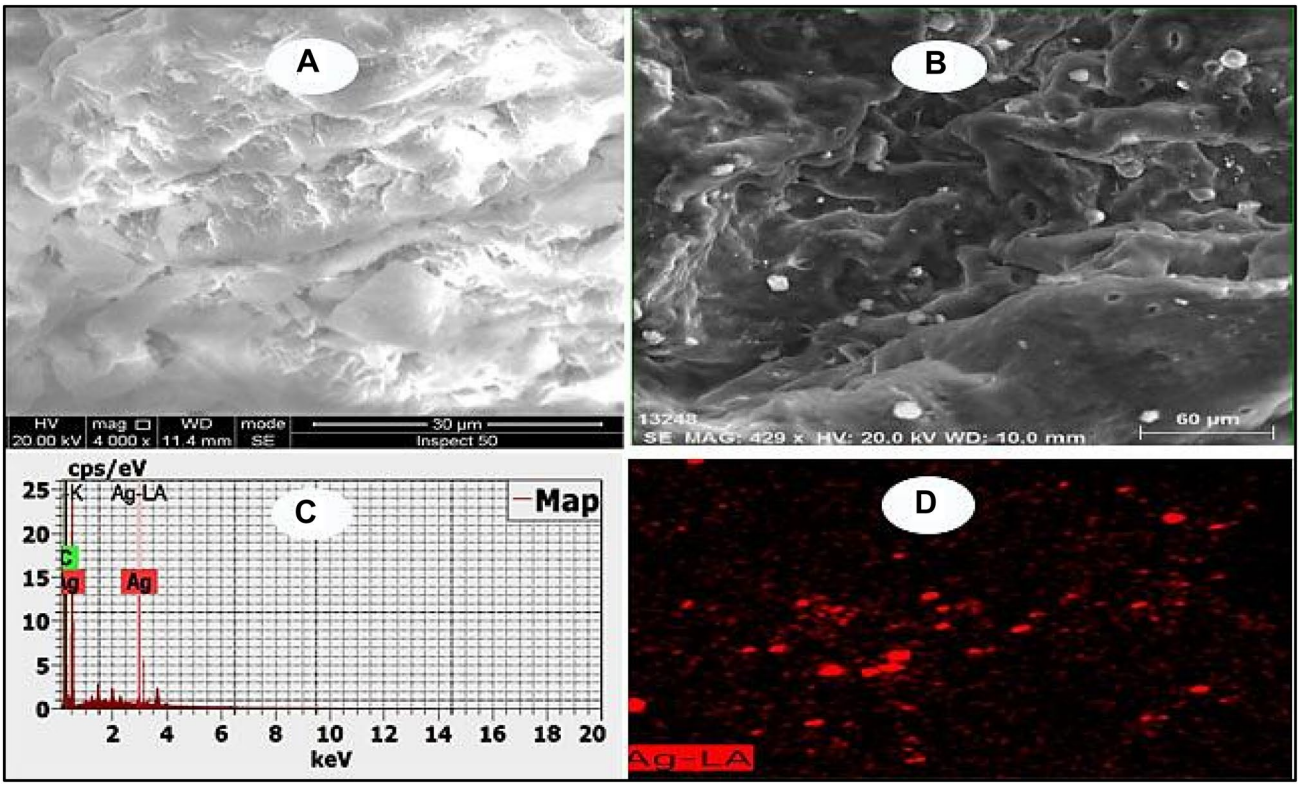

Fig. 2 Representative SEM/EDS spectra and LA imaging of orange peel. **A** Original orange peel; **B** adsorbed AgNPs on the surface of orange peel; **C** EDS analysis of orange peel after adsorption of AgNPs; **D** LA imaging of orange peel showing AgNPs on the surface

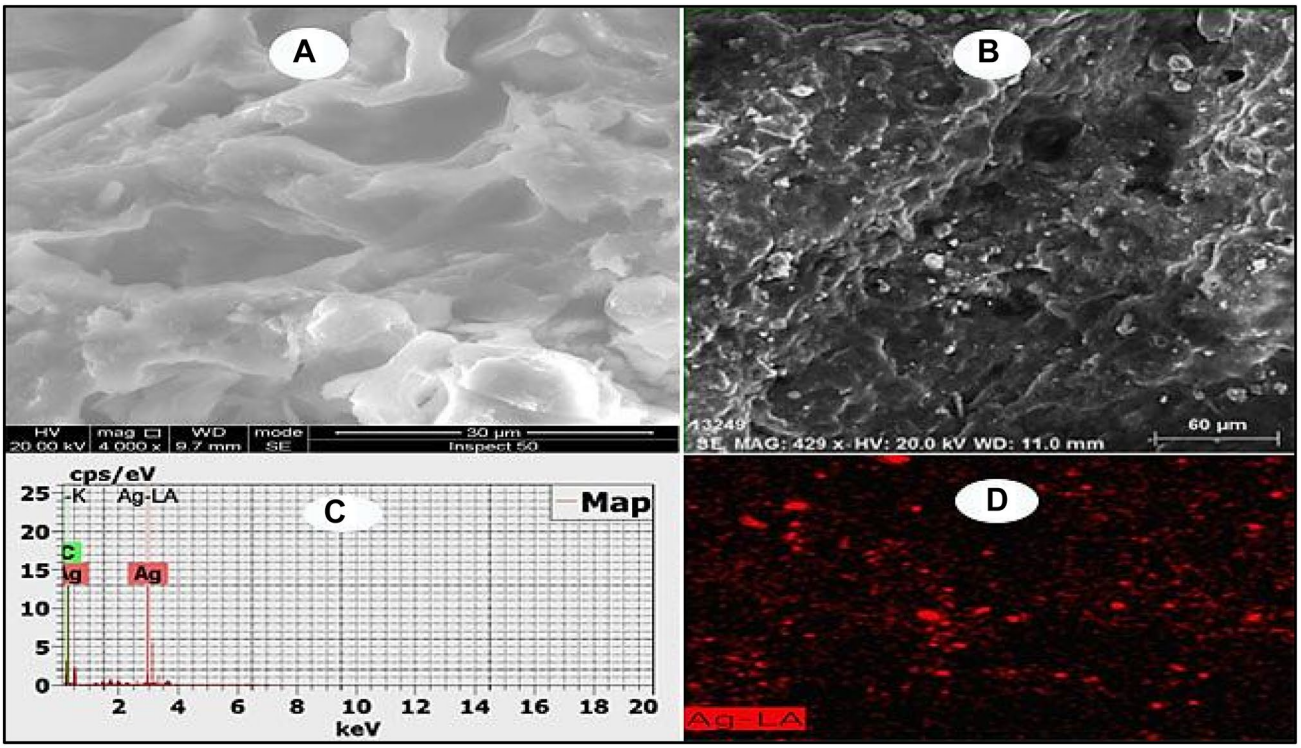

Fig. 3 Representative SEM/EDS spectra and LA imaging of banana peel. **A** Original banana peel; **B** adsorbed AgNPs on the surface of banana peel; **C** EDS analysis of banana peel after adsorption of AgNPs; **D** LA imaging of banana peel showing AgNPs on the surface

Results and Discussion

Figures 2 and 3 showed representative surface analyses of orange and banana peels respectively. As shown in Figs. 2 and 3A, the original peels showed greatly porous and clear surfaces. Both peels after the exposure to AgNPs (Figs. 2B and 3B) displayed partially covered surfaces with circular NPs. The data achieved from energy-dispersive X-ray spectroscopy (Figs. 2 and 3C) and laser ablation imaging (Figs. 2D and 3D), identified the recorded NPs on the surfaces of OP and BP as silver nanoparticles. Depending on the surface analyses of OP and BP, both peels were demonstrated great adsorbents efficiency to Ag NPs. The clearance efficiency of OP and BP is facilitated by their components' which have high adsorption ability (Annadurai et al. 2003). For example, OP is rich in pectin (51.1%), hemicellulose (12.6%), cellulose (11.1%), protein (7.8%), lignin (1.0%), and other constituents in small quantity (Liang et al. 2010). Similarly, BP contains a high source of pectin (10%–21%), hemicelluloses (6.4%–9.4%), cellulose (7.6%–9.6%), lignin (6%–12%), and galacturonic acid (Ahmad and Danish 2018). Oyewo et al. (2018) indicated that the excess amino acid groups in BP increase its potential, in comparison with other agricultural products, to adsorb metal.

Different levels of DNA damages were recorded in the present study as represented in Fig. 4.

Based on the measured % tail DNA, DNA damages in the liver and muscle tissues of all studied fish groups were considerably increased compared to the control groups (Fig. 5). The maximum DNA damage was recorded in hepatic and muscular tissues of AgNPs-exposed fish while the % of DNA damage showed significant decreases after OP and BP water treatment at all studied periods. Comparing the DNA damage within the same group, all studied groups (except muscle cells of AgNPs + BP) showed a marked decrease after 24 h. of exposure signifying the presence of an effective repairing mechanism.

The genotoxic potency of metallic NPs has major environmental concerns due to the ability of these NPs to invade the nucleus and elicit mitotic spindle malfunction, chromosomal breaks, or DNA damage through different mechanisms (Abdel-Khalek et al. 2016). Therefore, NPs-induced genotoxicity should be assessed carefully to protect the next generations from probable genetic disorders. Comet assay was

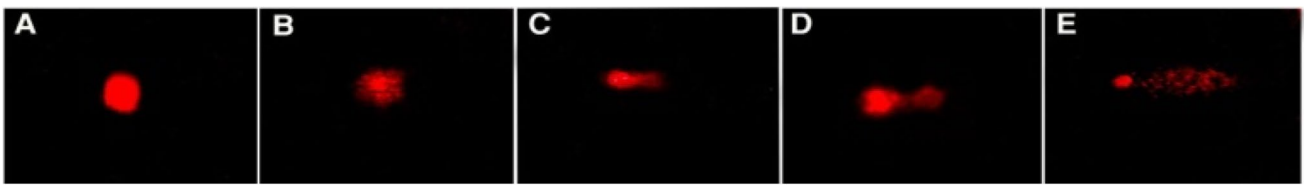

Fig. 4 Demonstrative images for comet assay showing different levels of DNA damage in terms of % of tail DNA. **A** Undamaged cell; **B** slightly damaged; **C** medium damaged; **D** highly damaged; **E** severely damaged

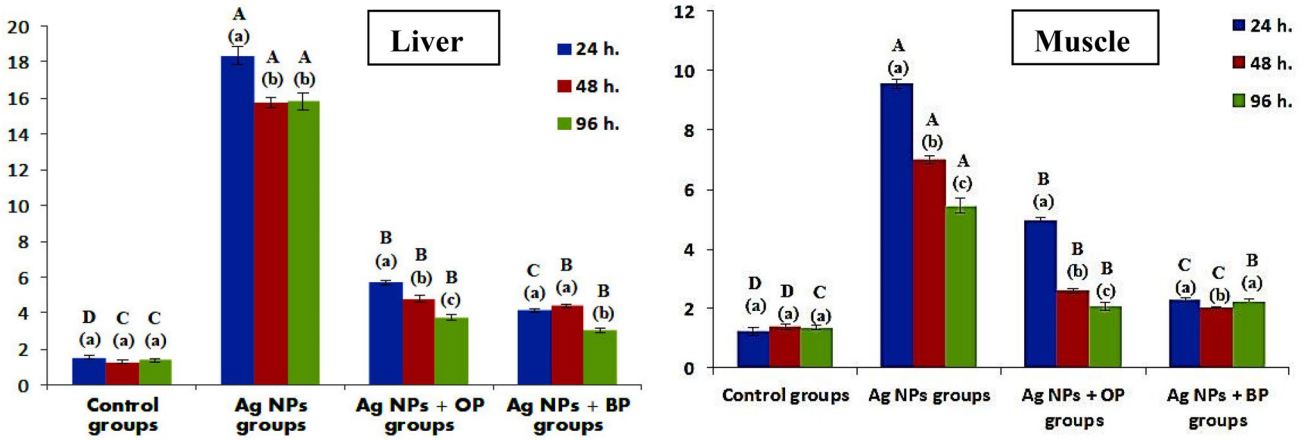

Fig. 5 The %DNA in the comet tails of the hepatic and muscular cells (n = 100 cells) of the studied fish groups after 24 h, 48 h, and 96 h. Data are represented as means of one hundred cells in each time interval of each group ± SE. The small letters represent Duncan's test ($p < 0.05$) between different time intervals within the same group. Columns with the same small letters are not significantly different; otherwise, they do. The capital letters represent Duncan's test ($p < 0.05$) between the same time interval among different groups. Columns with the same capital letters are not significantly different; otherwise, they do. The letters are arranged in descending order as A, B, C, and D

applied to the liver (major detoxification organ) and muscle (edible part) tissues of *O. niloticus* to provide a clear picture of AgNPs-genotoxic effects. Nabiev et al. (2007) identified that nano-sized particles could reach the nucleus through its nuclear pores in a nano-specific pathway and might physically intact with the DNA. Hence the possibility of AgNPs entering the nucleus and cause direct DNA damage is high. The present results showed a tissue- and time-specification of DNA damage with maximum % tail DNA in liver tissues after 24 h. of AgNPs exposure. These observations may be due to the higher tendency of hepatic tissues, compared with muscular tissues, to store and detoxify metallic NPs by various metabolic processes which increase the NPs-hepatic content and consequently the DNA damage (Abdel-Khalek et al. 2016). The genotoxicity was mediated either by direct DNA damage after Ag NPs-DNA interaction or indirectly due to the oxidative damage induced by reactive oxygen species (ROS) induction. Genotoxic substances-DNA interaction can lead to alkaline labile adducts and additional changes which could lead to severe DNA strand damage through enzymatic removal of damaged nucleotides (Simoniello et al. 2009). AgNPs have been recorded to cause genotoxic damage by oxidative stress initiation (Maurer and Meyer 2016). However, stress has been found temporary and reversible (Genter et al. 2012) and can be overcome after the production of antioxidant enzymes (Bacchetta et al. 2017). The activation of different antioxidant components in *O. niloticus* after applying the same conditions of the present study was ensured in the previous work of Abdel-Khalek et al. (2021) using different enzymatic and non-enzymatic biomarkers. These findings are consistent with the results of the present study as the % of DNA damage was significantly reduced at the end of the experimental time indicating the presence of an efficient repairing system and/or activation of a robust antioxidant defense mechanism. To verify the DNA damage and chromosomal abrasions, MN test was applied in the peripheral erythrocytes of the studied fish. As shown in Fig. 6, ten deformations were recorded and their frequencies were compared among different groups.

As represented in Table 1, the different studied groups showed a remarkable increase ($p < 0.05$) in all observed nuclear abnormalities compared to the control groups as represented by capital letters. The maximum induction of all studied nuclear anomalies was observed in AgNPs-exposed fish after 96 h. of exposure. Commonly, the clastogenic damage was time-dependent as nuclear deformations were increased with time and maximized after 96 h. Comparing the frequencies of the studied anomalies of treated and untreated water groups, all recorded nuclear anomalies were markedly decreased especially after BP water treatment.

To draw a complete picture of the genotoxic impacts of AgNPs, chromosomal aberrations as acentric and dicentric chromosomes, chromosomal fusions, and destruction were evaluated using MN test. The current work has assessed MN and other nuclear abnormalities in the erythrocytes of the studied fish as blood is the key route for all the foreign substances. Massarsky et al. (2014) found that erythrocytes of rainbow trout were more sensitive than hepatocytes after 48 h. of exposure to two forms of silver (AgNPs and ions). Several studies showed high frequencies of MN and other nuclear anomalies after metals exposure either in their nano- or bulk-forms (Abdel-Khalek 2016). Based on the recorded nuclear deformations, AgNPs showed clastogenic effects on the studied species and could modify chromatin consistency, alter the function of microtubules and cause chromosomal disruption. Clastogenic agents that entered erythrocytes may trigger spindle defects during anaphase separation and cause chromosomal fragments that cannot be connected to the main nucleus of the daughter cell (Fenech et al. 2011). Furthermore, Khan et al. (2017) related many nuclear defects (for example nuclear bud) to DNA double strand breaks, fragmentation, fusions, or defective chromosomal alignment after the exposure of *Labeo rohita* fish to different doses of AgNPs (17.78 ± 12.12 nm). The recorded intracellular cytoplasmic vacuolation of the present work showed a disturbance in the ionic balance as showed by Aki et al. (2012) who reported this irreversible cytoplasmic vacuolization as an early sign of cell death. Massarsky et al. (2014) showed that AgNPs could increase lipid peroxidation in fish after 48 h. of exposure, and suggested that the generated ROS may be extracellular or close to the cell

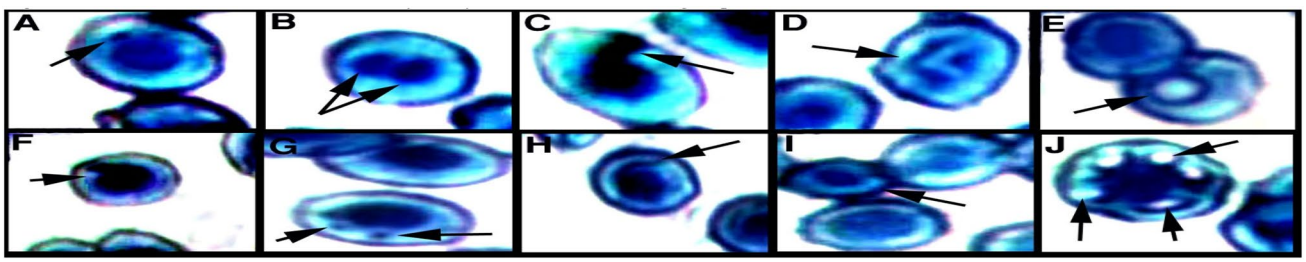

Fig. 6 The recorded nuclear alterations in erythrocytes of the studied fish groups after 24 h, 48 h, and 96 h. **A** Micro-nucleated erythrocyte; **B** bi-nucleated erythrocyte; **C** blebbed nucleus; **D** fragmented nucleus; **E** karyolysis; **F** notched nucleus; **G** erythrocyte with more than one MN; **H** nuclear thread; **I** microcytic cell; **J** vacuolated cytoplasm. ×1000 magnification

Table 1 The recorded nuclear alterations (n = 7000 cells; 1000 cells for 7 fish) in erythrocytes (‰) of the studied fish groups after 24 h, 48 h, and 96 h

	24 h	48 h	96 h	p_f (time)
Micronucleus (MN)				
Control groups	3.0a ± 0.44C	2.57a ± 0.53C	2.0a ± 0.44D	> 0.05
AgNPs exposed groups	30.29b ± 2.40A	47.71a ± 4.93A	47.0a ± 2.88A	< 0.05
AgNPs + OP exposed groups	15.14b ± 1.78B	20.14b ± 2.50B	27.71a ± 2.76B	< 0.05
AgNPs + BP exposed groups	10.71ab ± 0.99B	8.57b ± 0.78C	12.57a ± 1.17C	< 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Bi-nucleated cell				
Control groups	3.14a ± 0.55C	2.71a ± 0.42D	2.57a ± 0.43D	> 0.05
AgNPs exposed groups	36.71b ± 2.26A	43.57b ± 4.59A	58.86a ± 3.37A	< 0.05
AgNPs + OP exposed groups	18.57c ± 1.48B	24.71b ± 1.97B	30.86a ± 1.65B	< 0.05
AgNPs + BP exposed groups	15.43a ± 1.043B	16.14a ± 1.10C	18.43a ± 0.89C	> 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Blebbed nuclei				
Control groups	2.57a ± 0.57C	2.43a ± 0.48C	2.71a ± 0.42C	> 0.05
AgNPs exposed groups	24.71b ± 3.74A	33.71ab ± 5.03A	40.86a ± 6.12A	> 0.05
AgNPs + OP exposed groups	12.0b ± 1.72B	16.14ab ± 2.58B	21.71a ± 2.83B	< 0.05
AgNPs + BP exposed groups	6.43ab ± 0.61BC	5.57b ± 0.72C	8.57a ± 1.11C	> 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Fragmented nucleus				
Control groups	3.0a ± 0.38D	2.86a ± 0.40C	3.0a ± 0.49C	> 0.05
AgNPs exposed groups	27.57b ± 2.46A	37.14ab ± 3.38A	45.0a ± 4.19A	< 0.05
AgNPs + OP exposed groups	17.29b ± 1.27B	23.29ab ± 2.25B	28.29a ± 2.37B	< 0.05
AgNPs + BP exposed groups	9.1a ± 0.96C	9.0a ± 1.11C	9.43a ± 0.72C	> 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Karyolysis				
Control groups	2.86a ± 0.51B	2.29a ± 0.47C	2.43a ± 0.43C	> 0.05
AgNPs exposed groups	23.43b ± 2.36A	31.86ab ± 3.19A	38.57a ± 3.83A	< 0.05
AgNPs + OP exposed groups	6.14b ± 0.74B	8.43b ± 1.27B	13.0a ± 2.0B	< 0.05
AgNPs + BP exposed groups	6.14a ± 0.70B	5.29a ± 0.81BC	7.29a ± 0.97BC	> 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Notched nucleus				
Control groups	3.71a ± 0.61C	2.57a ± 0.48C	2.57a ± 0.69D	> 0.05
AgNPs exposed groups	29.29c ± 1.81A	39.71b ± 2.58A	48.0a ± 3.12A	< 0.05
AgNPs + OP exposed groups	10.43b ± 0.72B	13.71b ± 1.15B	19.57a ± 1.86B	< 0.05
AgNPs + BP exposed groups	9.14a ± 0.67B	9.43a ± 0.72B	11.86a ± 1.32C	> 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Cell with more than one MN				
Control groups	0.29a ± 0.18C	0.43a ± 0.20D	0.71a ± 0.29D	> 0.05
AgNPs exposed groups	15.0b ± 2.02A	20.14b ± 2.62A	28.86a ± 2.55A	< 0.05
AgNPs + OP exposed groups	5.57c ± 0.92B	13.29b ± 1.34B	19.71a ± 1.96B	< 0.05
AgNPs + BP exposed groups	5.57b ± 0.99B	6.86b ± 0.67C	10.14a ± 0.77C	< 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Nuclear threads				
Control groups	1.57a ± 0.37C	1.0a ± 0.31C	1.43a ± 0.37C	> 0.05
AgNPs exposed groups	7.57b ± 1.11A	10.0b ± 1.75A	17.0a ± 3.21A	< 0.05
AgNPs + OP exposed groups	3.57b ± 0.78B	6.57b ± 0.92B	11.86a ± 2.57AB	< 0.05
AgNPs + BP exposed groups	9.29a ± 1.30A	9.71a ± 0.87AB	10.0a ± 0.76B	> 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Microcytic cell				
Control groups	5.14a ± 1.47C	5.57a ± 0.95B	5.29a ± 1.02C	> 0.05
AgNPs exposed groups	20.0b ± 2.39A	23.43b ± 2.35A	33.86a ± 2.75A	< 0.05

Table 1 (continued)

	24 h	48 h	96 h	p_f (time)
AgNPs + OP exposed groups	12.57a ± 1.51B	19.0a ± 3.09A	18.29a ± 1.82B	> 0.05
AgNPs + BP exposed groups	3.29c ± 0.68C	5.29b ± 0.60B	7.43a ± 0.69C	< 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	
Vacuolated cytoplasm				
Control groups	1.86a ± 0.34B	1.86a ± 0.46B	2.29a ± 0.52C	> 0.05
AgNPs exposed groups	21.29b ± 1.73A	28.57ab ± 2.99A	32.29a ± 2.53A	< 0.05
AgNPs + OP exposed groups	15.14a ± 1.64A	19.71a ± 1.67A	21.43a ± 2.59B	> 0.05
AgNPs + BP exposed groups	2.43b ± 0.37B	3.86a ± 0.51B	4.86a ± 0.51C	< 0.05
p_f (groups)	< 0.05	< 0.05	< 0.05	

Data are represented as means of seven samples in each time interval of each group ± SE. The small letters represent Duncan's test ($p < 0.05$) between different time intervals within the same group. Rows with the same small letters are not significantly different; otherwise, they do. The capital letters represent Duncan's test ($p < 0.05$) between the same time interval among different groups. Columns with the same capital letters are not significantly different; otherwise, they do. The letters are arranged in descending order as A, B, C, and D.

membranes affecting their integrity and lead to a high intracellular and intranuclear invasion rate of NPs

Structural chromosomal damages require DNA double-strand breaks, replication of damaged DNA, or inhibition of DNA synthesis. Costa et al. (2015) reported an association between DNA damage (based on the comet assay parameters) and chromosomal deformities due to the progressive increase in chromosome instability and the inefficiency of DNA repair mechanisms, which may result in the accumulation of genetic lesions. The significant reduction in the recorded genotoxic and clastogenic effects after water treatment with OP and BP is consistent with the assumption of the current study and supports the efficacy of both peels to adsorb AgNPs from water and reduce their bioavailability. The large surface areas, high swelling abilities, richness in cellulose, pectin, and other compounds that have high adsorption ability are the key factors that limiting AgNPs bioavailability in water and reducing their genotoxic effects. Moreover, the negative surfaces of both fruit peels when pH exceeds 7 were recorded by Pathak et al. (2017), so the electrostatic attraction between the negatively charged adsorbent and the cationic ions (Ag^+) can also improve the effectiveness of silver ions removal. Further studies with different absorbents and doses are needed to mitigate the toxicological impacts of NPs and improve the health status of fish.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Consent to Participate All authors read and approved the final manuscript.

Consent for Publication Not applicable.

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