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Exploring Antioxidant and Genotoxic Activities of Silver Nanoparticles Synthesized from Karaerik Grape Leaves: A Green Approach

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Abstract

Nowadays, plant extract-mediated biosynthesis of nanoparticles has gained prominence as a pivotal research domain. Silver nanoparticles are traditionally synthesized using highly toxic and ecologically hazardous chemical and physical methods. The emerging green synthesis approach offers more eco-friendly alternatives while reducing production costs. Hence, the present study opted for a nature-friendly green synthesis method to produce silver nanoparticles. Silver nanoparticles were characterized using UV-visible spectroscopy (UV-VIS), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR). The reaction involved the treatment of $AgNO_3$ (5 mM) with an aqueous extract of Karaerik grape leaf. Subsequently, the potentially toxic, genotoxic, and antioxidant effects of purchased chemically produced silver nanoparticles ($AgNP_{(c)}$) and silver nanoparticles ($AgNP_{(b)}$) that we synthesized using the green method were investigated on *Drosophila melanogaster* transheterozygous larvae. The study employed 72 ± 4 hour-old larvae bearing the recessive *flr*^{3} and *mwh* determinant genes on their third chromosomes. Exposure scenarios included 1 mM Ethyl methanesulfonate (EMS), AgNPs (1.25, 2.5, and 5 mg/mL), and EMS+ AgNPs. Interestingly, the EMS+ AgNPs combination reduced total oxidant status while increasing total antioxidant status significantly compared to EMS alone. To assess genotoxic effects, mutant trichomes resulting from genetic changes in the development of wing imaginal discs were examined. Furthermore, the AgNP synthesized through green synthesis demonstrated antioxidant properties and displayed no genotoxicity. In conclusion, the research highlights the promising potential of green-synthesized silver nanoparticles, which provide an eco-friendly and safe method for various applications.

1. Introduction

The main reason nano-sized structures attract attention is that they show functionality with their unique properties, different from their diverse volumetric dimensions and structures [1]. Medical-oriented nanoparticles are recognized for their ability to facilitate disease diagnosis and subsequently develop effective treatment methods. The fact that nanoparticles can be used in the medical field in this way provides benefits in many areas, such as gene therapies with nanospheres, earlier diagnosis and treatment of cancer, and targeted drug delivery [2]. Nanosilver is also widely used in biomedical fields such as infusion ports, contraceptive devices, endovascular stents, peritoneal dialysis devices, urological stents, endotracheal tubes, contact lens coatings, surgical and dental instruments, endoscopes, electrodes, subcutaneous cuffs [3]. Traditional methods for synthesizing nanoparticles often utilize highly toxic chemicals, resulting in harmful side effects during their application. Therefore, there is a need for an

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alternative approach to reduce these hazardous consequences. Green synthesis ensures the production of safe and effective nanoparticles while also enabling affordable and non-toxic manufacturing processes [4]. Concerns regarding the genotoxic and cytotoxic effects of metal nanoparticles, utilized in various applications and synthesized through physical, chemical, and biological methods, are rising on. Research is being conducted on the applications of gold and silver nanoparticles, which are classified as metal nanoparticles [5]. It is also known that silver in nanoparticle form, which is antimicrobial, is used more frequently than other metals and has low toxicity in mammalian cells [6]. The advantages of the green synthesis method, which utilizes plant extracts, include the easy availability of plants, the absence of special conditions, the biocompatibility of waste produced, their suitability for large-scale production, and their low cost and environmental friendliness [7, 8]. A substantial portion of present nanotoxicology research relies on *in vitro* models that do not provide insights into the destiny of nanoparticles within host organisms, including aspects such as metabolism, accumulation, biodistribution, elimination, persistence, and more [9]. D. *melanogaster* is an ideal model organism for conducting mutation screens and assessing the biological activity of various chemical compounds, including nanoparticles [10, 11].

In this context, we have used *Vitis vinifera* L. cv. Karaerik, a subspecies of the black grape cultivar known as Cimin grape, grown endemic in the Üzümlü district of Erzincan province, to determine the antioxidant and genotoxic effects of silver nanoparticles obtained from the leaves of Karaerik using the green synthesis method on *D. melanogaster*. The somatic mutation and recombination test (SMART), a fast and convenient method with many advantages, was used.

2. Material and Method

2.1. Strains of *Drosophila*

In our experiments, two different *Drosophila* strains were used: *multiple-wing hair* males (*mwh/mwh*) and *flare* females (flr^3/*In*(*3LR*)TM3, *ri* p^p *sep I*(*3*)*89Aabx*^{34e}). These strains were acquired from the repositories at Erzincan Binali Yıldırım University's Basic Sciences Application and Research Center Laboratories. The *multiple-wing hair* marker is located on chromosome 3, and its unique phenotype is multiple trichomes per cell when homozygous. The *flare* marker is recessive and also located on chromosome 3. This produces abnormal epidermal point-like hairs on the wing. The genetic symbols and descriptions have been detailed extensively in previous publications [12, 13].

2.2. Chemicals

Ethyl methanesulfonate (EMS, 62-50-0) and silver nitrate ($AgNO_3$, 7761-88-8) were obtained from Sigma Aldrich. Silver nanopowder ($AgNP_{(c)}$, 28-48nm) was purchased from Nanografi company.

2.3. Fresh on plant extract

Karaerik grape leaf extract was chosen to synthesize silver nanoparticles (AgNPs) due to its cost-effectiveness, medicinal properties, and easy accessibility, making it a practical and advantageous option for the synthesis process.

Fresh leaves of *Vitis vinifera* L. cv. Karaerik (Vitaceae) were collected from grape farms in Üzümlü, Erzincan, Turkey, in September 2018. Dr. Ali Kandemir expertly performed the taxonomic identification of the plant species using "Flora of Turkey and the East Aegean Islands" [14- 16], and all plant specimens were subsequently deposited in the Herbarium of the Science and Art Faculty at Erzincan Binali Yıldırım University. The grape leaves were thoroughly washed to remove dust particles and then air-dried at room temperature for one week to eliminate residual moisture. To reduce silver ions (Ag^+) to silver nanoparticles (Ag^0), a grape leaf extract was prepared by placing 5 g of the washed and dried finely-cut leaves into 100 mL of sterile distilled water. This mixture was continually heated at 80°C at ambient temperature for 1 hour, with regular agitation. After approximately 10 minutes of heating, the aqueous solution's color transitioned from clear to light yellow. After being cooled to room temperature, the extract was filtered using Whatman No. 1 paper and centrifuged at 3500 rpm for 5 minutes to remove residues. The final extract was then stored at room temperature for future experiments.

2.4. Green synthesis methods of silver nanoparticles

A 1 mM aqueous $AgNO_3$ solution was prepared with distilled water. Then, 10 mL of Karaerik grape leaf extract was added to a 90 mL solution with 1 mM $AgNO_3$. The first indication of AgNP

synthesis is the visual color change in the reaction mixture. In this study, the initially colorless reaction mixture gradually turned brown. After reducing the $AgNO_3$ solution to silver nanoparticles, the synthesized AgNPs were first centrifuged at 10.000 g for 10 minutes. After the centrifugation, it was lyophilized for characterization and experimental studies.

2.5. Characterization of green synthesized silver nanoparticles

UV-visible spectroscopy (PerkinElmer Lambda 35) was used to scan spectra from 200-700 nm with 1 nm resolution to validate optical measurements. Structural and morphological characterization was performed using SEM (FEI-Quanta FEG 450) with 120,000X magnification and 30 kV voltage. Elemental analysis via XRD (Panalytical Empyrean) determined the crystal structure and purity of AgNPs. FTIR spectra (Thermo Scientific Nicolet 6700) in the 400-4000 cm^{-1} range identified oxide forms and biomaterials on NP surfaces.

2.6. Survival rate determination

To determine the LD_{50} dose of AgNPs obtained by the green synthesis method ($AgNP_{(b)}$) and commercially purchased in synthetic form ($AgNP_{(c)}$), concentrations were determined according to the LD_{50} doses determined after 24 h applications at various concentration ranges. Adult individuals were obtained from third-instar transheterozygote larvae (72±4 h) treated with AgNPs to obtain an adequate number of flies for the antioxidant assay and SMART. The survival rate was determined by calculating the number of adult individuals per 100 larvae. Larvae were treated with EMS (1 mM), $AgNP_{(b)}$, and $AgNP_{(c)}$ (1.25, 2.5, and 5 mg/mL) separately and together. In the EMS + AgNP groups, only the highest concentrations of $AgNP_{(b)}$ and $AgNP_{(c)}$ were used.

2.7. Antioxidant assay

Total oxidant status (TOS) and total antioxidant status (TAS) were determined using photometric methods with commercial kits provided by Rel Assay (Rel Assay Kit Diagnostics, Turkey), developed by O. Erel [17]. To standardize these data, Trolox, a water-soluble version of vitamin E, was used as a TAS calibrator, while hydrogen peroxide was used as a TOS calibrator. The oxidative stress index (OSI) was determined as a percentage of the TOS level relative to the TAS level.

2.8. Somatic mutation and recombination test

The *mwh* virgin males and *flr*^{3} females of mutant strains were crossbred, and after 8 hours, the eggs were collected. Third-instar transheterozygote larvae (72 ± 4 h) obtained from these eggs were subjected to chronic treatment with 1.25, 2.5, and 5 mg/mL AgNPs. For the SMART, the eggs were nourished with *Drosophila* Instant Medium (Carolina Biological Supply Company, Burlington, NC, USA), which was prepared by dissolving 1.5 g of powder in 7 mL of water, and AgNP was incorporated into this medium at the specified final concentrations. Distilled water was utilized for the negative control; ethyl methanesulfonate (1 mM) served as a positive control. EMS is an alkylating chemical that may cause point mutations, small deletions, and chromosomal breaks; it also has recombinogenic activity [18, 19]. All treatments were repeated three times. These treatment flasks were maintained at 25 ± 1°C and a relative humidity of approximately 65% until the adult subjects hatched. Following the treatment phase, newly emerged adults were collected and preserved in 70% ethanol. Their wings were removed, mounted on glass slides, and fixed with Faure's solution (30 g acacia gum, 20 mL glycerol, 50 g chloral hydrate, and 50 mL water). The slides were then examined under a light microscope at 400X magnification.

2.9. Data analysis

The results were reported as mean values along with their standard errors (SE) or percentages, where appropriate. Duncan's one-way range test was used to determine the statistical significance of the data. The data was analyzed using SPSS software (version 24 for WindowsTM). The genotoxic potential in the Somatic Mutation and Recombination test was assessed by comparing the spot frequency (small single, large single, and twin spots) per fly in each treated group to that of the negative control. For the frequencies of spots per wing, a multiple-decision procedure is used to decide whether a result is positive, weakly positive, inconclusive, or negative. Statistical analysis was performed using a two-tailed χ^2 test for proportions ($\alpha = \beta = 0.05$) following the method described by H. Frei, and F. E. Würgler [20].

3. Results and Discussion

3.1. Characterization of synthesized silver nanoparticles

$AgNP_{(b)}$ characterization was performed using SEM equipped with EDX, XRD, UV-vis, and FT-IR techniques. The UV-vis absorbance spectra of $AgNP_{(b)}$ are presented in Figure 1.

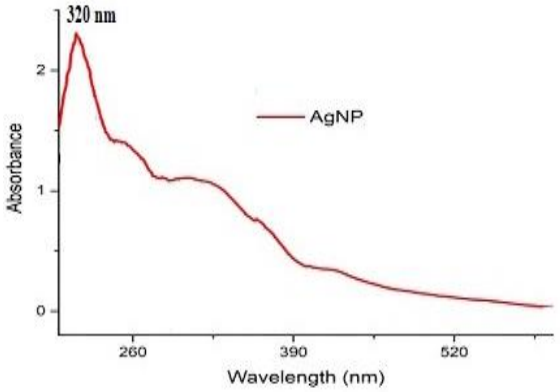

Figure 1. UV-visible absorption spectrum of $AgNP_{(b)}$

For the experiment, a 1 cm quartz cuvette was utilized. The study identified the maximum peak for $AgNP_{(b)}$ at 320 nm (Figure 1). Aromatic compounds from Karaerik grape leaf extract probably cause the peak at 320 nm.

The SEM images and the energy dispersive spectra of the AgNPs are shown in Figure 2. It is seen that AgNPs of different shapes were obtained in the case of various leaf extracts being used as reducing and capping agents. The extracts from *V. vinifera* gave rise to AgNPs that were mostly spherical and exhibited a polydisperse distribution. The size of the AgNPs ranged from 28.79 nm to 34.24 nm, with occasional instances of agglomeration being observed. This could be attributed to the quantity and nature of capping agents available in the various leaf extracts, as evidenced by the shifts and variations in the peak regions observed in the FTIR analysis.

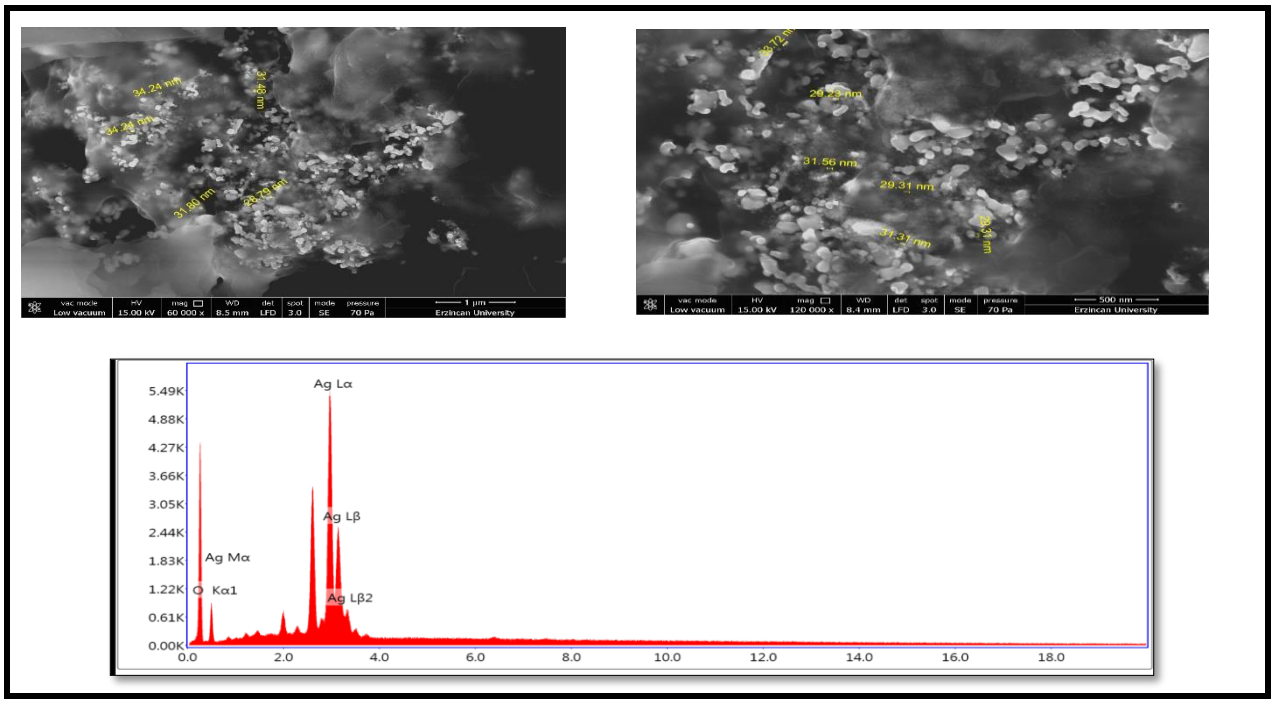

Figure 2. SEM and EDAX analysis of silver nanoparticles

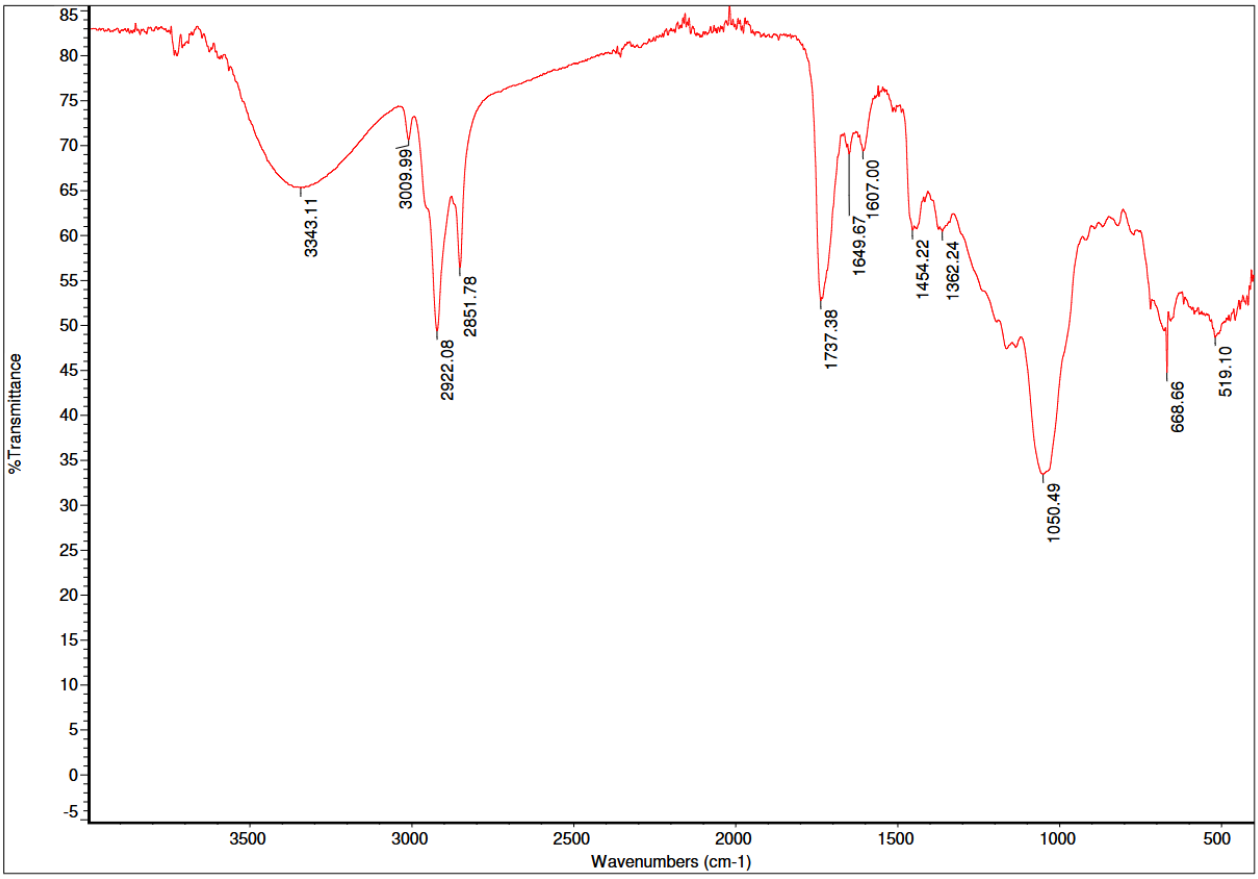

Figure 3. FTIR spectra of $AgNP_{(b)}$

FTIR analysis to characterize the AgNPs obtained from plant extract is shown in Figure 3. These distinctive bands suggest the presence of structures within the extract, primarily including polysaccharides/sugars, phenolic compounds, and proteins, with a particular emphasis on flavonoids.

Elemental analysis was conducted using energy-dispersive X-ray spectroscopy on the Panalytical, Empyrean. A powder X-ray diffraction (XRD) spectrum was used to identify the crystal shape and purity of AgNPs.The findings are depicted in Figure 4. No diffraction peaks associated with impurities were detected in the XRD patterns, indicating the product's high purity. Furthermore, the sharp and narrow nature of these peaks signifies that the crystallization of AgNPs is well-defined.

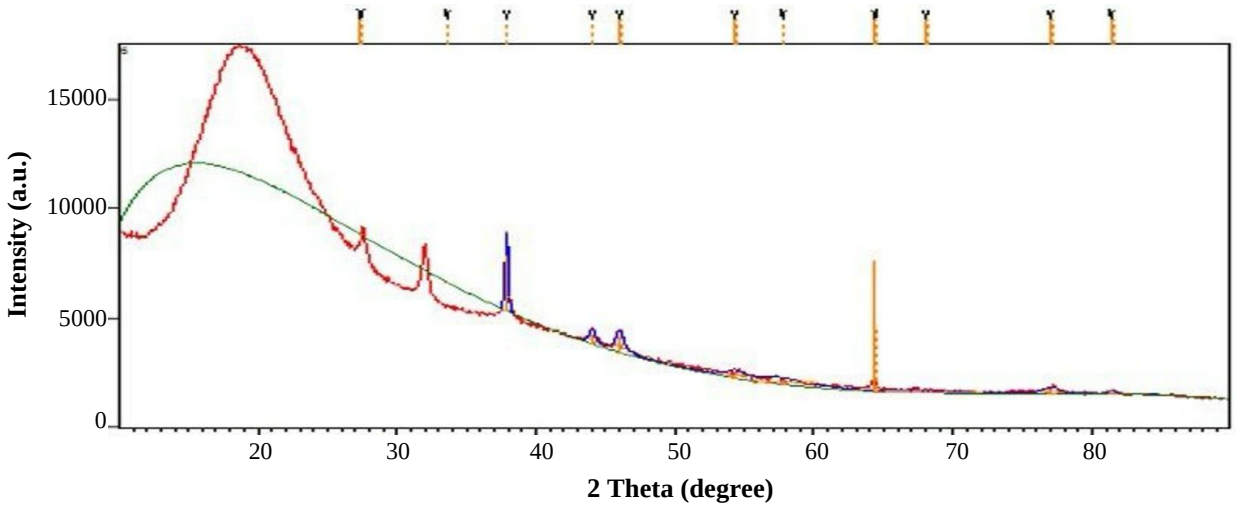

Figure 4. XRD patterns of $AgNP_{(b)}$

3.2. Survival rate determination

The data from the experiments, which aimed to investigate the impact of AgNP application on the survival rate, are presented in Figure 5. The findings showed a reduced survival rate in larvae treated with 1.25, 2.5, and 5 mg/mL AgNP extracts compared to the control group.

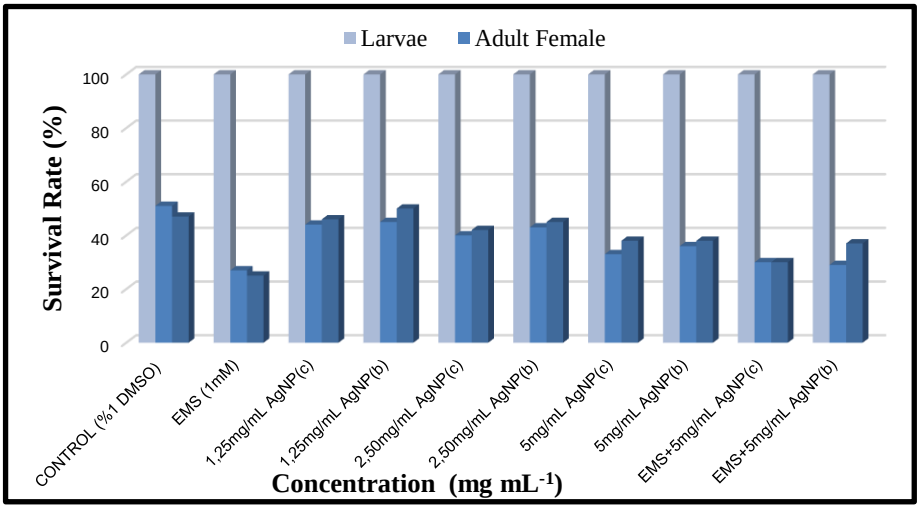

Figure 5. Percentage survival rates of $AgNP_{(c)}$ ve $AgNP_{(b)}$ in *D. melanogaster*

3.3. Antioxidant assay

The oxidative parameters total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were assessed in adult *D. melanogaster* to investigate the impact of AgNPs on transheterozygous larvae. All AgNP-treated groups showed higher TOS and lower TAS values when compared to the EMS group (Table 1). Results were statistically significant ($p<0.05$). In addition, a high OSI value (OSI=TOS/TAS) indicated adverse effects (Table 1).

Table 1. Oxidative stress parameter data were measured in *D. melanogaster* groups treated with $AgNP_{(c)}$ and $AgNP_{(b)}$

Experimental groups	TOS (μmol H_2O_2 Equiv. L^{-1})	TAS (mmol Trolox Eqvui. L^{-1})	OSI (AU)
Control (%1 DMSO)	11.15±3.51[a]	1.24±0.07[a]	8.95±2.11[a]
EMS (1 mM)	19.26±6.48[b]	0.07±0.01[b]	263.83±40.05[b]
1.25 mg/mL $AgNP_{(c)}$	11.08±4.12[a]	1.32±0.09[c]	8.33±3.42[a]
1.25 mg/mL $AgNP_{(b)}$	10.83±3.67[c]	1.41±0.10[d]	7.66±2.22[c]
2.5 mg/mL $AgNP_{(c)}$	9.28±1.16[d]	1.42±0.08[d]	6.49±1.54[d]
2.5 mg/mL $AgNP_{(b)}$	8.82±0.18[d]	1.50±0.02[e]	5.87±1.10[e]
5 mg/mL $AgNP_{(c)}$	7.75±0.21[d, e]	1.59±0.11[f]	4.84±0.23[f]
5 mg/mL $AgNP_{(b)}$	6.33±0.18[f]	1.67±0.07[g]	3.77±0.65[g]
EMS+5 mg/mL $AgNP_{(c)}$	13.14±2.15[g]	0.24±0.01[h]	54.29±7.17[h]
EMS+5 mg/mL $AgNP_{(b)}$	12.79±2.32[g]	0.29±0.02[i]	42.77±5.31[i]

[a-i]: Letters in the same column indicate significant differences at the 0.05 level.

3.4. Somatic mutation and recombination test

It was compared whether $AgNP_{(c)}$ and $AgNP_{(b)}$ had any genotoxic effects on *D. melanogaster* wings with *mwh/flr*^{3} and *mwh/TM3* genotypes at the determined doses. For this purpose, the results of $AgNP_{(c)}$ and $AgNP_{(b)}$ at different concentrations (1.25, 2.5, and 5 mg/mL) and the negative control group (distilled water) were statistically compared. From the data obtained, it was observed that the number of wing spots in the $AgNP_{(c)}$ and $AgNP_{(b)}$ application groups increased

in parallel with the increasing concentration compared to the negative control group (Table 2). When the results were analyzed statistically, the numerically observed increases were not significant and were evaluated as negative (-) ($p>0.05$). In addition, it was determined that $AgNP_{(b)}$ was not genotoxic, and the clone induction frequencies of $AgNP_{(c)}$ were higher than $AgNP_{(b)}$ at all concentrations.

Table 2. Genotoxicity of the $AgNP_{(c)}$ ve $AgNP_{(b)}$ in the *D. melanogaster* wing spot test results obtained with *mwh/flr*^{3} and *mwh/TM3* wings

	Experimental groups	(N)	Small single spots (1–2 cells) (*m = 2*)			Large single spots (>2 cells) (*m = 5*)			Twin spots (*m = 5*)			Total *mwh* spots (*m = 2*)			Total spots (m = 2)			CIF
			No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	
mwh/flr^3	Distilled water	80	23	(0.28)		3	(0.03)		2	(0.03)		22	(0.27)		28	(0.35)		1.12
	EMS (1mM)	80	188	(2.35)	+	110	(1.37)	+	26	(0.32)	+	190	(2.37)	+	324	(4.05)	+	9.72
	$AgNP_{(c)}$ (1.25 mg/mL)	80	27	(0.33)	-	2	(0.03)	-	2	(0.03)	-	25	(0.31)	-	31	(0.38)	-	1.28
	$AgNP_{(k)}$ (2.5 mg/mL)	80	30	(0.37)	-	3	(0.03)	-	1	(0.01)	-	31	(0.38)	-	34	(0.42)	-	1.58
	$AgNP_{(c)}$ (5 mg/mL)	80	35	(0.43)	-	5	(0.06)	-	3	(0.03)	-	37	(0.46)	-	43	(0.53)	-	1.89
	$AgNP_{(b)}$ (1.25 mg/mL)	80	24	(0.30)	-	1	(0.01)	-	0	(0.00)	-	24	(0.30)	-	25	(0.31)	-	1.22
	$AgNP_{(c)}$ (2.5 mg/mL)	80	26	(0.32)	-	1	(0.01)	-	0	(0.00)	-	26	(0.32)	-	27	(0.33)	-	1.33
	$AgNP_{(b)}$ (5 mg/mL)	80	28	(0.35)	-	1	(0.01)	-	1	(0.01)	-	29	(0.36)	-	30	(0.37)	-	1.48
mvh/TM3	Distilled water	80	18	(0.22)		2	(0.03)					20	(0.25)		20	(0.25)		1.02
	EMS (1mM)	80	147	(1.83)	+	87	(1.08)	+				234	(2.92)	+	234	(2.92)	+	11.98
	$AgNP_{(c)}$ (1.25 mg/mL)	80	26	(0.32)	-	3	(0.03)	-				29	(0.36)	-	29	(0.36)	-	1.48
	$AgNP_{(b)}$ (2.5 mg/mL)	80	29	(0.36)	-	2	(0.03)	-	*			31	(0.38)	-	31	(0.38)	-	1.58
	$AgNP_{(c)}$ (5 mg/mL)	80	33	(0.41)	-	1	(0.01)	-				34	(0.42)	-	34	(0.42)	-	1.74
	$AgNP_{(b)}$ (1.25 mg/mL)	80	20	(0.25)	-	0	(0.00)	-				20	(0.25)	-	20	(0.25)	-	1.02
	$AgNP_{(c)}$ (2.5 mg/mL)	80	23	(0.28)	-	0	(0.00)	-				23	(0.28)	-	23	(0.28)	-	1.17
	$AgNP_{(b)}$ (5 mg/mL)	80	25	(0.31)	-	0	(0.00)	-				25	(0.31)	-	25	(0.31)	-	1.28

N: Number of wings, No: number of clones, Fr.: frequency, D: statistical diagnosis according to Frei and Würgler (1985), CIF: Frequency of clone formation per 10^5 cells, *: balancer chromosome *TM3* does not carry the *flr*^{3} mutation.+: positive, -: negative, i: inconclusive, m: multiplication factor, probability levels $\alpha=\beta=0.05$.

The results of our study suggest that AgNPs exhibit antigenotoxic effects in a dose-dependent manner, as evidenced by the oxidative stress parameters. Specifically, all AgNP-treated groups demonstrated higher TOS and lower TAS values than the EMS group, with statistically significant results ($p<0.05$). Our findings also indicate that biologically synthesized AgNPs produce more favorable results than chemically synthesized ones. This could be attributed to the fact that biologically synthesized AgNPs often exhibit better biocompatibility and reduced cytotoxicity, which might enhance their protective effects against genotoxicity. The presence of biomolecules in biologically synthesized AgNPs can enhance their stability and reduce potential adverse effects, leading to a more effective response in mitigating oxidative damage. This observation could be explained by the notion that low to moderate levels of oxidative stress may

induce adaptive responses in cells, leading to enhanced DNA repair mechanisms and increased antioxidant defenses. Therefore, while AgNPs increase oxidative stress markers, their presence may also stimulate cellular protective mechanisms that reduce the genotoxic impact compared to the control group.

The biological pathway for synthesizing nanoparticles has now been shown to save energy and produce less harmful waste [21]. By applying green principles in chemistry to nanotechnology, it is anticipated that nanotechnological processes can produce new products using environmentally friendly materials [22]. Plant metabolites and plant extracts are used in such processes, including peptides or biological macromolecules such as proteins, nucleic acids, carbohydrates, and lipids [23]. One of the objectives of this research is to emphasize the need for alternative methods to minimize the use of hazardous procedures in NP synthesis. The biological synthesis technique is one of the most often used ways of producing silver nanoparticles. This method employs traditional chemistry processes to reduce or eliminate the use and production of hazardous substances, benefiting both the environment and the economy. Chemical methods for synthesizing AgNPs utilize reagents that are non-toxic and environmentally friendly [24]. The green synthesis method is a new way to produce AgNPs [25]. Sahin and Gubbuk [26] analyzed the silver nanoparticles synthesized using the green synthesis method, employing turmeric, ginger, cinnamon, and carob extracts with antioxidant properties. That demonstrated that the obtained AgNPs serve as effective reducing agents. When silver interacts with the aromatic compounds in the extracts, it generates $AgNP_{(b).}$ This eco-friendly method not only minimizes the use of hazardous reagents but also showcases the potential to produce nanoparticles using sustainable materials [27]. Acay et al. [28] showed that AgNPs are effective against hospital pathogens by characterizing AgNPs produced by green synthesis from *Vitis vinifera* leaf extract by UV-vis spectrophotometer, SEM, EDX, FTIR, TGA-DTA, and XRD methods. According to Gnanajobitha et al. [29], fruit proteins and metabolites are integral to stabilizing silver ions throughout the synthesis of silver nanoparticles. Roy et al. [4] obtained spherical and crystalline AgNPs with an average size of 18-20 nm by green synthesis method using *Vitis vinifera* fruit extract in a study they conducted. It has been reported that these nanoparticles show effective antibacterial activity against *B. subtilis* and *E. coli*. Asaduzzaman et al. [30] investigated the effects of 17 nm silver nanoparticles synthesized by the green synthesis method using the Bangladesh grape plant on shrimp and *Ehrlich ascites* carcinoma cells. They found that although it had a toxic effect on shrimps, it showed strong antibacterial and anticancer activity in *E. ascites* carcinoma cells. Silver nanoparticles induce cytotoxicity and genotoxicity in a size- and coating-dependent manner, with smaller particles causing greater toxicity and genotoxicity than larger ones. These studies suggest that smaller silver nanoparticles (≤20 nm) are generally more cytotoxic than larger ones due to their higher cellular uptake and bioavailability [31, 32].

Drosophila melanogaster, employed as a living model organism, particularly in nanotoxicity, has recently been acknowledged as an appropriate organism for studying nanomaterial-induced toxicity [33]. Demir et al. [34] showed that when AgNPs were applied to 3rd instar larvae at concentrations that ranged from 0.1 to 10 nm, small yet notable increases in the frequency of total spots were observed in their genotoxic analysis studies using the *Drosophila* wing spot assay. In the present study, which showed that silver nanoparticles can induce genotoxic activity in the wing spot assay, it was also determined that it contrasted with silver nitrate, where negative findings were obtained. Gorth et al. [35] evaluated silver nanoparticles by examining their size and assessing the toxicity of both nanoscale and microscale silver particles in *Drosophila* egg development. While the pupalization rates of *Drosophila* eggs exposed to the 20-30 nm AgNPs did not show a statistically significant decrease, it was determined that this rate decreased in eggs exposed to the 500-1200 nm AgNPs. At 10 ppm AgNP exposure, pupae exposed to only the 20-30 nm AgNPs can mature very significantly, while it has been reported that they mature at a lower rate in the 500-1200 nm and 100 nm AgNP groups compared to the control. As a result, it has been noted that nanoscale AgNPs are less toxic to *Drosophila* eggs than micro-sized silver particles. In another *in vivo* study using the Somatic Mutation and Recombination Test (SMART), the genotoxicity of AgNPs on *D. melanogaster* was evaluated, with larvae exposed to 4.7 nm AgNPs at 25, 30, and 50 µg/mL and to 42 nm AgNPs at 250, 500, and 1000 µg/mL. The study found that speckle formation, which indicates mutagenic and recombinogenic effects in the phenotype, remained unchanged at

spontaneous frequencies for all doses, but AgNPs of both sizes caused pigmentation defects and reduced locomotor activity in adult flies [36].

4. Conclusion and Suggestions

In the present study, the comparative genotoxic evaluation of $AgNP_{(c)}$ and $AgNP_{(b)}$ was conducted on third-instar larvae that were exposed to NPs, and $AgNP_{(b)}$ did not exhibit any significant genotoxic activity in the wing spot assay. This study employed a straightforward, rapid, environmentally friendly, and cost-effective approach to synthesize silver nanoparticles using Karaerik grape leaves. The nanoparticles were characterized using FTIR, XRD, UV-vis spectroscopy, SEM, and EDAX. These Ag nanoparticles exhibited robust preventative properties, making them a valuable asset as potent antioxidants when functionalized with *V. vinifera*. These studies suggest that biologically synthesized silver nanoparticles (AgNPs) generally exhibit better biological activity and lower toxicity to living cells than chemically synthesized AgNPs. Regarding these attributes, the synthesized nanoparticles hold considerable promise for diverse applications within the biomedical sector. Our findings indicate that silver nanoparticles have substantial potential for use in numerous industries, particularly in the food and pharmaceutical sectors. Further studies are needed to elucidate the precise molecular pathways involved in this protective response and to confirm the superiority of biologically synthesized AgNPs over their chemically synthesized counterparts.

Contributions of the authors

Conceived and designed the experiments: Deniz A. Ç.; Performed the experiments: All authors; Writing-review & editing: All authors; Supervision & funding acquisition: Deniz A. Ç.

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Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

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