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EFFECTS OF SILVER NITRATE NANOPARTICLES ON THE OXIDATIVE STATUS IN SPRAGUE DAWLEY RATS

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ABSTRACT

This study investigated the toxic effects of silver nanoparticles (8.67-26.3 nm) on body weight, organs body weight ratio and oxaditive status of male Sprague Dawley rats over a period of three months. In this experimental study, 100 male Sprague Dawley rats were categorized in four groups including control group and three experimental groups (n=25 in each group). The rats in the experimental groups were orally intubated 5 mg/kg, 25 mg/kg and 50 mg/kg of AgNPs solution by gavage, five days a week. Samples of blood were taken from the rats for oxidative stress assessments. Afterwards, lungs, kidney, liver and brain removed and weighted to calculate organs body weight ratio. The results demonstrated a statistically no significant change in final body weight nor organs relative weight % , oxidative stress condition were investigated with increase MDA level and decrease SOD, CAT and TAC level in serum.

Keywords: Silver nano-particles, body weight, malondialdhyde, catalase, total antioxidants capacity.

INTRODUCTION

Nanotechnology was developed for more than two decades, and this unique technology attracted the interest of scientists around the world (Singh *et al.,* 2017). Nanoparticles are defined as particles with dimensions <100 nm and have attracted much attention due to their unique properties. Their physical (e.g. plasmonic resonance, fluorescent enhancement) and chemical (e.g. catalytic activity enhancement) properties derived from the high quantity of surface atoms and the high area/volume relation, as their diameter decreases, their surface area increases dramatically and as a consequence there is an increase over the original properties of their bulk materials (Khan *et al.,* 2017).

Silver nanoparticles (AgNPs) are one of the most important classes and most commonly used nanomaterials due to their unique chemical, physical and biological properties different from those of bulk material with the same composition (Völker *et al.,* 2013).

AgNPs are extensively used in healthcare products, women's hygiene products, the food industry, paints, cosmetics, medical devices, sun screen, bio-sensors, clothing, and electronics (Edwards, 2009).

Silver nanoparticles toxic effects include cytotoxicity via apoptosis and necrosis, lethality, oxidative stress, DNA and cell membrane damage, mitochondrial malfunction, inflammation and decreased cellularproliferation (Zhang *et al.,* 2012).

Thus the presence of the antioxidant enzymes as a defense mechanism is critical and their use in research as a marker for the oxidative status is extensive. Owing to that, this study investigated the serum levels of MDA, SOD, CAT and the TAC to better understand the effect of the AgNPs prolonged and consecutive oral administration on oxidative status of the body.

For that this study aimed to study the toxic effects of long term exposure of silver nanoparticles on male Sprague Dawley rats through evaluation of toxic effects of different doses on the oxidative status.

MATERIALS AND METHODS

Chemicals and Reagents:
Silver nanoparticles were provided by Faculty of Pharmacy, Alazhar University, Assiut, Egypt, it was prepared by chemical reduction method. Size of AgNPs was measured by transmission electron microscope (TEM) model JEOL-JEM-100 CX II in Electron Microscopy unit, Assuit University. AgNPs

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sizes were ranged from 8.67-26.3 nm as shown in (Photo. 1). Superoxide dismutase (SOD), malondialdhyde (MDA), catalase (CAT), total antioxidant capacity (TAC) kits were obtained from Biodiagnostic and Research Reagents, Egypt**.**

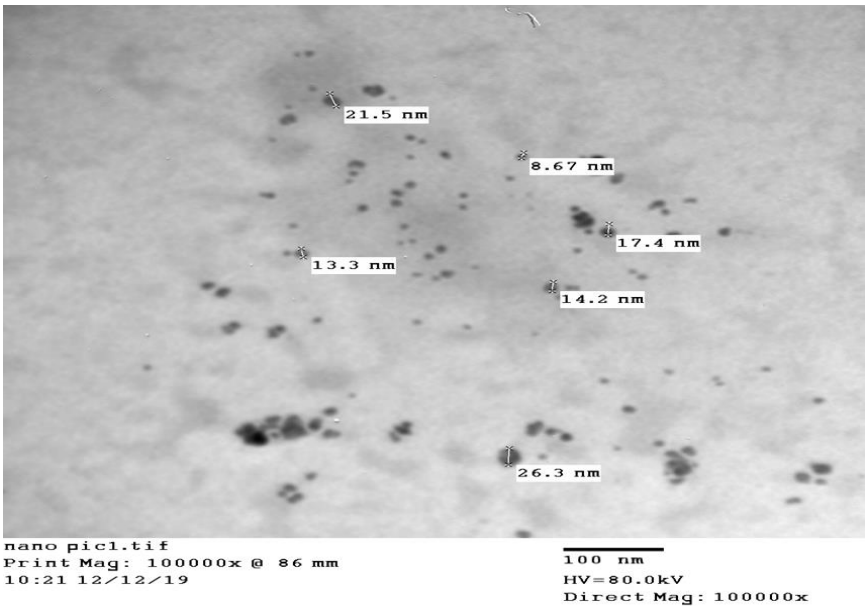

Fig. 1: TEM image of AgNPs showing spherical shapes of AgNPs with different size ranged between 8.67- 26.3 nm.

Animals: One hundred male albino rats (6-8 weeks old, weighing 80-100 g)were purchased from the laboratory animals' house of Al-Wasiemi medical center, Inc., Cairo, Egypt, at the age of weaning and used for the experiment after 2 weeks of acclimatization. Animals were housed in polypropylene cages with sawdust for bedding, provided rat feed (commercial pellet) and water *ad libitum*. Animal facilities were controlled for temperature (22±3 °C) and relative humidity (40–60%), and operated under a 12-hours light-dark cycle. Animal experiments were conducted according to the national guidelines of proper care and use of animals in the laboratory research.

Experimental design:
One hundred male rats Sprague Dawley rats were categorized in four groups including control group and three experimental groups (n=25 in each group). The rats in the experimental groups were orally intubated 5mg/kg, 25 mg/kg and 50 mg/kg of AgNPs solution by gavage, five days weekly for three months. The doses in this study were chosen according to Patlolla *et al.* (2015b). Seven rats from each group were taken for three time intervals after 1st, 2nd and 3rd months.

Rats were scarified by anesthesia using diethyl ether for samples collection after 4, 8 and 12 weeks for blood and tissue samples collection. Blood samples were collected directly from the descending aorta in vacutainer tubes without anticoagulant to obtain serum after centrifugation at 4500 rpm for 30 minutes preserved at -20ºC for oxidative stress analysis. Liver, brain, kidney and lung were removed carefully and weighed.

Methods:
Body weight: The body weight of each rat was recorded immediately before sacrifice. Brain, liver, kidneys and lungs were removed, stripped from fatty tissues and weighed. Relative organ weight (organs weight coefficient) for each rat was calculated and tabulated according to the following formula (absolute organ weight/body weight x 100).

Oxidative stress changes: The oxidative changes were measured by using the commercial kits as following: (**1**) Serum lipid peroxide (malondialdhyde, MDA) was estimated according to Ohkawa *et al.* (1979). (**2**) Catalase (CAT) enzyme was estimated according to Aebi (1984). (**3**) Superoxide dismutase (SOD) was estimated according to Nishikimi *et al.* (1972). (**4**) Total antioxidants capacity (TAC) was estimated according to Koracevic *et al.* (2001).

Statistical analysis: The results were analyzed statistically using one-way analysis of variance (ANOVA) with Dunnett multiple comparison tests as post-tests. These analyses were carried out using the computer SPSS program for windows, version 22.0 Difference between and among the groups were considered significant difference if $p \leq 0.05$ (Green and Salkind, 2003). All data were expressed as mean ± SE.

RESULTS

Body weight of rats showed no significant change in all groups during the whole period of the experiment compared to control groups. Relative lungs, kidneys, brain and liver weight % showed no significant change in all groups during the whole period of the experiment compared to control group (table 1).

Table 1: Relative lungs, kidneys, brain and liver weight % in all groups during the whole period of the experiment compared to control group.

Exposure time (month)	Tissue	G1 Control	Silver nanoparticles (AgNPs) exposed groups (mg/kg bw)		
			G2 (5)	**G3** (25)	**G4** (50)
1	Body weight	124.1±1.85	125.2±3.13	126.7±2.02	125.8±3.03
	Lung	0.60± 0.17	0.74±0.14	0.69±0.12	0.58±0.20
	Kidneys	0.81± 0.19	0.87±0.30	0.85±0.23	0.89±0.28
	Liver	3.73± 0.57	3.71±0.14	3.68±0.15	3.29±0.61
	Brain	1.23± 0.27	1.20±0.18	1.20±0.29	1.24±0.31
2	Body weight	141.7±3.24	137.8±6.13	145.4±3.39	145±5.10
	Lung	0.81±0.19	0.64±0.05	0.63±0.04	0.66±0.07
	Kidneys	0.84±0.03	0.93±0.12	0.79±0.03	0.81±0.02
	Liver	3.35±0.18	3.74±0.16	3.73±0.12	3.30±0.09
	Brain	1.15±0.04	1.07±0.03	1.08±0.03	1.04±0.02
3	Body weight	163.7±8.88	162.1±2.03	165.1±11.62	163.8±6.43
	Lung	0.51±0.015	0.57±0.02	0.67±0.15	0.56±0.10
	Kidneys	0.84±0.03	0.78±0.03	0.81±0.02	0.82±0.02
	Liver	3.39±0.06	3.48±0.07	3.53±0.10	3.49±0.07
	Brain	1.0±0.05	0.97±0.017	1.17±0.191	1.04±0.092

Serum malondialdehyde (MDA) showed a significant increase during the whole period of the experiment in G3 and G4 while G2 showed a significant increase in the 2nd and the 3rd months only when compared with control group (table 2 and fig. 2).

Table 2: Effect of AgNPs on serum levels of MDA (nmol/ml) of male albino rats.

Oxidative stress parameter	Exposure time (month)	G1 Control	Silver nanoparticles (AgNPs) exposed groups (mg/kg bw)		
			G2 (5)	**G3** (25)	**G4** (50)
MDA	1	35.12 ± 2.83^{a}	34.05 ± 1.14^{a}	55.75 ± 1.94^{b}	56.48 ± 1.76^{b}
	2	38.86 ± 2.75^{a}	59.88 ± 1.76^{b}	66.85 ± 2.24^{b}	88.11 ± 1.66^{c}
	3	37.89 ± 2.42^{a}	68.12 ± 0.56^{b}	83.78 ± 1.54^{c}	102.41 ± 5.47^{c}

- Data are presented as mean ± S.E (N=7).
- Values with different letters mean significant different at $p \leq 0.05$.

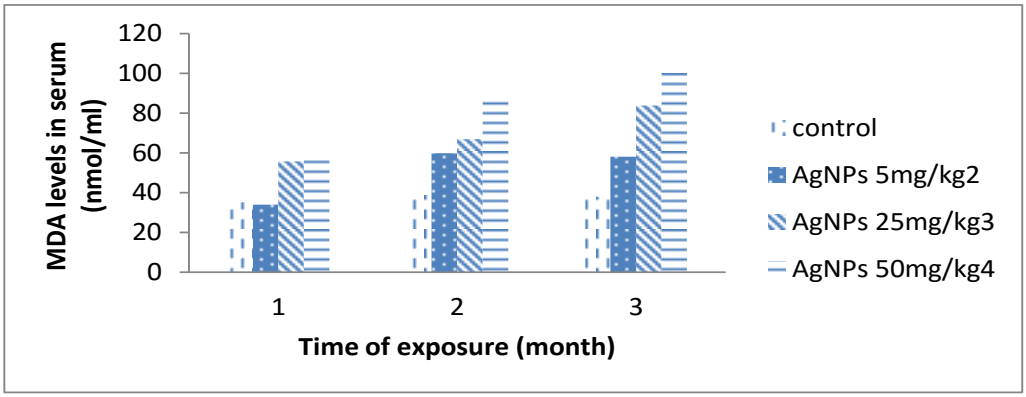

Fig. 2: Effect of AgNPs on serum levels of MDA (nmol/ml).

Serum superoxide dismutase (SOD) showed a significant decrease in G2 only in the 3rd month while in G3 andG4 showed a significant decrease during the whole period of the experiment when compared with control group (table 3 and fig. 3).

Table 3: Effect of AgNPs on serum levels of SOD activity (U/ml) of male albino rats.

Oxidative stress parameter	Exposure time (month)	G1 Control	Silver nanoparticles (AgNPs) exposed groups (mg/kg bw)		
			G2 (5)	**G3** (25)	**G4** (50)
SOD	1	546.51 ± 4.10^{a}	532.48 ± 1.57^{a}	358.00 ± 13.01^{b}	204.07 ± 5.26^{c}
	2	522.85 ± 16.79^{a}	528.41 ± 16.1^{a}	338.58 ± 3.33^{b}	175.11 ± 10.5^{c}
	3	545.85 ± 17.62^{a}	391.29 ± 3.29^{b}	198.38 ± 57.55^{c}	126.18 ± 4.33^{c}

- Data are presented as mean ± S.E (N=7).
- Values with different letters mean significant different at $p \leq 0.05$.

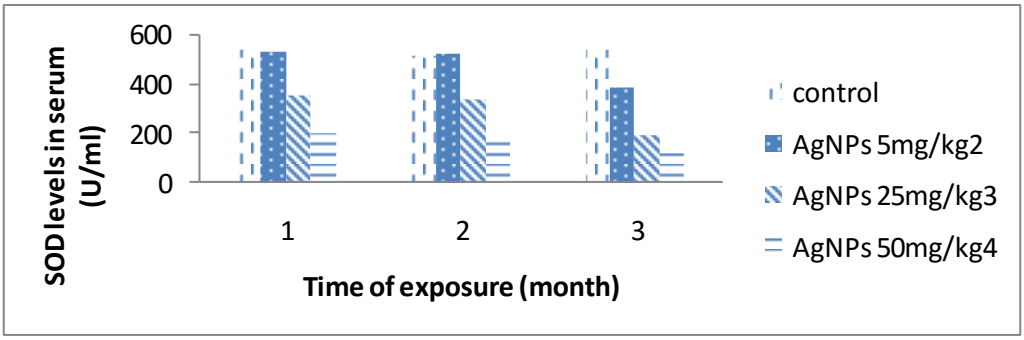

Fig. 3: Effect of AgNPs on serum levels of SOD activity (U/ml).

Serum catalase level showed non-significant decrease in G2 during the 1st and 2nd months while the 3rd month showed a significant decrease comparing to the control group. As for G3 and G4 the two groups showed a significant decrease during the whole period of the experiment when compared with control group but during the 1st and 2nd months the two groups showed no significance compared to each other (table 4 and fig. 4).

Table 4: Effect of AgNPs on serum levels of CAT (U/L) of male albino rats.

Oxidative stress parameter	Exposure time (month)	G1 Control	Silver nanoparticles (AgNPs) exposed groups (mg/kg bw)		
			G2 (5)	**G3** (25)	**G4** (50)
CAT	1	397.98 ± 3.13^{a}	395.66 ± 2.07^{a}	315.71 ± 14.08^{b}	221.39 ± 1.76^{c}
	2	401.01 ± 3.36^{a}	394.33 ± 3.35^{a}	175.34 ± 1.99^{b}	175.08 ± 3.089^{b}
	3	398.83 ± 4.63^{a}	313.38 ± 9.99^{b}	99.61 ± 4.42^{c}	100.20 ± 3.20^{c}

- Data are presented as mean ± S.E (N=7).
- Values with different letters mean significant different at $p \leq 0.05$.

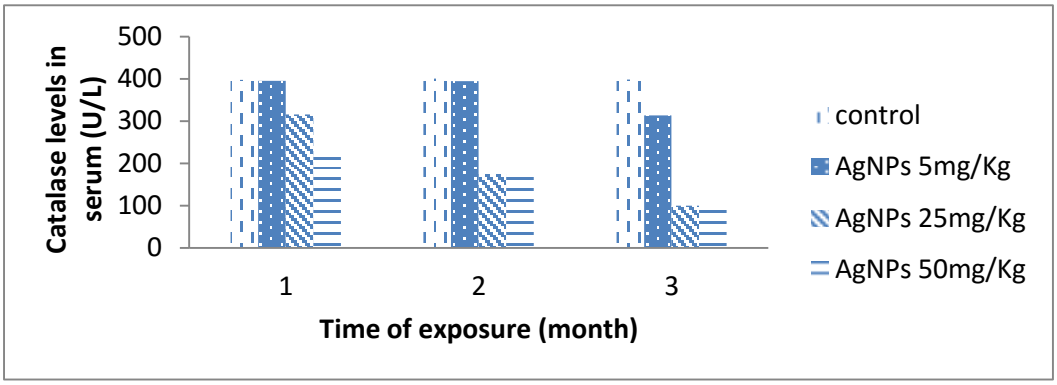

Fig. 4: Effect of AgNPs on serum levels of CAT of male albino rats.

Serum total antioxidants (TAC) showed a significant decrease in all groups during the whole period of the experiment except at the 1st and the 2nd months in G2 showed no significance when compared with control group (table 5 and fig. 5).

Table 5: Effect of AgNPs on TAC (mM/L) of male albino rats.

Oxidative stress parameter	Exposure time (month)	G1 Control	Silver nanoparticles (AgNPs) exposed groups (mg/kg bw)		
			G2 (5)	**G3** (25)	**G4** (50)
TAC	1	1.83 ± 0.05^{a}	1.50 ± 0.14^{a}	$1.43 \pm .05^{b}$	0.91 ± 0.03^{c}
	2	1.78 ± 0.02^{a}	1.44 ± 0.08^{b}	0.67 ± 0.01^{b}	0.72 ± 0.01^{b}
	3	$1.78 \pm .013^{a}$	0.91 ± 0.05^{b}	0.51 ± 0.01^{b}	0.20 ± 0.02^{c}

- Data are presented as mean ± S.E (N=7).
- Values with different letters mean significant different at $p \leq 0.05$.

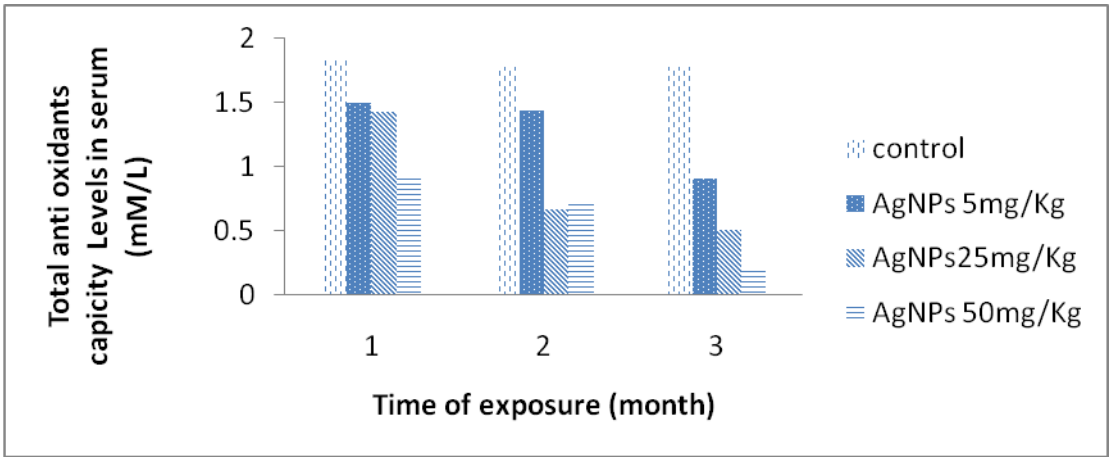

Fig. 5: Effect of AgNPs on serum levels of TAC of male albino rats TAC (mM/L).

DISCUSSION

Over the last couple of decades, silver has been engineered into nanoparticles with dimensions ranging from 1 to 100 nm. AgNPs have recently gained interest for a range of biomedical applications, owing to their potent antibacterial activity (Chen and Schluesener, 2008). However, AgNPs are still one of the most controversial materials due to their potential toxicity in biological systems (Dziendzikowska *et al.*, 2012).

1- Body and organs weight:

In the present study there were no significant changes in the body weight, absolute and relative organs weight after exposing rats orally to 5, 25 and 50 mg/kg b.w 5 days weekly for 3 months. This result is similar to study by Espinosa *et al.* (2013) who used two different sizes of AgNPs (14 nm and 36 nm) administered orally and reported that there was no differences in final body weight of rats among treated groups when treatment time was finished. Also, the study of Kim *et al.* (2010) who used different doses of AgNPs (30, 125 and 500 mg/kg) administered orally, they stated non-significant change in the body weight of male rats except after 4 weeks of exposure to high doses and no significant change in organs weight (lungs, kidneys, liver and brain) in relation to body weight. Another study with different rout of exposure using intraperitoneal injections by different doses of approximately 8.7 nm AgNPs (1, 2 and 4 mg/kg b.w) daily for 28 days stated no difference concerning the total body weight but there was a significant change in the liver weight in relation to body weight (El Mahdy *et al.*, 2015). Odeyemi *et al.* (2019) worked with Wister rats exposing them to oral acute toxicity by administration of 500, 1000 and 2000 mg/kg body weight getting no significant difference between the treatment groups compared with the control group for mean organ-to-body weight ratio except in the liver of rats treated with high doses.

2- Oxidative stress changes:

Like other nanoparticles, AgNPs also provoke oxidative stress into the cell through ROS generation (Limbach *et al.*, 2007). The dangerous effect of ROS on the viability of the cell and sanity of DNA is a well-known fact (Simonian and Coyle, 1996).

The results of this study revealed that AgNPs significantly increased serum level of MDA (Lipid peroxidation) when compared to control group which may denote increased oxidative stress especially with the high doses. The results stand in

support with other studies which showed increased serum and tissue levels of MDA after AgNPs administration in rats and mice in comparison to control non treated group (Adeyemi and Faniyan, 2014; Moradi *et al.*, 2018). Moreover, the results of this study showed significantly reduced serum levels of SOD, CAT and TAC after oral administration of AgNPs for 3 months. These results were in agreement with the result reported by Ansar *et al.* (2017) who administered AgNPs to rats intraperitoneally (5 mg/kg/day) while it disagreed with the study of Adeyemi and Faniyan, (2014) in which they reported an increase level of SOD after exposure to different doses of AgNPs.

Increased serum level of MDA together with reduced serum levels of GSH, SOD and TAC indicates accumulation of reactive oxygen species (ROS) and oxidative damage. These results are consistent with other studies that reported ROS induction and oxidative stress has beenimplicated as reasons of toxicity following AgNPs administration (Patlolla *et al.*, 2015a; Skalska *et al.*, 2016).

Once nanoparticles enter the body, they may become systemically available regardless of administration route and owing to their extremely small particle size, they may be retained in organs and cause toxic effects (*Xue et al.*, 2012).

From this study we can conclude that silver in the form of nanoparticles causes changes in the exposed rats especially in the oxidative status. These changes in the oxidative parameter such as decrease in catalase and superoxide dismutatse enzymes and total antioxidants capacity, and on the other hand increase in MDA may enhance the liberation of the free radicals which could result in oxidative stress. This oxidative stress suppress of the immune system which the main defense mechanism against the any abnormal effect inside the body (either infection or effect of xenobiotics) for human and animals. So, good efforts must be done to decrease the levels of this metal in the environment by using of many adsorbable materials that decrease reaching of silver (specially that of low molecular sizes) to the human and animal bodies to avoid its harmful effects.

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تأثير جزيئات الفضة متناهية الصغر علي الاجهاد التأكسدي في ذكور الجرذان البيضاء

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تم تصميم هذه الدراسه لتقييم السمية المحتملة لجزيئات الفضة متناهية الصغر علي الاجهاد التأكسدي في ذكور الجرذان البيضاء . أستخدم في هذه الدراسة مائة جرذ ذكر أبيض ، قسمت إلي أربع مجموعات كل منها به خمس وعشرون. أستخدمت المجموعة الأولي (G1) كمجموعة ضابطة للتجربة حيث تم تركها دون اي معاملات و المجموعة الثانية (G2) تعرضت الي جزيئات الفضة متناهية الصغر بجرعة 5 ملليجرام/كيلو جرام من وزن الجسم عن طريق الفم باستخدام انبوب المعدة ٥ ايام في الاسبوع لمدة اثنتى عشر اسبوعا. أما المجموعة الثالثه (G3) تعرضت الي جزيئات الفضة متناهية الصغر بجرعة 25 ملليجرام /كيلو جرام من وزن الجسم عن طريق الفم باستخدام انبوب المعدة ٥ اياماسبوعيا لمدة اثنتى عشر اسبوعا. وأخيرا تعرضت المجموعة الرابعة (G4) الي جزيئات الفضة متناهية الصغر بجرعة 50 ملليجرام /كيلو جرام من وزن الجسم عن طريق الفم باستخدام انبوب المعدة ٥ اسبوعيا لمدة اثنتي عشر اسبوعا . تمذبح سبعة فئران من كل المجموعات التجريبية بعد أربعة وثمانية واثنتي عشر اسبوعا من بدء التجربة لجمع عينات الأنسجة والدم .

تم في هذه الدراسة جمع عينات الدم بدون مضاد للتخثرلفصل المصل لقياس مستويات بعض الدلالات البيوكيميائية (الليبيد بيروكسيد , MDA سوبر أوكسيد ديسميوتيز ,SOD كتاليز ,CAT توتال انتي اوكسيدينت TAC) باستخدام جهاز الاسبكتروفوتومتر. تم جمع عينات الأنسجة (الكبد والمخ والكليتين والرئتين) وحساب أوزانهم.

أظهرت نتائج هذه الدراسة ان التعرض لجزيئات الفضة متناهية الصغر ادت إلي:- حدوث تغيرات غير معنوية في وزن الجسم (المجموعة الثانية والثالثة والرابعة) مقارنة بالمجموعة الضابطة، كما أوضحت الدراسة أن التعرض لجزيئات الفضة متناهية الصغر أدت الي تغييرات غير معنوية في أوزان الكبد والمخ والكليتين والرئتين بالمقارنة مع المجموعة الضابطة.

أوضحت الدراسة البيوكيميائية عن حدوث زيادة غير معنوية في مستوي MDA في المجموعة الثانية في الشهر الأول مقارنة بالمجموعة الضابط في حين حدثت زيادة معنوية خلال الشهر الثاني والثالث أما للمجموعتين الثالثه والرابعه فكانت الزياده معنوية في الشهر الاول والثاني والثالث مقارنة بالمجموعة الضابطة.

أظهرت قياسات ال Catalase تغيير غير معنوي للمجموعة الثانية خلال الشهر الاول والثاني وانخفاض معنوية خلال الشهر الثالث مقارنة بالمجموعة الضابطه في حين اظهرت المجموعة الثالثه والرابعة انخفاض معنوية في الشهر الاول والثاني والثالث مقارنة بالمجموعة الضابطه مع العلم انه في الشهر الاول والثاني الانخفاض كان غير معنوي بين المجموعتين .

أظهرت قياسات ال TAC و SOD تغيير غير معنوي للمجموعة الثانية خلال الشهر الاول والثاني وانخفاض معنوي خلال الشهر الثالث مقارنة بالمجموعة الضابطه في حين اظهرت المجموعة الثالثه والرابعة انخفاض معنوي في الشهر الاول والثاني والثالث مقارنة بالمجموعة الضابطه.

خلصت هذه الدراسه الى ان استخدام جزيئات الفضة متناهية الصغر أدى الى تغيرات فى مضادات الأكسده مما نتج عنه اجهاد تاكسدى والذى بدوره سوف يقلل ويضعف الجهاز المناعى فى الحد من انتاج المشتقات الحره وبالتالى عدم القدره على حماية الجسم من الملوثات البيئيه وفى مقاومة الأمراض. لذلك يجب الحد من استخدام مثل هذه المواد مثل الفضه مع الانتباه الى الجرعه المعطاه منها وكذلك وقت التعرض لهل لما قد تسببه من أضرار صحيه على كلِ من الأنسان والحيوان.