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Mitigation of Genotoxic and Cytotoxic Effects of Silver Nanoparticles on Onion Root Tips using some Antioxidant Scavengers

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THE TARGET of this study is to use *Allium cepa* roots as model to evaluate the potential use of three antioxidants scavengers [vitamin C (VC), vitamin E (VE) and butylated hydroxytoluene (BHT)], either singly or in combination, in mitigation of genotoxic and cytotoxic effects of silver nanoparticles (AgNPs). The results indicated that AgNPs manifested mitodepressive and genotoxic symptoms, represented as decrease in mitotic index (MI), elevated total chromosome aberrations (TCA) and tail DNA (tDNA) associated with oxidative stress markers represented by increase in malonaldehyde (MDA) and hydrogen peroxide (H_2O_2) contents. Each of VC, VE and 1:1:1 mix of VC, VE and BHT ameliorated the harmful effects of AgNPs with best performance recorded for VE. Though, BHT decreased AgNPs-generated MDA and H_2O_2; it enforced the mitodepressive and genotoxic properties associated with application of nanoparticles. The results also reflected superiority of pretreatment over co-treatment with antioxidants in mitigation of the AgNPs-associated hazards.

Keywords: Antioxidants, Chromosome aberrations, Comet assay, Mitotic index, Onion, Silver nanoparticles.

Introduction

Nowadays, metallic nanoparticles (NPs) have great attention due to their new physico-chemical characteristics compared with those of the corresponding bulk-sized metals (Khatoon et al., 2017; Fouad & Hafez, 2018a). Such characteristics afford them bi-toxicity of both metal and NPs, which trigger an alert to the world toward their ecological risks (Anjum et al., 2013).

Among metal-NPs, silver nanoparticles (AgNPs) are presumed to spread in the environment due to their expanding in divergent applications. They are used as herbicide (Eldarier et al., 2020), in crop disease protections to manage insects, viruses and microbial pathogens (Khan & Rizvi, 2014; Mohy El-Din & El Said, 2016; Prasad et al., 2017a; Gupta et al., 2018) and for the amelioration of yield, efficiency and sustainability of agricultural crops (Thuesombat et al., 2014; Prasad et al., 2017b). As plants are the main motifs of ecosystem; they can unfortunately bio-accumulate and enter AgNPs into the food web to be transferred to herbivores and omnivores (Nair et al., 2010; Anjum et al., 2013; Saha & Gupta, 2017). This is why several concerns have been reported regarding their prospective impacts on environment, safety and health care (Ma et al., 2010; Pokhrel & Dubey, 2013; Yu et al., 2013; Korani et al., 2015; Lee & Jun, 2019).

Reports have shown that AgNPs can be easily absorbed by plants, then translocated through their cells and consequently transacted with their cellular components and organelles provoking redox-signaling avalanche of free radicals, reactive oxygen species (ROS) (Kumari et al., 2009, 2011; Qian et al., 2013; McShan et al., 2014). The disproportion between the generated ROS and the stimulated antioxidant defense compounds causes oxidative stress that assaults biomolecules like lipids, proteins and DNA (Akeem et al., 2011; Moucheshi et al., 2014). This cyto-genotoxicity upshot on plant cells may increase the recurrence of chromosomal aberrations, cell death and decrease the mitotic index (Babu et al., 2008; Kumari et al., 2009; Stampoulis et al., 2009;

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Panda et al., 2011; Patlolla et al., 2012; Tanti et al., 2012; Fouad & Hafez, 2018b). The severe toxicity of AgNPs to the plant depends on their size, and concentration, chemical properties, the coating and the carrier used as well as the plant species (Anjum et al., 2013; Thuesombat et al., 2014; Saha & Gupta, 2017).

Plants can overcome the oxidative damages caused by AgNPs toxicity to certain limits via their natural antioxidant defense mechanisms (Moucheshi et al., 2014). Recently, investigations were carried on to find double-edged mechanisms ~~to~~ that detoxify AgNPs and preserve their exclusive properties for the great of human progress without wrangling (Gupta et al., 2018). So to maintain, minimize or avert this cyto-genotoxicity, the defense must be boosted by supplying enough antimutagenic compounds such as phenolics and vitamins. These substances are reputed to have antioxidant activity (Hoda et al., 1991; Bronzetti et al., 2001; Cai et al., 2001; Cai et al., 2004) that procure them the ability to protect cells through their efficacy as reducing agents and free radical scavengers (Carr & Frei, 1999).

Vitamin C (VC), Vitamin E (VE) and 3,5-di-tert-butyl-4-hydroxytoluene (BHT) were chosen for this study for their scavenging activities. VC acts as a natural antioxidant, fortifying the cells from the harmful effects of ROS through its reducing ability (Carr & Frei, 1999; Hacisevki, 2009) and also helps to activate VE (Sies & Stahl, 1995). VE is a robust biological antioxidant (Traber, 2007) that sustains normal mitotic division and preserves the integrity, elasticity and function of the cell plasma membranes (Skrzydlewska et al., 2001; Coulter et al., 2006). BHT is a synthetic phenolic antioxidant used as safe food additive and preservative in some countries as USA, Europe, India (Kahl & Kappus, 1993; Lanigan & Yamarik, 2002). It acts as reducing agent as it reacts slowly with free radical to stop the generation of ROS produced from oxidative stress (Papas, 1993; Lambert et al., 1996).

Vitamin C (VC) and Vitamin E (VE) In addition, VC and/or VE are also used in agriculture as natural foliage spraying agents to improve the productivity of some crops under non stress condition (Nosser, 2011; Shabana et al., 2015) or to alleviate the biochemical and physiological damages performed by stresses (Darvishan et al., 2013; Salama et al., 2014; Hussein & Alva, 2014; Semida et al., 2016; Sadiq et al., 2017). Both vitamins are known as natural antioxidants, protecting the cells against the harmful effects of ROS (Carr & Frei, 1999; Traber, 2007; Hacisevki, 2009). In addition to natural antioxidants, 3,5-di-tert-butyl-4-hydroxytoluene (BHT) is a synthetic phenolic antioxidant used in agriculture as a stabilizer to increase the efficiency of organic biocides (Schultz et al., 2006; Hsu et al., 2007; Nesci et al., 2011; Khaskheli et al., 2014). On the other hand, BHT is used as a stabilizer to increase the efficiency of organic biocides, like pesticides and fungicides, to control serious agricultural diseases due to its lipophilic, low degradation and non-volatile properties, so its majority would stay stacked to crops (Schultz et al., 2006; Hsu et al., 2007; Nesci et al., 2011; Khaskheli et al., 2014).

Allium cepa L., commonly known as onion, is a popular, efficient, inexpensive and reliable plant used for cyto-genotoxicity screening of water-soluble and non-soluble chemical compounds in the environment. This test was approved by the international program on chemical safety (IPCS), for the *in situ* evaluation and monitoring of potential bio-hazardous substances, as AgNPs, on both mitotic division and chromosomes (WHO, 1985; Kumari et al., 2009; Khanna & Sharma, 2013; WHO/IPCS, 2018). Some reports were gathered dealing with AgNPs and their cyto-genotoxicity towards cell division and chromosomes using this international assay (Babu et al., 2008; Kumari et al., 2009; Panda et al., 2011; Ghosh et al., 2012; Tanti et al., 2012; Fouad & Hafez, 2018b). While, other studies correlated the AgNPs-cytotoxicity on onions with the DNA damages produced by ROS using comet analysis (Kumari et al., 2011; Panda et al., 2011; Ghosh et al., 2012). Thus, this study aimed to investigate the potential uses of VC, VE and BHT, singly or in combinations, in mitigation of the cytogenetic impacts of AgNPs on the root tips of *Allium cepa*.

Materials and Methods

Pant material

Common *Allium cepa* L. bulbs (2n= 16) were kindly afforded from the Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt. Healthy uniform young bulbs were selected and adopted for culturing. The selected bulbs were peeled carefully from their outer dried scales without damaging the primordial roots and then nurtured in 50ml beakers filled with

distilled water (DW). All cultures (triplicates) were maintained in dark at 25±2ºC for 48hrs, with renewing the DW twice a day. When the newly formed roots were 2.5cm in length, bulbs were ready for treatments and DW was substituted with treatment solutions.

AgNPs characterization

AgNPs were commercially acquired from Nanotech, Cairo, Egypt, as PVP-coated particles dispersed in deionized water. The roughly spherical NPs with an average size of 20-30nm were viewed with transmission electron microscope [JEOL (JEM-1400)] (Fig. 1), in Electron Microscopy Lab., Faculty of Agriculture Research Park (FARP), Cairo University. The treatment concentration was pertly prepared by diluting the AgNPs stock solution (1000mg/L) as reported by Fouad & Hafez (2018b).

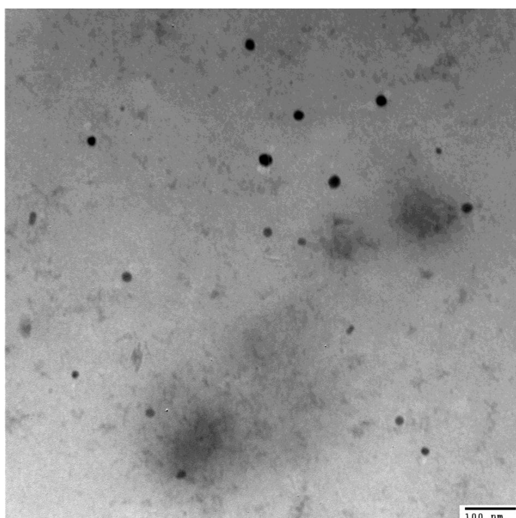

Fig. 1. TEM image showing size and shape of AgNPs.

Chemicals

Butylated hydroxytoluene (BHT, $C_{15}H_{24}O$, Sigma), Vitamin C (Ascorbic acid, VC, $C_6H_8O_6$, Merck) and vitamin E solution (α-Tocopherol, VE, $C_{29}H_{50}O_2$, Merck) were used as antioxidant scavengers. Experimental doses (300 and 600mg/L) were freshly diluted from stock solutions (1000mg/L) and were selected from our preliminary pilot experiments.

Treatments

Three experimental groups were constructed to evaluate the alleviation capability of the antioxidants (VC, VE, BHT and a 1:1:1 mixture of them) towards the cyto-genotoxicity of 15mg/L AgNPs for 9hrs, concluded from our previous study (Fouad & Hafez, 2018b). The first was performed to study the effect of antioxidants by soaking the onion root tips for 24hrs in the antioxidants (300 and 600mg/L) then placed in DW for 9hrs. The same design was used in the second experiment except replacing DW with 15mg/L AgNPs to evaluate the protective ability of antioxidants against AgNPs. In the third group, the emerging roots were soaked for 24hrs in DW then exposed simultaneously to 15mg/L AgNPs and antioxidant treatments as described in the previous experiments. Positive control samples were done by soaking the onion root tips in DW for 24hrs then 15mg/L AgNPs for 9 h, while negative control was carried out by continuous exposure to DW for 24 plus 9hrs. Root tip samples from each treatment were excised, gathered, washed thoroughly with DW, and placed on blotting paper before cytological analysis.

Cytological analysis

At the end of the treatments, the collected root tips from each treatment were fixed separately in ethanol-glacial acetic acid mixture (3:1, v/v) for 48hrs. Then, the fixative was replaced with ethanol (70%, v/v) and finally stored in refrigerator at 4ºC for 48hrs. Just before examination, fixed tips were hydrolyzed in 1N HCl at constant 60ºC for 4-5min. The hydrolyzed-tips were washed with DW and then placed in Feulgen stain for an hour. The intensely stained meristematic area was dissected and squashed in presence of few drops of 45% acetic acids. Five thousand cells per treatment were examined for chromosomal aberrations by light microscope (Leica DM750) at 1000x magnification and photographically recorded using Digital Camera (Leica ICC50 HD). To assess the outcome of the treatments on cell divisions, mitotic indices (MI) and total chromosome aberrations (TCA) were calculated; where MI was estimated as % dividing cells from total cells and TCA was evaluated as % of dividing cells.

Comet assay

DNA damage level was estimated in tissues of controls and treated onion root tips using alkaline comet assay as described by Tice et al. (2000) with minor modifications. A piece of each root tissues was homogenized gently into cold mincing solution to release the nuclei. The mincing solution consists of Hanks' balanced Salt Solution (HBSS) without calcium and magnesium

cations (Sigma) supplemented with 20mM EDTA and 10% Dimethylsulphoxide (DMSO). 10µl aliquot of cell suspension containing about 10000 cells was shuffled with 75µl (0.5%) low melting point agarose (Sigma) and spread on a frosted glass slide pre-immersed in 1% normal melting agarose. Subsequently, the solidified slides were nursed for 24hrs in chilled lysis buffer (2.5M NaCl, 100mM EDTA, and 10mM Tris, pH 10) freshly supplied with 10% DMSO and 1% Triton X-100 at 4ºC in dark. Next, the slides were kept for 20min in fresh alkaline buffer (pH 13): 300 mM NaOH and 1mM EDTA. The uncoiled DNA was electrophoresed for 20min at 25V (0.90 V/cm) and 300mA. The products were neutralized in 0.4M Trizma base and finally fixed in chilled absolute ethanol. The slides were air dried and stored at room temperature until they were analyzed. For each sample and controls, the extent of DNA fragments departure from the nucleoid was documented by capturing and scoring image of 50 cells stained with ethidium bromide (×400 magnification) using Komet 5 image analysis software established by Kinetic Imaging, Ltd (Liverpool, UK). The expanse of DNA damage was evaluated by calculating % tail DNA (tDNA).

Lipid peroxidation

Lipid peroxidation was assayed by analyzing malonaldehyde (MDA) formation using thiobarbituric acid (TBA) technique as reported by Stewart & Bewley (1980). Liquid nitrogen-powdered root tips, of controls and treatmentss were homogenized in Tris–HCl buffer (100mM, pH 7.4) containing PVP (1.5% m/v). All homogenates were filtered and then centrifuged at 10000 *g* for 20min. Each 1 ml supernatant was combined with 4ml TBA (0.5% m/v) dissolved in 20% (m/v) trichloroacetic acid solution. Then the samples were incubated at 90ºC for 30min. After cooling the tubes, the samples were centrifuged at 10000*g* for 20min. Absorbance of all supernatants were recorded at 532 and 600nm (nonspecific absorbance at 600nm was subtracted) and finally the MDA content was calculated as nmol/g fresh weight using its extinction coefficient (155mM^{-1} cm^{-1}).

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) content in control and in treated-root tips was determined according to Loreto & Velikova (2001). Root tips (0.5g) were homogenized with 2.5ml of 0.1% (w/v) freshly prepared trichloroacetic acid in an ice bath. The homogenate was centrifuged for 15 min at 4°C and 12,000 g. The supernatant (0.5 ml) was collected in a new tube and mixed with 0.5 mL of potassium phosphate buffer (10mmol/L, pH 7.0) and 1 mL of KI (1 M). The absorbance of the supernatant was recorded at 390nm. The amount of H_2O_2 (nmol/g of fresh weight) was estimated by comparing with a standard curve using different concentrations of H_2O_2.

Statistical analysis

Results of each treatment were manifested as mean of triplicates ± standard deviation (SD). For each investigated parameter, the least significant difference (LSD) at P= 0.05 was evaluated using SPSS version 14 software.

Results

Results of the present investigation reflected no significant effect of VC on all studied parameters, compared with the corresponding negative controls. On the other hand, VE was advantageous that was more obvious at the higher concentration. Compared with the corresponding control treated with DW, exposure to VE at 600mg/L was associated with 10% increase in MI (Fig. 2a) and 31.2, 38.7, 53.8 and 36.2% decrease in TCA%, tDNA% and contents of MDA and H_2O_2 content, respectively (Fig. 2b, 3, 4). BHT, at 300mg/L had no significant effect on all studied parameters. Doubling BHT concentration was associated with unhealthy symptoms including significant decrease in MI and significant increase in TCA%, TDNA%, compared with the corresponding negative control, though the antioxidant role designated with significant decrease in MDA content. Some examples of the abnormal cell divisions produced by BHT treatments were illustrated in Fig. 5. Representatives of DNA damages stimulated by the presence of BHT were shown in Fig. 6. Except 36.2% significant decrease in MDA content, compared with DW-control; recorded at 600mg/L, the mixture of the previous antioxidants had no significant effect on any of the studied parameters.

Results of the present investigation demonstrated that, treating the roots with 15mg/L AgNPs (positive control) reflected significant mitodepressive effect symbolized in 43% reduction in MI, compared with negative control (Fig 2a). The genotoxicity of AgNPs treatment appeared in the form of increase of TCA% and

tDNA% reaching ~2.2 and 2.27 folds of the corresponding DW- treated control (Fig. 2b, 3). AgNPs provoked chromosome aberrations (Fig. 5) where stickiness, spindle disturbances (multipolar, star-shaped, c-mitosis, chromosome vagrant and loss) and clastogenic aberrations (bridges and breaks) appeared to be the most dominant ones. Figure 6 showed some examples of the DNA damages motivated by AgNPs. The mitodepressive and genotoxic effects recorded following AgNPs treatment were associated with oxidative stress symptoms designated with significant increase in MDA and H_2O_2 contents that reached 2.84 and 1.53 folds of the negative control (Fig. 4).

Exposure to VC at 300mg/L for 24hrs prior AgNPs treatment ameliorated the harmful effects of the latter, in terms of significant increase in MI (Fig. 2a) and decrease in MDA and H_2O_2 contents (Fig. 4), compared with AgNPs-treated control. Such ameliorating effect of VC was intensified at 600 mg/L and expanded to appear in terms of significant decrease in TCA% (Fig. 2b) and TDNA % (Fig. 3). However, neither of the recorded parameters reached the corresponding negative control at both VC concentrations. This partial ameliorating effect was significantly decreased upon application of AgNPs simultaneously with VC at 600mg/L and was completely vanished using VC at 300 (Fig. 2-4).

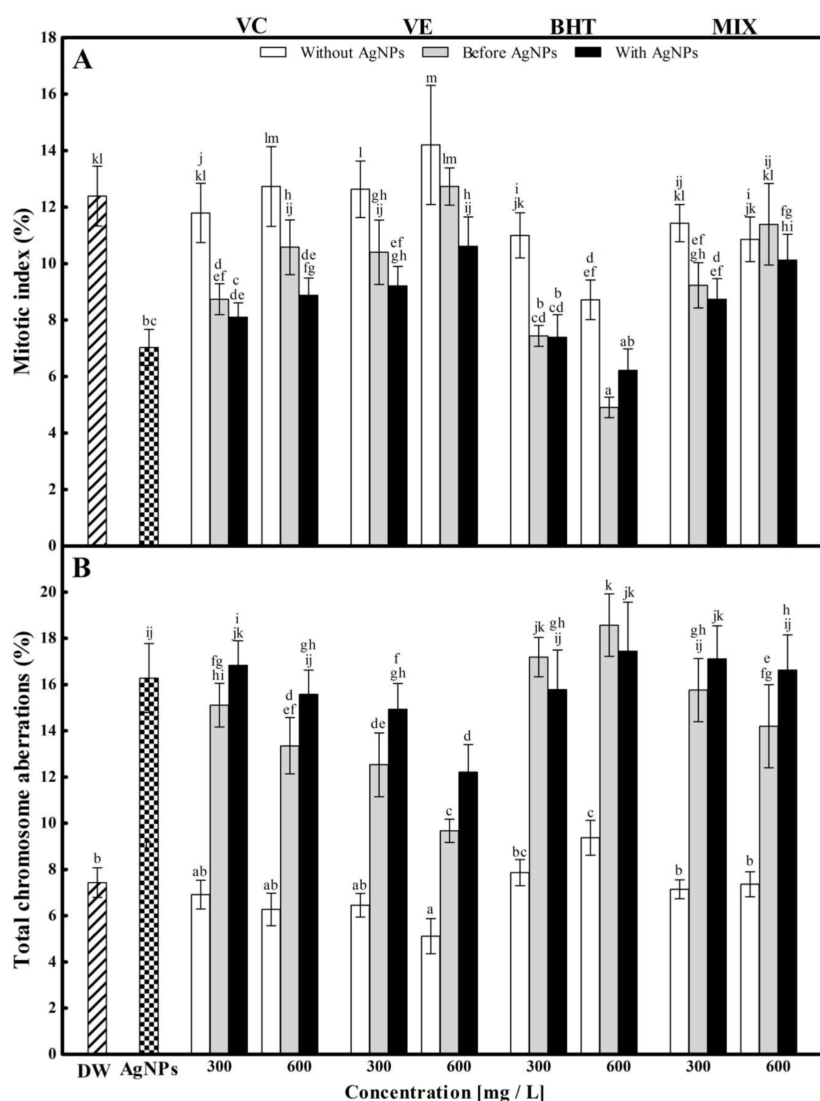

Fig. 2. Effect of antioxidants at different combinations with 15mg/L AgNPs on (A) Mitotic index and (B) Total chromosome aberrations in onion root tips [Values are represented as mean ± SD of triplicates, SD bars with different letters are significantly different, according to the LSD test, at P< 0.05].

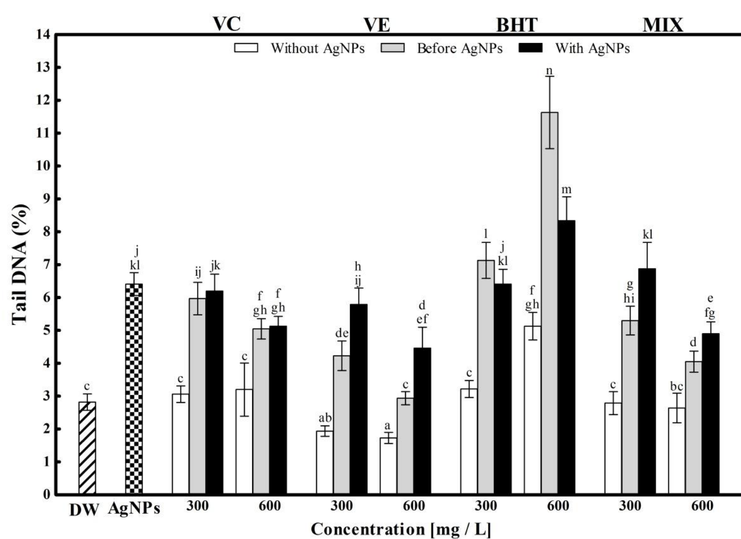

Fig. 3. Effect of antioxidants at different combinations with 15mg/L AgNPs on tail DNA in onion root tips [Values are represented as mean ± SD of triplicates, SD bars with different letters are significantly different, according to the LSD test, at P< 0.05].

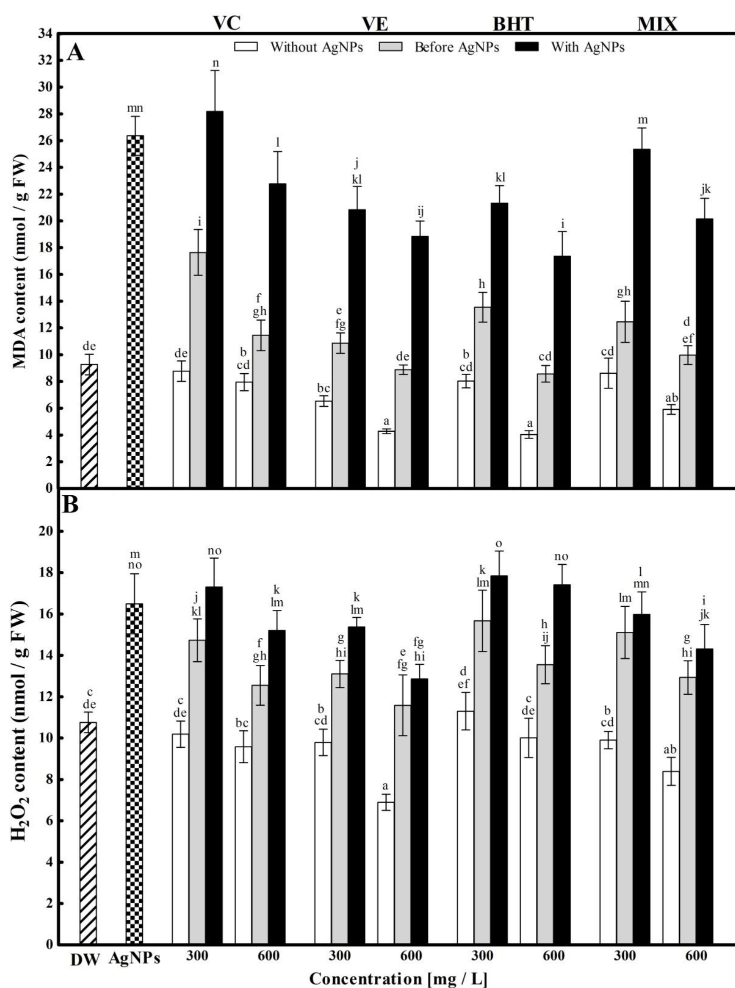

Fig. 4. Effect of antioxidants at different combinations with 15mg/L AgNPs on MDA and H_2O_2 contents in onion root tips [Values are represented as mean ± SD of triplicates, SD bars with different letters are significantly different, according to the LSD test, at P< 0.05].

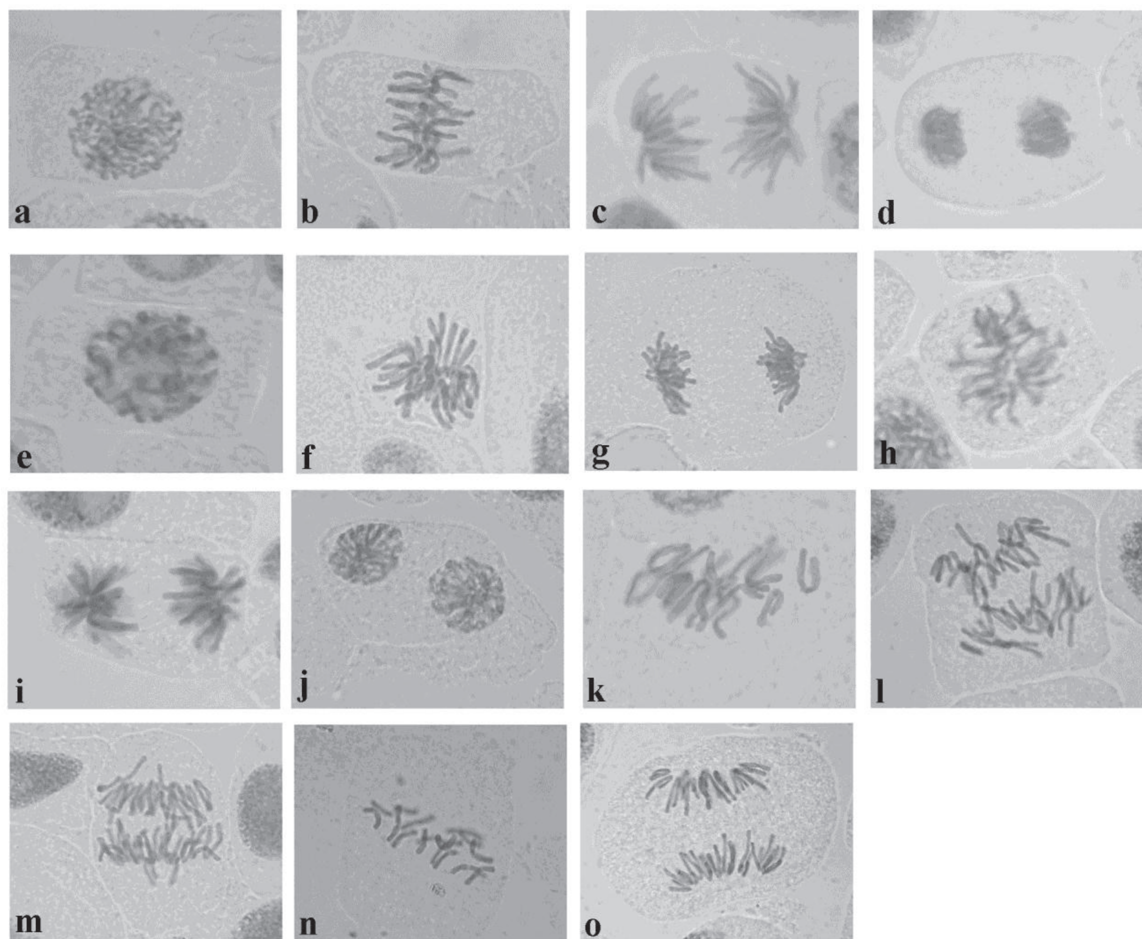

Fig. 5. Representative examples of normal and abnormal cell divisions in onion root tips subjected to the various treatments. (a-d): Normal prophase, metaphase, anaphase, and telophase, respectively, under control conditions (DW); e: Stickiness in prophase; f: Stickiness in metaphase; g: Stickiness in anaphase; h: Star-shaped metaphase; I: Star-shaped anaphase; j: Star-shaped telophase; k: Lost chromosome in metaphase; l: Lost chromosome in anaphase; m: Vagrant and bridge; n: c-metaphase; o: Multipolar, after treatment with AgNPs, BHT or different combinations of AgNPs and antioxidants.

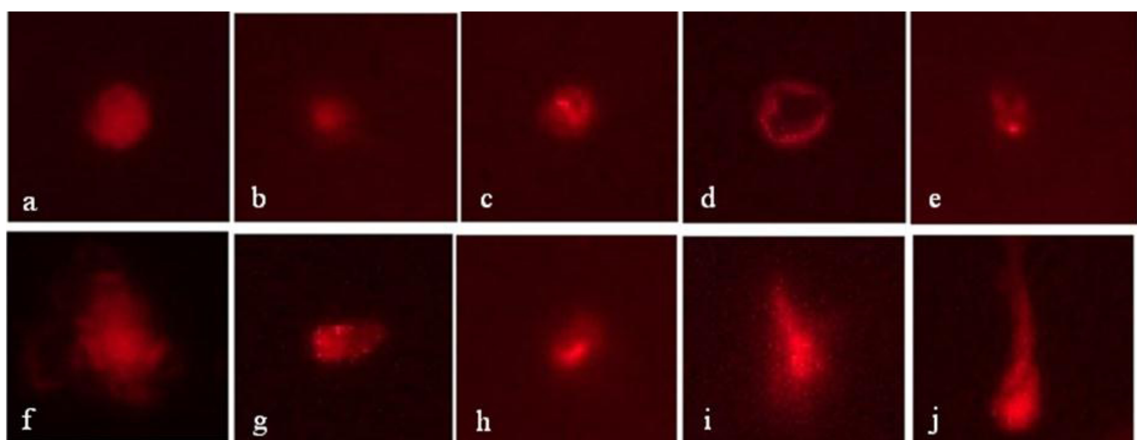

Fig. 6. Representative comet assay images illustrating some examples of DNA damages in onion root tips subjected to the various treatments; a) Undamaged DNA (control) and (b–j) Various grades of DNA damages motivated by the presence of AgNPs, BHT or different combinations of AgNPs and antioxidants.

Twenty four hours-soaking in VE provided an effective protection against subsequent AgNPs treatment. Compared with the corresponding positive control, VE at 300mg/L induced 47.9% significant enhancement in MI (Fig. 2a) and 20.6 to 58.8% decrease in other cellular damage-related symptoms parameters (Fig, 2b, 3, 4). Except occurrence of chromosomal aberrations (Fig. 2b), doubling VE concentration provided a full protection against AgNPs–induced damage as demonstrated by reaching other parameters to negative control level. Applying both AgNPs and VE in the same time attenuated the previously recorded protecting role of VE. Such protection was indistinguishable at 300mg/L observing tDNA and H_2O_2 contents.

Except the 48.6% decrease in MDA content (Fig. 4a), compared with the positive control, BHT at 300 mg/L failed to ameliorate the effect of subsequent exposure to AgNPs (Fig. 2, 3, 4b). Doubling BHT concentration intensified the mitodepressive effect of AgNPs manifested in 30.2% decrease in MI, compared with positive control. Genotoxicity of AgNPs was also exaggerated in terms of 14.1 and 81.4% increase in TCA% and tDNA%, respectively, compared with AgNPs-treated control. However, BHT at 600mg/L decreased production of MDA that reached negative control level and H_2O_2 that reached 82.2% of corresponding control exposed to AgNPs. Applying BHT along with AgNPs modified the previous results. Compared with positive control, about 19.1% decrease in MDA content was recorded; otherwise BHT at 300mg/L had no significant effect. Except 30.1% increase in %tDNA and 34.1% decrease in MDA content, doubling BHT concentration had no significant effect, compared with control exposed to AgNPs.

Compared with results recorded following exposure to AgNPs, 24hrs pretreatment with 300mg/L of 1:1:1 mixture of VC, VE and BHT ameliorated harmful effects of AgNPs in terms of 31.3% increase in MI (Fig. 2a) and 17.3 and 52.7% decrease in tDNA% (Fig. 3) and MDA content (Fig. 4a), respectively. Such amelioration was strengthened upon applying 600mg/L of the antioxidant mixture to be complete recovery of MI and MDA content to negative control values as well as 12.8, 36.8 and 21.6% significant decrease in TCA%, tDNA% and H_2O_2 content, respectively, compared with positive control. Such effect was restricted to 24.3% enhancement in MI, compared with AgNPs-treated control, upon using 300mg/L of the antioxidants mixture in co-treatment protocol. Doubling the concentration of antioxidants resulted in better ameliorating effect symbolized in 44% increase in MI and reduction in TCA% and contents of MDA and H_2O_2 contents, compared with positive control.

Discussion

It was obvious from our previous study that AgNPs, at 15mg/L for 9hrs, manifested genotoxicity (in terms of chromosomal aberrations) more than cytotoxicity (in terms of mitodepressive activity) on the onion cells (Fouad & Hafez, 2018b). This conducts our work to track its effect on DNA damages and the total chromosomes anomalies in absence or presence of natural and/or synthetic antioxidant scavengers.

Our experiments showed that AgNPs increased damages at chromosomal (stickiness, spindle and clastogenic aberrations) and DNA levels in association with a decline in MI. This cytogenetic toxicity was also reported by Pulate et al. (2001), Kumari et al. (2009) and Prokhorova et al. (2013). In addition, Babu et al. (2008) reported that AgNPs have mitoclassic, mitodepressive and clastogenic properties, so it can 1) alter the mitotic spindles orientation, and interacts with the SH of tubulin causing different spindle disturbances, 2) interweave of the chromatin fibers that may connect the chromosomes forming stickiness and 3) perform breaks that cause the loss of some chromosomal fragments. The observed damages in the present investigation may be attributed to ROS formation accompanied with AgNPs-induced oxidative stress and the subsequent damage of cellular macromolecules including DNA, proteins and lipids (De Veylder et al., 2007) and the direct physical impacts of nanoparticles on cellular structure (Fouad & Hafez, 2018b).

Treatment of the roots with VC alone showed no divergence from the negative control which was reflected as normal cell divisions, healthy DNA and insignificant change in MDA and H_2O_2 contents. In this context, Düsman et al. (2012) indicated that there is no statistical significant change in MI by treating the onion roots with synthetic VC (300 and 600mg/L) for 24hrs, comparing with that of control (water). In addition, Hoda et al. (1991) reported that VC supplementation can ameliorate the plant metabolism and it is non-toxic and non-

mutagenic. Moreover, Barakat (2003) reported that treating the roots of three wheat cultivars with 100ppm VC appeared to increase the MI more than control. Soliman et al. (2017) also found that treating *Vicia faba* roots for 24 or 48hrs with VC increases the MI% (1.5-1.6 folds) over the control.

Based on the results of this investigation, treating the root tips for 24hrs with VC before AgNPs, mitigated significantly the toxic effect provoked by AgNPs. This protective role may be attributed to the effective reducing anti-mutagenic antioxidant property of VC. It works as an electron donor that scavenges AgNPs-generated free radicals, as indicated with decrease in MDA and H_2O_2 contents, to safeguard cellular macromolecules as DNA from the toxic impacts of free radicals (Carr & Frei, 1999; Hacisevki, 2009; McShan et al., 2014).

The application of VE alone to the roots exhibited high significant increment in MI, decrease in TCA% and DNA damage reflecting an amelioration in the health of dividing cells, cell division and DNA over those of control and VC. In addition, VE decreased significantly MDA and H_2O_2 contents. Comparable results were observed by Bronzetti et al. (2001) who indicated that VE did not have toxic or mutagenic impact, but it ameliorate the plant performance (Hoda et al., 1991; Bronzetti et al., 2001; Barakat, 2003).

Exposing the root tips with VE (600mg/L) before applying AgNPs revoked completely the toxic effect performed by AgNPs on MI and DNA damages reaching again their negative control status. Similarly, VE alleviated completely the level of both MDA and H_2O_2. In addition, this treatment decreased significantly the TCA% but did not reach the normal level. The efficient shielding property of VE may be due to its scavenging ability putting it as a trap for AgNPs-generated free radicals preventing their potential damage for cellular macromolecules (El-Nahas et al., 1993; Griffiths & Lunec, 2001; Halliwell, 2001). The significantly better ameliorating effect of VE, compared with VC, may be attributed to the wider spectrum of free radicals scavenged by VE, compared with VC (Beilsk, 1982). However, neither of vitamins could totally eliminate the impact of AgNPs on occurrence of chromosomal aberrations that may be attributed to the direct physical impacts of nanoparticles on cellular structure (Fouad & Hafez, 2018b).

Our experiment showed that applying BHT, especially at 600mg/L to the root tips of onion for 24hrs, significantly decreased the MI, which was accompanied with elevated TCA and DNA damage. The cyto-genotoxicity of BHT was observed in wheat seedlings (Bakeeva et al., 2001) and *Allium cepa* root tips (Pandey et al., 2014). The later research group attributed the decrease in MI caused by BHT to the decrease in ATP level, suppression of DNA synthesis or obstruction in the G2-phase preventing the cell from leaving interphase and dividing mitotically. They also indicated that the abnormalities reflect the irreversible cytotoxic effects of BHT due to its partial inhibition impact on the cytoskeletal structure that involved mitosis leading to cell death. Besides, BHT treatment declined the MDA level but H_2O_2 remain unchanged (as negative control). The decrease in MDA content may be attributed to the antilipid peroxidation potential of BHT (Griffiths et al., 2000; Sasse et al., 2009; Mandade et al., 2011).

Exposing the root tips to BHT for 24hrs before AgNPs treatment intensified the mitodepressive and genotoxic effects of the latter while ameliorated MDA and H_2O_2 generated following AgNPs treatment. In this respect, Grillo et al. (1999) reported that BHT has a bi-sword action as it can induce chromosomal damages and protect the cells against oxidative damage induced by other toxicants. The safeguard ability of BHT against the toxicity of many chemical agents may be due to its anti-oxidant activity, which involves the overcome of ROS and lipid soluble radicals (Black, 2004).

Results of our investigation indicated that treating the root tips with 1:1:1 mixture of VC, VE and BHT insignificantly affect MI, TCA and DNA damages that all remain as positive control. This could be due to presence of too small concentrations of each antioxidant unable to produce detectable changes. It may be also attributed to antagonism between the negative impact of BHT and the positive one of VC and VE on the previous parameters. Results of both MDA and H_2O_2 contents reflected normalized values insignificantly different from the corresponding values recorded using each antioxidant solely.

Pretreatment with mixture of antioxidants reduced significantly the toxic effect of AgNPs concerning MI, TCA % and tDNA %. Such

results revealed the role of both of VC and VE in counteracting the BHT-enforced damage of AgNPs. Activation of VE in presence of VC to encourage normal cell divisions and to safeguard elasticity and function of cell membranes against the danger of ROS was recorded by several research groups (Burton et al., 1995; Sies & Stahl, 1995; Traber, 2007).

Results of the present investigation reflected general superiority of pretreatment of roots with antioxidant on co-treatment with AgNPs in ameliorating toxicity of the latter. It can be attributed to establishment of preformed defense system enabling cells to cope with incoming AgNPs hazards. The defense system was manifested in general improvements in cell division in cells exposed to VC, VE and mixture of antioxidants, compared with DW-treated control.

Conclusion

In conclusion, the natural antioxidants (VC and VE) appeared to ameliorate the cytotoxic and genotoxic impacts of AgNPs with superiority to VE that may be attributed to its higher efficiency as ROS scavenger. The synthetic antioxidant BHT has a bi-sword action as it can induce chromosomal damage as well as reduces ROS generation. 1:1:1 mixture of the previous antioxidants produces average effect reflecting attenuation of VC and VE effects in presence of BHT. Consequently, it is recommended to apply the natural antioxidants (and not the synthetic) either singly or in combination to mitigate the AgNPs related cyto- and genotoxicity. To maximize the protective role of VC and VE, it is recommended to apply them prior to the exposure of AgNPs.

Conflict of Interest: The authors declare no conflict of interest.

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تخفيف السمية الخلوية و السمية الجينية لجسيمات الفضة متناهية الصغر فى جذور نبات البصل بإستخدام بعض مضادات الأكسدة

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تهدف الدراسة إلى إستخدام جذور البصل كنموذج لدراسة قدرة ثلاثة من مضادات الأكسدة (فيتامين ج، فيتامين هـ و مادة بيوتيل هيدروكسيتولوين) على تقليل السمية الخلوية والسمية الجينية لجسيمات الفضة متناهية الصغر. أوضحت النتائج قدرة جسيمات الفضة على الحد من قدرة الخلايا على الإنقسام والذى تجلى فى النقص فى معامل الإنقسام كما ظهرت أعراض السمية الجينية عقب التعرض لتلك الجسيمات متمثلة فى زيادة معدل حدوث التشوهات الصبغية ونواتج تلف الحامض النووى. وقد صاحب الأعراض السابقة زيادة فى دلالات الإجهاد التأكسدى مثل زيادة محتوى المالون ألدهيد وفوق أكسيد الهيدروجين. كان لإستخدام فيتامين ج، فيتامين هـ ومادة بيوتيل هيدروكسيتولوين بالإضافة إلى خليط متساوى منها أثرا طيبا فى حماية الخلايا من الأثار المصاحبة للتعرض لجسيمات الفضة وقد تفوقت المعاملة بفيتامين هـ على باقى المعاملات فى حماية الخلايا. أظهرت النتائج أنه بالرغم من قدرة مادة بيوتيل هيدروكسيتولوين على تقليل إنتاج المالون ألدهيد و فوق أكسيد الهيدروجين الناتج من التعرض لجسيمات الفضة إلا أنها زادت من السمية الجينية لتلك الجسيمات وقدرتها على الحد من من قدرة الخلايا على الإنقسام. وأخيرا وضح من نتائج الدراسة أفضلية المعاملة المسبقة للخلايا بمضادات الأكسدة قبل التعرض لجسيمات الفضة على المعاملة المتزامنة لها فى الحد من الأثار الضارة لتلك الجسيمات.