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EVALUATION OF SILVER NANOPARTICLES GENOTOXICITY IN *HERMETIA ILLUCENS* USING COMET ASSAY

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ABSTRACT

Silver nanoparticles (AgNPs) are commonly used in various sectors such as food, cosmetics, medicine, and insect control. Otherwise, the toxicological effects of this promising technology should be studied to ensure its safety. This research aimed to investigate the potential toxicity of different concentration of AgNPs (0-20 mg/mL) on the cuticle cells of insects using alkaline comet assay. The level of DNA damaged was significantly higher in insects treated with 5-20 mg/mL AgNPs comparing to that from the control one with the fold of 1, 1.7, 1.9, 4.3, respectively in the tail length. A strong positive correlation occurred between concentration of AgNPs and all comet assay parameters were occurred with linear prediction equations. The possible deleterious impacts of AgNPs on the *Hermetia illucens* (L.) were discussed. Also, the potential using of comet assay as an accurate and cost-effective monitoring tool of fate of using nanoparticles was proposed.

Key words: Alkaline comet assay, DNA damage, biomarkers, silver nanoparticles, environmental fate, *Hermetia illucens*, oxidative stress, impact assessment, monitoring tool, cost-effective methodology, toxicity, nanoparticles safety

Nanoparticles are frequently used in a wide range of application. Nanoparticles are in high demand due to their unique physical and chemical properties as well as their ease to control. All these advantages are distinct from both free molecules and larger-sized particles. There are more than two million studies focused on the potential application of this promising technology (Yousef et al., 2019). Briefly, silver nanoparticles (AgNPs) are widely used such as an insecticide, a dye eliminator, an antifungal textile, and an antimicrobial or anticancer agent (Das et al., 2020; Xu et al., 2020). So, the potential genotoxicity of AgNPs contact application should be monitored. The ability of organisms to monitor the effect of releasing AgNPs into environment, the remediated ability of using fate, transport, or final disposal of nanoparticles, and the potential considered as environmental stressor were slightly studied (Ohore et al., 2021). Especially, insects are common in terrestrial ecosystems such as flies, and grasshoppers can be used as a sensitive assessment tool to stressor (Abdelfattah et al., 2017). In this context, environmental stress can increase the production of reactive oxygen species (ROS) in organisms (Abdelfattah et al., 2021). When ROS overload and exceed normal level, lead to oxidative stress causing deleterious effect to macromolecules of living organisms, including DNA damage, protein carbonylation, lipid peroxidation, and enzyme inactivation (Halliwell, 1999; Abdelfattah, 2016; Renault et al., 2016; Abdelfattah et al., 2017; Yousef et al., 2019; Abdelfattah, 2020; Nassar et al., 2020; Abdelfattah and Renault, 2021; Abdelfattah et al., 2021).

DNA damage may involve removing the bases that leads to strand breaks and consequently mutation (Abdelfattah et al., 2017). Single strand breaks of DNA damage can be measured using single cell gel electrophoresis (SCGE), or alkaline comet assay. This method is considered as one of the simplest, most sensitive and reliable method for detecting DNA strands breakages. The features of comet assay technique allow early detection of the stressor deleterious. Recently, the comet assay has become more common as a tool to study genotoxicity of various stressors in different animals, and in the last decade, also in insects (Abdelfattah et al., 2017). Hence, the aim of the study was to evaluate the damage level of DNA, using alkaline comet assay in the cuticle cells of black soldier fly (BSF) *Hermetia illucens* (L.), which exposed to different concentrations of AgNPs(l.) (0-20 mg/ mL).

MATERIALS AND METHODS

Polyvinylpyrrolidone (PVP)-coated AgNPs of mean sizes 20-30 nm was provided from Nanotech, Cairo, Egypt. Characterization of AgNPs was carried previously by Hafez and Fouad (2020). Besides that,

H. illucens was supplied from Entomology Department, Faculty of Science, Cairo University, Egypt. Before the experiment, the insect reared under the rearing condition (12:12 L: D; 34˚±2; 60% RH) in the cages size 30*30*40 cm for 100 adults. The feeding habits of adult are nectar feeding. So, AgNPs was applied in contact to adult by emersion the experimental cages 10*10*5 cm in different concentrations of AgNPs solutions (0, 5, 10, 15, 20 mg/ ml) for 48 hours post application. For each sub-group, 50 insects were dissected, after application, to isolate cuticle tissues for further analysis and were stored at −20 °C until use. Each experiment was done in three replicates.

The Single Cell Gel Electrophoresis assay (SCGE), known as the Comet assay was used to assess the DNA strand breaks according to Duroudier et al. (2021). The analysis of DNA damage was performed using OPTIKA B-350 fluorescent microscope (OPTIKA, Ponteranica, Italy), with a CCD camera. The image analysis system (Comet IV software) was used to quantify the single strand breaks of DNA by different parameters. Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp). A non-parametric test was carried out using the k independent Kruskal-Wallis test to compare between the effects of different concentration of AgNPs on comet parameters. Generalized estimating equation (GEE) was used to examine the effect of nanoparticles concentration on DNA damage. Reproduced and residual correlations between AgNPs concentration and all comet parameters of adult *H. illucens* were done based on Pearson's regression analysis.

RESULTS AND DISCUSSION

In the present study, the alkaline comet assay was used to evaluate the genotoxicity of promising nanoparticles contact as a recommendation of Souza et al. (2021) that include the fate of nanoparticles should be studied. *Hermetia illucens*, were treated with different concentration of AgNPs (0-20 mg/ ml) (Figs. 1 A-F, 2; Table 1). The contact effect of AgNPs can increase the production of ROS in the cells or tissues of living organisms and leads to oxidative stress (Ratan et al., 2020). The level of stress was evaluated in this study indirectly using oxidative damage of DNA in the cuticle tissue of *H. illucens*. This analysis was done according to previous accepted literature (Abdelfattah et al., 2017). The results revealed a significant increase of DNA damage in cuticle tissue of treated insect with different concentration of AgNPs compared to the control insects (Figs. 1, 2).

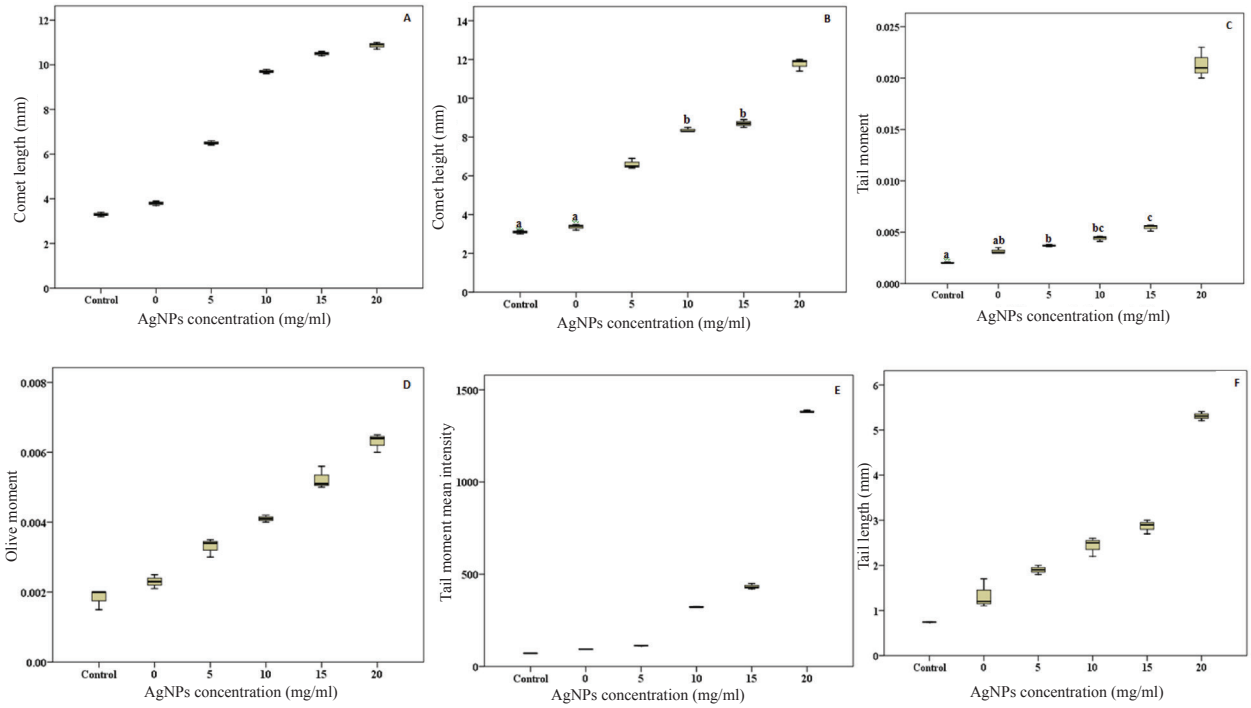

Fig. 1. Alkaline comet assay of cuticle cells males of *H. illucens* treated with AgNPs. Median values marked with different small letters significantly different (Kruskal-Wallis test, $p < 0.05$).

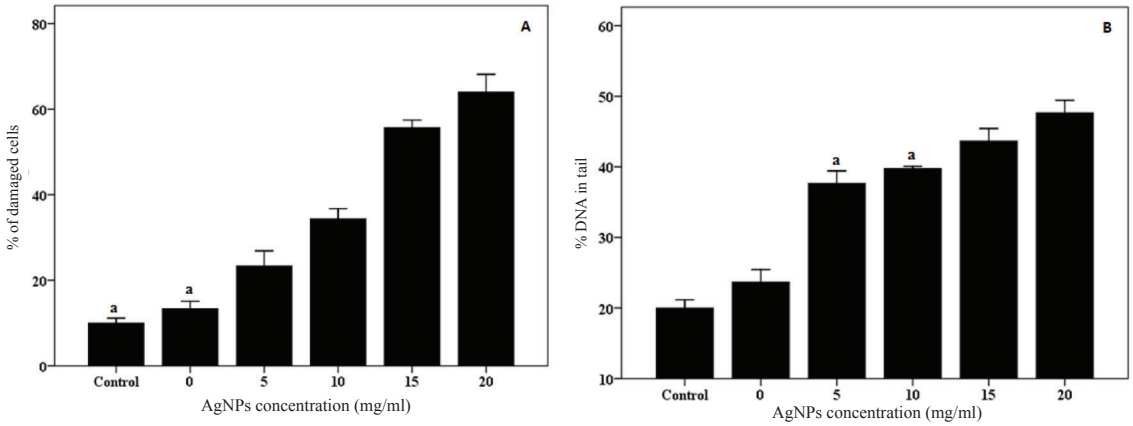

Fig. 2. DNA damage cells and DNA in tail % of cuticle cells males of *H. illucens* treated with AgNPs. Median values marked with different small letters significantly different (Kruskal-Wallis test, $p < 0.05$)

Table 1. Generalized estimating equation (GEE), regression equation, and correlation factor to analyze the interaction among concentration of AgNPs and intercept on comet parameters from cuticle of males *H. illucens*.

Item	QIC	Wald Chi-square	r	*Estimated equation*	R^2	*df*	*P value*
Concentration effect							
Comet length	12.14	22540	0.96	Y= 0.67X	-0.08	5	<0.0001
Comet Height	12.5	9156	0.97	Y= 0.60 X	0.24	5	<0.0001
Olive moment	12.1	1006	0.98	Y= 0.0005 X	0.69	5	<0.0001
Tail moment	12.2	2237	0.76	Y= 0.0008 X	0.60	5	<0.0001
Tail moment mean intensity	573.3	93402	0.83	Y= 50.5 X	0.71	5	<0.0001
% DNA in tail	32.7	2293	0.95	Y= 2.9 X	-0.29	5	<0.0001
% damaged cells	76.6	3700	0.97	Y= 3.4 X	0.85	5	<0.0001
Tail length	12.3	10968	0.92	Y= 0.24 X	0.66	5	<0.0001
Intercept							
Comet length	12.14	122427	-	-	-	1	<0.0001
Comet Height	12.5	30385	-	-	-	1	<0.0001
Olive moment	12.1	6356	-	-	-	1	<0.0001
Tail moment	12.2	2779	-	-	-	1	<0.0001
Tail moment mean intensity	573.3	93402	-	-	-	1	<0.0001
% DNA in tail	32.7	19532	-	-	-	1	<0.0001
% damaged cells	76.6	5604	-	-	-	1	<0.0001
Tail length	12.3	5034	-	-	-	1	<0.0001

The insect cuticle acts as the main and first site of defense. Also, it plays a key role in some essential activities (Ortiz-Urquiza and Keyhani, 2013). The various stressors can contact cuticle cells and lead to oxidative stress (Yousef et al., 2017; Abdelfattah and Renault, 2021; Abdelfattah et al., 2021). Investigations by Yasur and Rani (2015) showed differences in the activities of antioxidant and detoxifying enzymes, carboxylesterases (CarE), glucosidases (Glu) and glutathione S-transferases (GST) in the larval stage of lepidopteran gut after PVP-coated AgNPs treatment. Also, the activities of superoxide dismutase, catalase, and peroxidase were elevated in the larval bodies due to the AgNPs treatments. Besides that, the application of AgNPs at high concentrations led to induce heat shock protein 70, oxidative stress and apoptosis in *D. melanogaster* (Ahamed et al., 2010). These results agree with those of the present study which emphasized a strong positive correlation between AgNPs concentration and all comet parameters of treated insects also, a high level of significance of concentration and intercept effect of AgNPs treatment (Table 1). All these findings suggest

that nanoparticles exposure may induce oxidative stress, which can be indirectly detected through evaluation of macromolecules damage or the activity of antioxidant enzymes. The deleterious damage of DNA may occur as results of increasing levels of ROS. Also, in recent reports nano-Ag treatment led to damage the digestive system with symptoms of oozing of inner gut contents of lepidopteran larva, in addition to reduction the insect growth with prolonged larval period and larvae became sluggish, finally led to death.

Also, prolonged larval growth occurred in *S. litura* and *A. janata*, as a result of silver nanoparticles treatments was observed (Yasur and Rani, 2015). The present results corroborate with those of Lobo et al. (2010), who reported that DNA is considered as key target of free radical attack in the living cells. Abdelfattah et al. (2017) observed genotoxicity effect of different environmental stressors on different tissues of males and female grasshopper *Aiolopus thalassinus.* The relationship between comet parameters and different concentration of AgNPs treatment in the present study showed a unified pattern of a positive correlation. Asharani et al. (2008) revealed that AgNPs had a potential genotoxic effect and may cause chromosomal aberrations, DNA damage, and cell proliferation in cell lines of zebra fish. Nair and Choi (2011) focused on the exposure effect of different concentration of commercial silver nanoparticles (0.2, 0.5, and 1 mg/ l) on the aquatic midge *Chironomus riparius* (Meigen). The results emphasized the up and down expression of antioxidant enzyme glutathione-S-transferase (GST) genes as a result of nanoparticles application. Another study found that 4 mg/ l AgNPs concentration failed to show acute toxicity on *C. riparius,* These findings emphasize that the toxicity mechanisms of AgNPs depend on signaling transduction pathways which are associated with synthesis of proteins and activation of gonadotrophin releasing hormone; based on down-regulation of the ribosomal protein gene (CrL15) regulating ribosomal assembly, and upregulation of the gonadotrophin releasing hormone gene (CrGnRH1) or the Balbiani ring protein gene (CrBR2.2), respectively. Another study evaluated the effects of AgNPs on reproductive and pulmonary cells viability, lipid peroxidation, and total oxidative DNA damage. The results proved a strong cytotoxic activity of AgNPs at low concentrations (2-13 µg/ ml) and caused an overproduction of reactive oxygen species (ROS) (Zapór, 2016).

Many studies have evaluated the impact of AgNPs application, and these proved that the deleterious effect of AgNPs may occur as a result of direct cell membrane attachment, membrane integrity disruption, ROS generation, membrane permeability changes, proteins interaction, and DNA replication interference (Yu et al., 2013). Nanoparticles can bind to sulphur and phosphorus group and lead to degradation of proteins and DNA, respectively. So, the macromolecules damage occurred as a result of exoskeleton penetration by nanoparticles (Benelli, 2016). The present study concludes that contact treatment of AgNPs caused damaging effects on the DNA of *H. illucens* cuticle cells especially at highest concentration 20 mg/ ml as a result of ROS production. So, the genotoxicity study of AgNPzs- based products should be done before approval pf the products by decision makers.

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