

Possible Role of Selenium Nano-particles on Gentamicin-Induced Toxicity in Rat Testis: Morphological and Morphometric study

Original
Article

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ABSTRACT

Background: Gentamicin (GM) is a broad spectrum antibiotic that is used worldwide. Exact mechanism of its testicular toxicity is still unknown. The present study aimed at studying the possible protective role of selenium nano-particles (SeNPs) on histological, biochemical, and morphometric changes in rat testis.

Aim of Study: The current study was designed to elucidate the possible role of SeNPs on GM induced toxicity in rat testis.

Materials and Methods: Twenty adult albino rats were divided into four groups, control, sham control, GM group, GM+ SeNPs group. The body weight (BW), testis weight, epididymis weight, seminal vesicle weight, prostate weight and serum testosterone level were determined. Testis sections were subjected to histological, morphometric, biochemical and statistical studies.

Results: GM induced significant (sig) reduction in weight of testes, accessory sex organs, sperm count, motility and testosterone level. The drug also caused sig decrease in the activity of testicular catalase (CAT) and reduced glutathione (GSH) content, and sig increase in malondialdehyde (MDA) and H₂O₂ levels. Sig increase in basement membrane (BM) thickness, area % of caspase3 immunoreexpression (IE) and in Ki67 count were recorded. Injection of SeNPs improved sig the previously mentioned changes.

Conclusion: GM induced morphological, morphometric and biochemical changes on reproductive organs particularly testis of adult male rats. SeNPs administration with GM ameliorated these adverse effects that might be due to its antioxidant activity.

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Key Words: Antioxidant, gentamicin, nanoparticle, selenium, testis.

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INTRODUCTION

Antibiotics such as GM, neomycin, streptomycin and ofloxacin are used frequently by urologists, andrologist and in *vitro* fertilization infection treatment or when high concentration of leukocytes is present in the semen of these patients^[1]. GM is an aminoglycoside broad spectrum antibiotic that has been approved in more than 100 countries worldwide. It is used especially against Gram-negative bacteria and streptococcus aureus^[2].

Nephrotoxicity, ototoxicity are a well-known adverse drawbacks of GM, although the exact mechanism assumed in that toxicity is not fully understood, but oxidative stress and reactive oxygen species (ROS) generation might be involved in the pathogenesis of toxicity^[3]. The spermatozoa membrane is full of polyunsaturated fat, which make it susceptible to oxidative stress induced damage^[4].

Selenium (Se), due to its toxicity it was ignored as a therapeutic agent. Se is an essential trace element, which is very important antioxidant to human health and its deficiency might cause serious health effects^[5]. It was

proved that Se deficiency might affect the mitochondria in goat spermatozoa as it has a protective effect on mitochondria membrane integrity^[6].

The physicochemical properties of nanoparticles, such as size, surface charge, and hydrophobicity, affect their mucosal absorption characteristics as smaller particles showed higher cellular uptake^[7]. SeNPs has a very wide range in biomedical applications as a nutritional and health improving supplements^[8]. SeNPs possesses a better antioxidant ability than other chemical forms of selenium with reducing the risk of selenium toxicity^[9].

Previously, it was assumed that SeNPs were useful to prevent cisplatin (CIS)-induced gonadotoxicity^[10]. As treatment with SeNPs significantly improved the serum testosterone, sperm quality and reduced CIS-induced free radical toxic stress and spermatid DNA damage in male rats.

The aim of the present study was to elucidate the possible role of SeNPs on GM induced toxicity in rat testis.

MATERIALS AND METHODS

Materials

Chemicals

GM manufactured by Memphis Co. for Pharm. and Chem. Ind. (MEMCO), Egypt; under authority of Schering-Plough Corporation, U.S.A, Cairo, Egypt. In the form of ampoules of 2 ml 40 mg/mL of gentamicin sulphate.

SeNps (40–45 nm particle size) were obtained from the Nano-Tech Egypt for Photo-Electronic, 6 October, Al Giza Egypt, as a sterilized solution, dispersed in phosphate-buffered saline (PBS) and ready for use. Transmission electron microscopy (TEM) was used for characterization of nanoparticles shape and size, on JEOL JEM-2100 high resolution microscope at an accelerating voltage of 200 kV. (Figure 1).

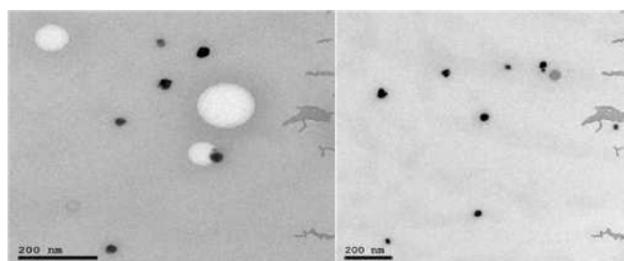


Fig. 1: Shows the TEM images of Nano selenium.

Animals

The current study was carried out on 20 adult albino rats, 3-5 months old weighing (180-220g). The rats were obtained from Animal House of Kasr-Alainy, Faculty of Medicine, Cairo University. Rats were housed for one week for environmental adaptation under standard laboratory conditions at 22-24 C with 12 hours light /dark cycle. They were fed on a constant adequate nutrition diet and allowed free access to drinking water ad libitum. The experimental work was conducted in accordance with the guidelines of the Committee of Laboratory Animals at Kasr-Alainy.

Rats were divided into four groups as follow

Control group: consist of five rats received nothing.

Sham control group: consist of five rats received SeNPs 0.5 mg/kg intraperitoneal (IP) for 6 successive days^[11].

GM-treated group: consist of five rats received GM 100 mg/kg IP for 6 successive days^[2].

GM+ SeNPs group: consist of five rats received both gentamicin and selenium.

SeNPs will be administered IP to rats 1 hr after the GM treatment with same dose and duration mentioned above. All rats were sacrificed by Cervical dislocation after light ether anaesthesia^[12].

Methods

- Determination of the body weight (BW) of all rats in different groups.
- Before sacrifice, blood was collected from the tail veins of animals belonging to each group using capillary tubes for assessment of serum testosterone.
- Median abdominal incision was performed and organs were dissected. Testes, epididymis, seminal vesicle and prostate were removed and weighed. The absolute organ weights and relative weights (organ weight /body weight X100) were measured for each rat and recorded. Both testes were fixed in 10% formol saline for 24 hours.
- Epididymal sperm motility percentage: abnormalities and Live- sperm Percentages: A drop of freshly undiluted semen solution from the cauda epididymis was mixed with one drop of physiological saline on the slide. The progressively motile sperm percentage was evaluated microscopically under high power lens^[13].
- Epididymal Sperm Count: epididymal spermatozoa were counted by a modified method^[14]. The count was done through mincing of epididymis in 5 ml saline, then in a rocker for 10 min at room temperature. About 10 μ l of semen was transferred to a chamber of Neubaur haemocytometer (Depth 0.1 mm; LABART, Germany) then put for 5 min before counting with light microscope at high power lens.
- Quantitative polymerase chain reaction (qPCR): formalin-fixed paraffin-embedded (FFPE) testes specimens by qPCR were performed^[16]. Reverse Transcription is carried out with the SuperScript First-Strand Synthesis System for reverse transcriptase (RT)-PCR^[17]. The following ribonucleic acid (RNA)/primer (Table 1) mixture was prepared in each tube: 5 μ g total RNA and 3 μ l random hexamers. The specimens were put at 65°C for 5 min and ice for only one min. Reaction master mixture was prepared for each reaction then added to the RNA/primer mixture and mixed briefly, after that placed at room temperature two minutes. 1 μ l (50 units) of SuperScript II RT was added to each tube, mixed and incubated at 25°C for 10 min. The tubes were incubated at 42°C for 50 min, heat inactivated at 70°C for 15 min, and then chilled on ice. 1 μ l RNAase H was added and incubated at 37°C for 20 min. The 1st strand complementary deoxyribonucleic acid (cDNA) was stored at -20°C until use for real-time PCR. The primer concentrations were normalized, gene-specific and reverse primer pair were mixed. A

copy of the setup file was saved and all PCR cycles were deleted [used for later dissociation curve analysis]. 50°C 2 min, 1 cycle, 95°C 10 min, 1 cycle, 95 °C 15 seconds -> 60 °C 30 seconds -> 72 °C 30 seconds, 40 cycles and 72°C 10 min, 1 cycle. A real-time PCR reaction mixture can be either 50 µl or 25 µl. After PCR is finished, the tubes were removed from the machine.

Table 1: The primer sequence of the studied Steroidogenic acute regulatory gene (STAR) and 3 Hydroxy steroid dehydrogenase (3HSD) gene

	Primer sequence
STAR	Forward: 5'-GGGCATACTCAACAACCAG-3' Reverse: 5'-ACCTCCAGTCGGAACACC-3'
3HSD	Forward :5-TGTGCCAGCCTTCATCTAC-3' Reverse :5'-CTTCTCGGCCATCCTTTT-3'
Beta actin	Forward primer:5'--GGTCGGTGTGAACGGATTGG -3 Reverse primer:5'- ATGTAGGCCATGAGGTCCACC-3

• Oxidative and antioxidative parameters

1. Measurement of Malanodialdehyde (MDA) concentration, Glutathione (GSH) concentration and Catalase (CAT) activity: half the specimens were kept in the deep freezer for homogenization. 1 gm of muscle was homogenized in 10 ml normal saline by using homogenizer (Ortoalresa, Spain). Then centrifuged at 1000 X g for about 10 minutes. It was collected in and kept in the deep freezer (at -20°C) reduced GSH, CAT and MDA^[18].
2. Estimation of Hydrogen Peroxide (H₂O₂) levels were measured by Pick^[19]. 100 uL of tissue homogenate was prepared in Tris-HCl buffer (20 mM, pH7.4) and 100 uL of assay solution (containing 0.2 mL phenol red,0.2 g/L and 0.2 mL of horse radish peroxidase, 20 U/mL in potassium phosphate buffer, 0.05 M, pH 7.0, and 9.6 mL of 0.9% NaCl) was taken and reaction was started by the addition of 10 uL of 1.0 N NaOH. Absorbance was recorded at 600 nm in a microplate using ELISA reader. Hydrogen peroxide standard curve was plotted by taking different concentrations of H₂O₂ for assay of H₂O₂, ranging from 20 to 100 mmol in a total volume of 100 uL and processed in the same way. Results are expressed as mmol H₂O₂ formed/mL homogenate.

• Paraffin blocks were prepared and 5µm thick sections were subjected to the following studies

1. Histological and histochemical study: Hematoxylin and eosin (H and E) stain^[20]. PAS stain^[21].
2. Immunohistochemical study: Caspase3 immunostaining^[22], the marker for apoptosis. 7ml of rabbit polyclonal Ab (RB-1197-R7) (Lab Vision Corporation, USA) was prediluted ready to use solution stored at 2-8°C. The +ve tissue control was a specimen of human tonsil. Caspase 3 +ve cells showed cytoplasmic reaction.

Ki 67 immunostaining^[23] polyclonal rabbit antirat 1ry Ab (MKI67/NB110-897171) mg/ml, nuclear reaction, proliferation marker, dilution 1:100-1:500. +ve control tonsil, -ve control ommit application of 1ry Ab, apply for 60 minutes.

• Morphometric study: Using Leica Qwin 500 LTD (Cambridge, UK) computer assisted image analysis system, thickness of the basement membrane (BM) in PAS stained sections and count of +ve nuclei in Ki 67 were done in immuno-stained sections using interactive measurements menu. The area % of caspase3 immuno expression (IE) and optical density of caspase3 IE were estimated using binary mode. The measurements were done in 10 high power fields.

• Statistical study: Quantitative data were summarized as means and standard deviations and compared using one-way analysis-of-variance (ANOVA). Any significant ANOVA was followed by post hoc Tukey test to detect which pairs of groups caused the significant difference. *P-values* <0.05 were considered statistically sig^[24]. Calculations were made on Statistical Package of Social Science (SPSS) software version 16.

RESULTS

Mean BW, testes and reproductive organs weight

No clinical signs of toxicity were noticed neither in GM group nor in GM+SeNPs groups. However, a sig decrease was found in BW, absolute and relative weights of testes, seminal vesicles, prostates and epididymis in GM group when compared to the other groups. In contrast, the previously mentioned weights of reproductive organs were recorded to be significantly increased in the GM + SeNPs treated group when compared with GM group. (Table 2, Histogram 1).

Hormonal, sperm count and motility changes

Serum testosterone, sperm count and motility were sig decreased in both GM-treated and combined GM+SeNPs groups compared with control (Table 3, Histogram 1).

Oxidative and antioxidative parameter changes

Measurement of the serum contents GSH, CAT were sig decreased in GM group compared with other three groups. While, MDA and H₂O₂ were sig increased in GM than other groups. Star and 3HSD genes in GM group, SeNPs groups were decreased sig compared with the control group. On the other hand, sig increase was detected in SeNPs+GM group compared to GM group (Table 3, Histogram 1).

Histological and histochemical changes

Histological examination showed normal appearance of seminiferous tubule arrangement and cellular lining (spermatogonia, spermatocytes and spermatids) with minimal connective tissue in between in both control and Sham groups (Figures 2a,b,c). While in GM group, wide spacing between the tubules appeared and some tubules revealed minimal spermatogenic cell lining

(Figure 2d). High magnification demonstrated lining spermatogonia only (Figure 2e) and other tubules with obvious vacuoles among the lining cells (Figures 3g,3h). In Gm+SeNPs, the tubules were apparently normal with congested vessels in between (Figure 2f). Restoration of normal spermatogenic lining with residual minimal vacuolation and congested vessels were seen (Figures 3g).

Sections in the testis of rats stained by PAS showed multiple seminiferous tubules with apparently normal BM with normal thickness in control (Figures 4a, 4b). In GM group thickened BM of some tubules, showed wavy BM, folded BM and thinning of BM with focal disruption (Figures 4c,4d,4e, 4f). In GM+SeNPs group some tubules showed thinning of the BM (Figure 5g) and others revealed partially thickened BM (Figure 5h).

Immunohistochemical changes

Weak positive reaction of caspase 3 IE was detected in few interstitial cell in control group (Figure 6a), with cytoplasmic reaction of Leydig cells (Figure 6b). In GM

group increased density of IE in the interstitial cells of some areas (Figure 6c), +ve IE in some cells lining some tubules (Figure 6d) with increased density and distribution of IE in other areas (Figure 6e). In GM+SeNPs group minimal increase in density and IE were found in some interstitial cells (Figure 6f). Regarding Ki67+ve nuclear IE was detected in few interstitial cells in control group (Figure 7a). While in GM group +ve nuclear IE in some interstitial and spermatogenic cells were observed (Figure 7b), in GM+SeNPs group +ve nuclear IE in was evident in multiple interstitial and spermatogenic cells (Figure 7c).

Morphometric changes

The mean BM thickness in PAS stained sections, mean area % of caspase 3 IE and optical density of caspase3 IE detected indicated a sig increase in the GM group compared to other groups and in Se-treated group compared to the control groups. Regarding count of Ki67 +ve nuclei a sig increase in GM group was evident as compared to control groups and sig increase in GM+SeNPs group as compared to other groups (Table 4, Histogram 2).

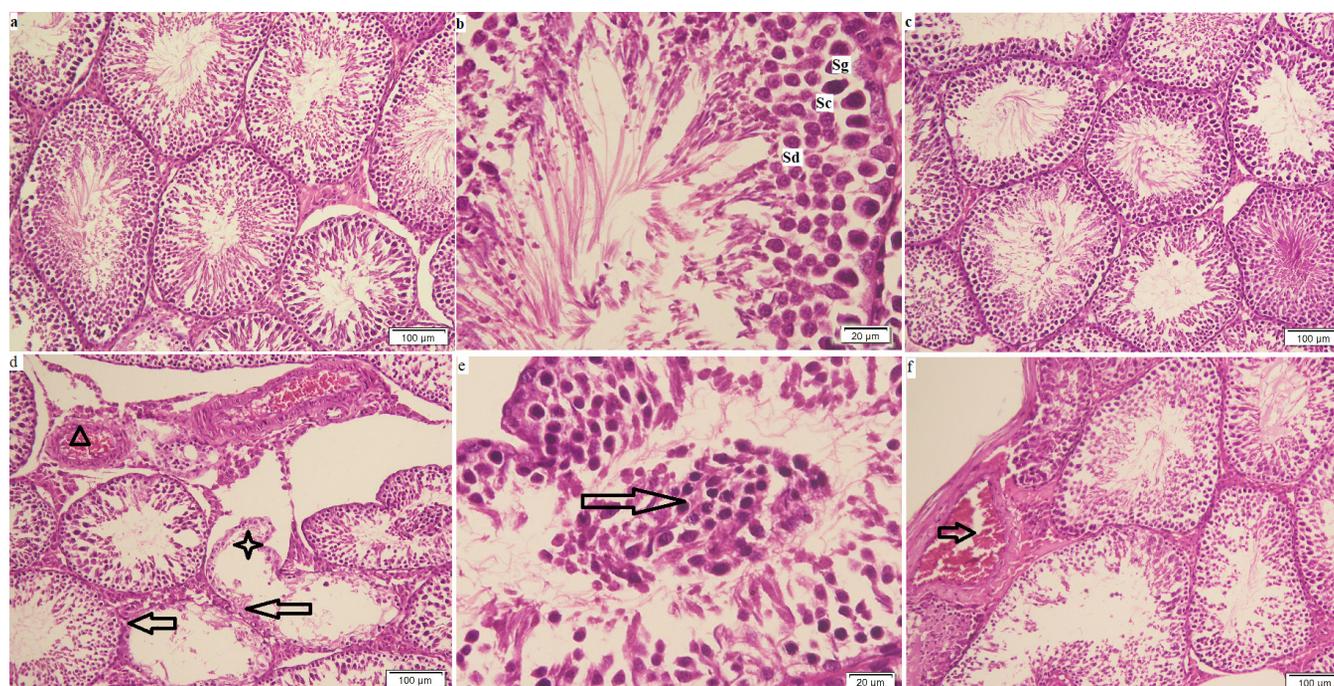


Fig. 2: Sections in the testis of rats (H and E) representing: a) and c) control and sham control apparently normal seminiferous tubules with minimal connective tissue in between (H and Ex100). b) higher magnification of control group showing apparently normal spermatogonia (Sg), 1ry spermatocytes (Sc) and spermatids (Sd) (H and Ex400). d) GM group showing a distorted tubule with reduced lining (star), two tubules with reduced lining (arrows) and two congested vessels (triangle) (H and Ex100). e) GM group showing dislodged spermatogenic cells inside the lumen of a seminiferous tubule (arrow) (H and Ex400). f) GM+SeNPs showing apparently normal tubules and a congested vessel (arrow) (H and Ex100).

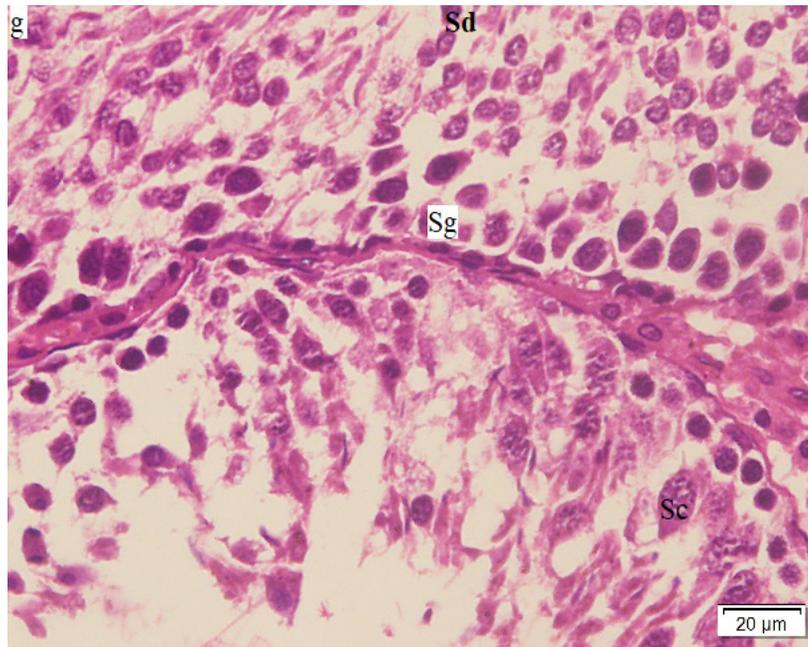


Fig. 3g: Sections in the testis of rats (H and Ex400) representing: g) GM+SeNPs showing restoration of normal spermatogenic lining spermatogonia (Sg), Iry spermatocytes (Sc) and spermatids (Sd).

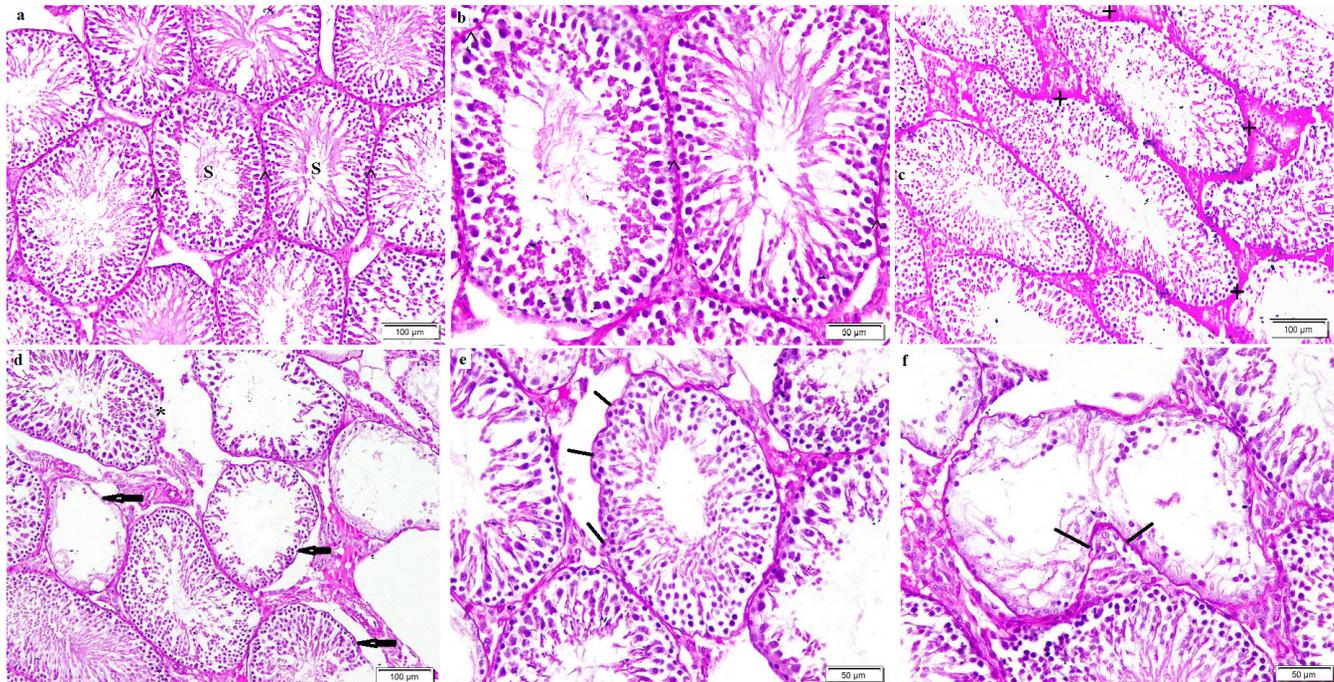


Fig. 4: Sections in the testis of rats (PAS) representing: a) control rat showing multiple seminiferous tubules (S) with apparently normal basement membrane (BM) (^)(PASx100). b) control rat showing normal thickness of the BM (^)(PASx200). c) GM group showing thickened BM of some tubule(+)(PASx100). d)GM group showing thinned BM (arrows)of some tubules and focal disruption (star) of BM of a tubule (PASx100).e) and f) GM group showing a wavy BM (lines) of a tubule and a folded BM (lines) of another respectively (PASx200).

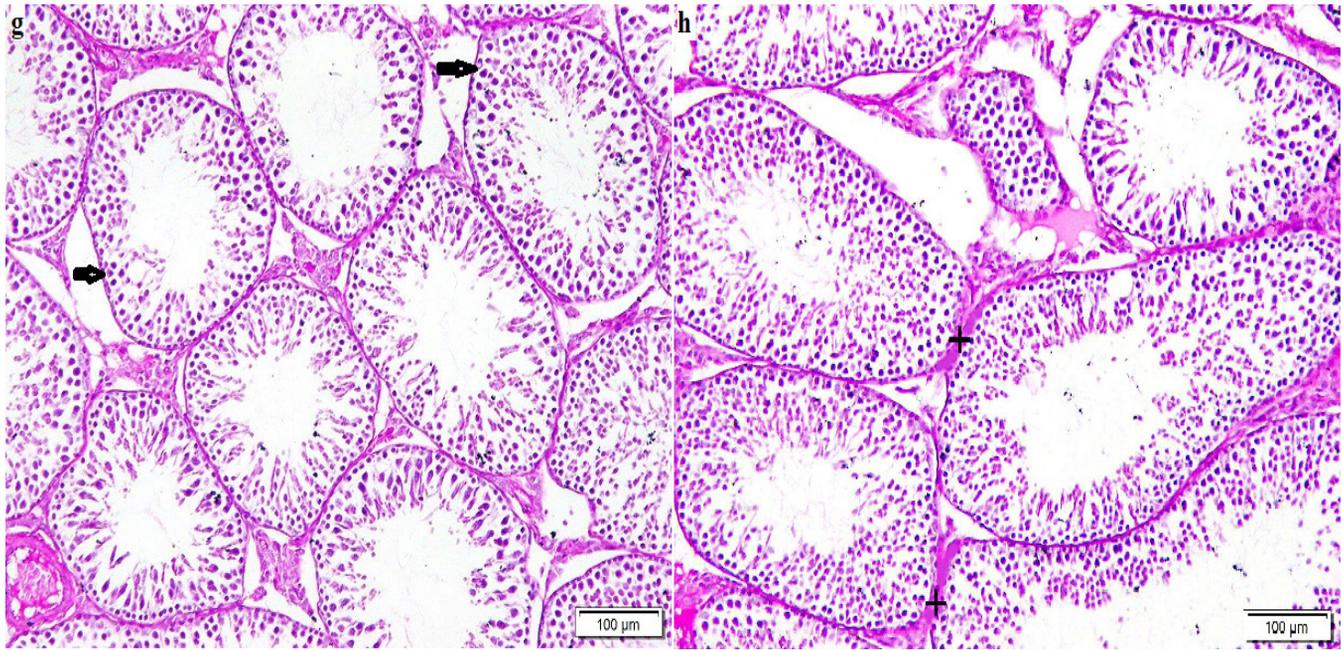


Fig. 5: Sections in the testis of rats (PASx100) representing: g) GM+SeNPs group showing some tubules with thin BM (arrows). h)GM+SENP group showing some tubules with partially thickened BM(+).

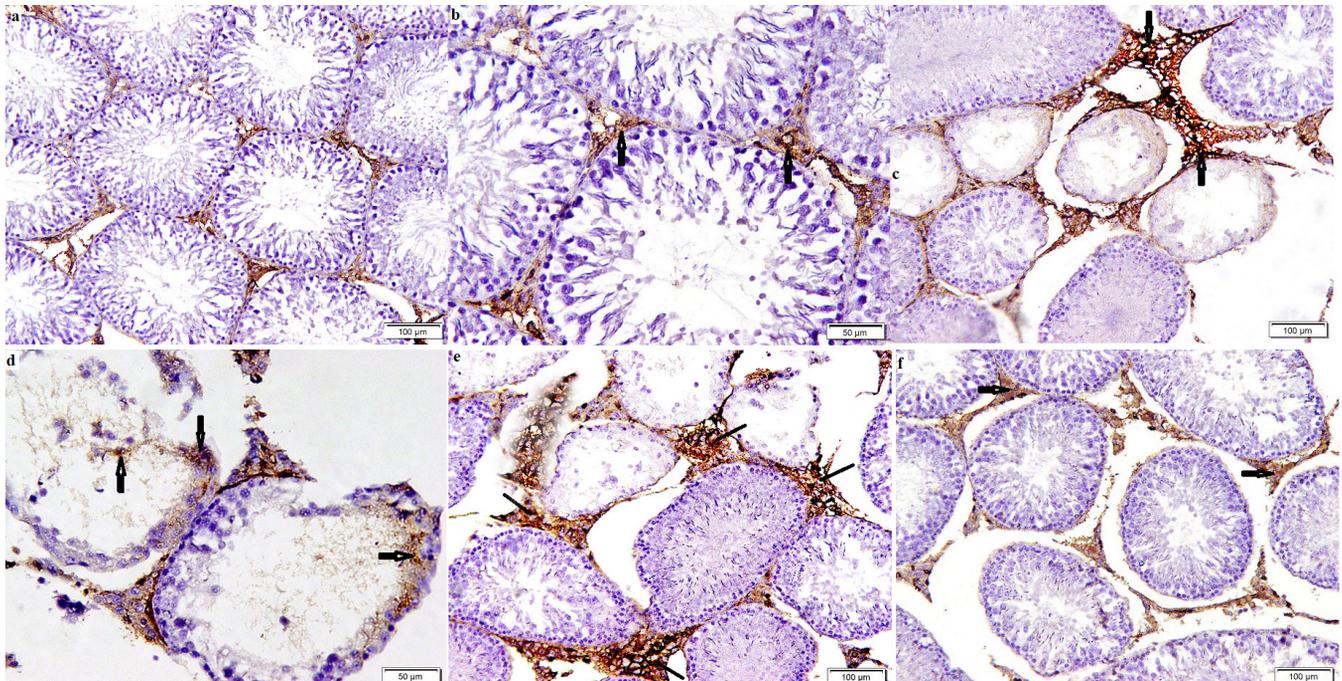


Fig.6: Sections in the testis of rats (Caspase3 immunostaining) representing: a) control rat showing weak +ve caspase3(IE)(arrow head) in few interstitial cells (caspase3 immunostaining x100).b)control rat showing cytoplasmic reaction of Leydig cells(arrows)(caspase3 immunostaining x200).c)GM group showing increased density of IE in multiple interstitial cells(arrows) (caspase3 immunostaining x100). d)GM group showing +ve IE in the cells lining two tubules (arrow)(caspase3 immunostaining x200).e)GM group showing increased density and distribution of IE(lines)(caspase3 immunostaining x100). f)GM+SeNPs group showing minimal increase in IE and density reaction (arrows) in some interstitial cells(caspase3 immunostaining x100).

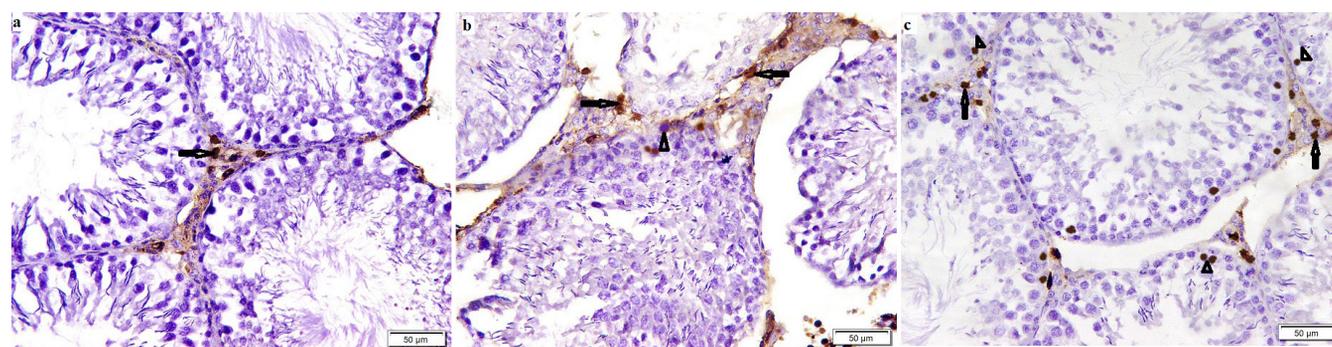


Fig 7: Sections in the testis of rats (Ki67 immunostaining , x 200) representing: a)control rat showing +ve nuclear IE in few interstitial cells (arrow).b) GM group showing +ve nuclear IE in some interstitial cells (arrows) and spermatogenic cells (triangle).c) GM+SeNPs group showing +ve nuclear IE in multiple interstitial cells (arrows) and spermatogenic cells (triangles).

Table 2: Absolute and relative reproductive organ weights in different groups.

	Control	Sham control	GM group	Gm+SeNPs	P value
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
BW	157.2±8.17	157.4±5.98	137.2*±3.11	158.2±6.76	<0.001
Testes weight	1.1±0.07	1.1±0.13	0.7*±0.03	1.0±0.03	<0.001
Epididymis weigh	1.1±0.06	1.1±0.11	0.8*±0.04	1.0±0.06	<0.001
Prostate weight	0.3±0.02	0.3±0.02	0.2*±0.01	0.3±0.02	<0.001
Seminalvesicle weight	0.7±0.02	0.7±0.03	0.3*±0.02	0.5*±0.08	<0.001
RelativeTestes weight	0.7±0.02	0.7±0.07	0.5*±0.03	0.6*±0.04	<0.001
Relative epididymis weight	0.7±0.07	0.7±0.07	0.6*±0.04	0.7±0.06	≤0.001
relative prostate weight	0.2±0.01	0.2±0.02	0.1*±0.01	0.2±0.02	<0.001
relative seminal vesicle weight	0.4±0.02	0.4±0.02	0.2*±0.02	0.3*±0.04	<0.001

* statistically sig different than other three groups.

Table 3: Showing oxidative and antioxidative parameter and gene expression changes.

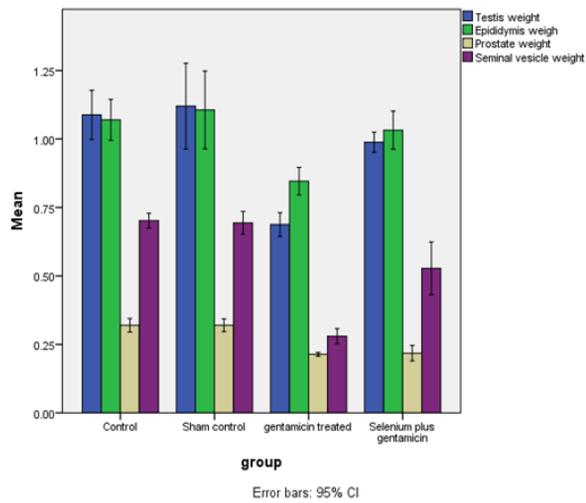
	Control	Sham control	GM group	SeNPs+GM	P value
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Sperm count X10 ⁶	33.2±0.08	33.1±0.10	21.7*±0.07	28.4*±0.05	<0.001
Sperm motility %	45.1±0.11	45.3±0.11	17.7*±0.09	29.1*±0.11	<0.001
Serum testosterone (unit)	1.5±0.11	1.4±0.08	0.8*±0.02	1.2*±0.08	<0.001
GSH (unit)	59.6±4.98	56.0±0.16	22.6*±0.10	49.2*±0.13	<0.001
CAT (unit)	121.7±0.13	121.6±0.10	76.2*±0.13	118.0*±0.18	<0.001
H ₂ O ₂ (unit)	3.2±0.09	3.0±0.18	12.8*±0.10	7.4*±0.11	<0.001
MDA (unit)	10.0±0.13	10.0±0.21	43.6*±0.23	21.5*±0.11	<0.001
STAR (unit)	1.1±0.08	1.1±0.11	0.2*±0.01	0.7*±0.01	<0.001
HSD3 (unit)	1.1±0.08	1.2±0.10	0.2*±0.01	0.7*±0.01	<0.001

* statistically sig different than other three groups.

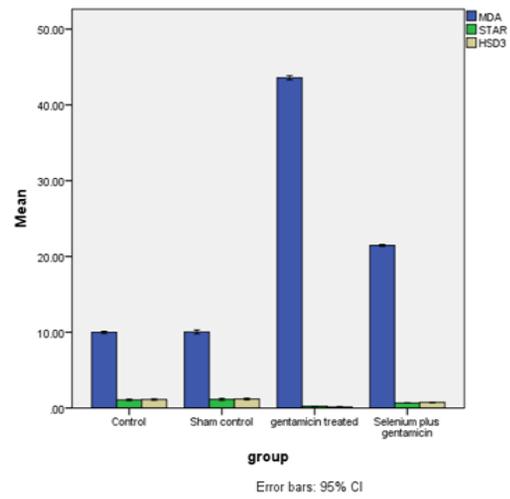
Table 4: Mean thickness of BM, area% of caspase3 IE, optical density of caspase3 IE and count of Ki67 +ve nuclei.

Group	Thickness BM	Area%	Optical density	Count of Ki67 +ve
Control	0.15±0.02	2.00±0.04	0.20±0.02	2.80±0.53
Control-Sham	0.16±0.01	2.03±0.05	0.25±0.01	2.90±0.50
GM	0.89±0.08*	9.43±0.21*	0.74±0.11*	5.80±1.09 [^]
SeNPs	0.29±0.03 [^]	4.68±0.16 [^]	0.43±0.12 [^]	11.50±2.49*

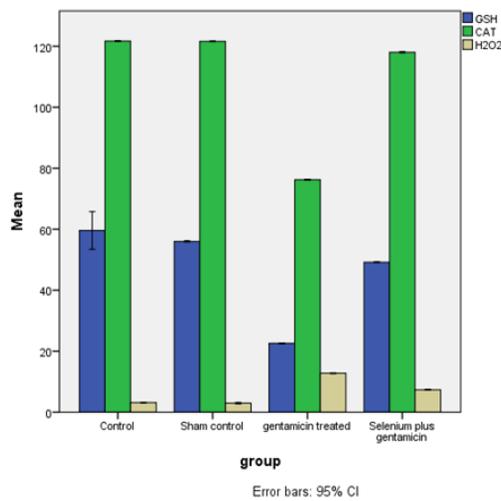
Sig $P \leq 0.05$. - [^] sig compared to control groups . - * sig compared to the other groups.



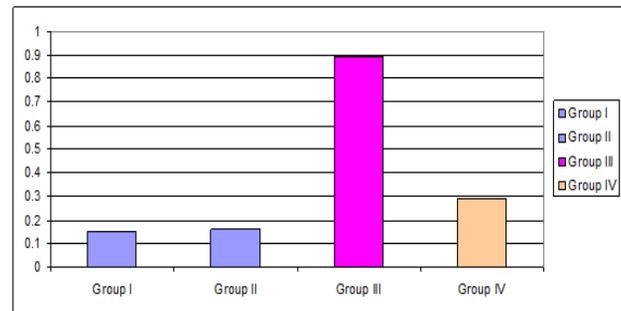
Histogram 1a: Mean organs weight



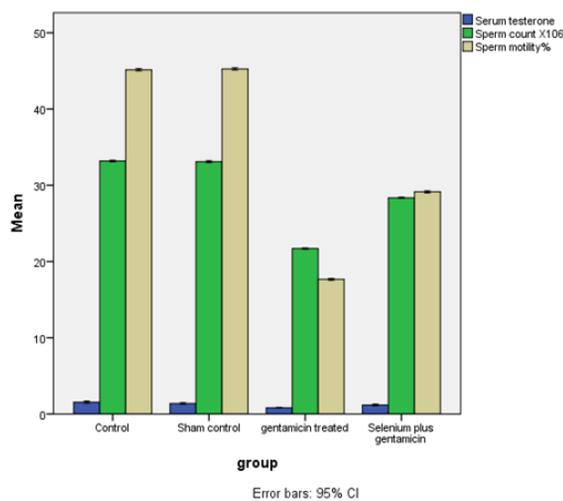
Histogram 1d: Mean of MDA, STAR and and HSD genes



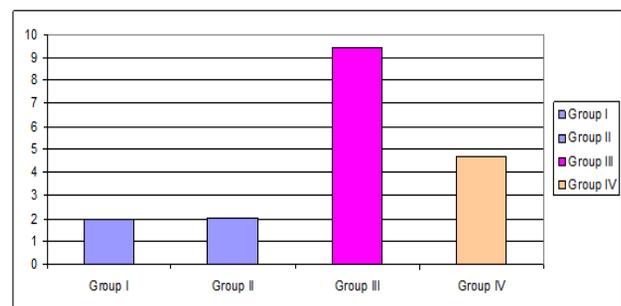
Histogram 1b: Mean GSH,CAT,H₂O₂



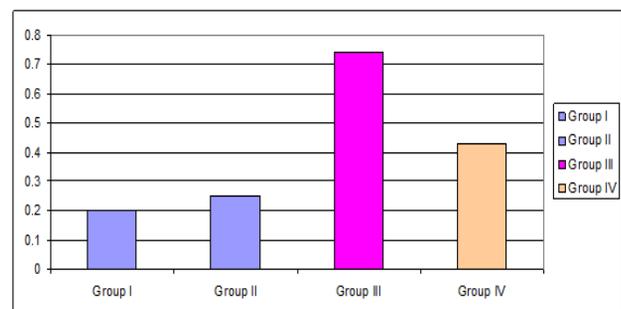
Histogram 2a: Thickness of BM



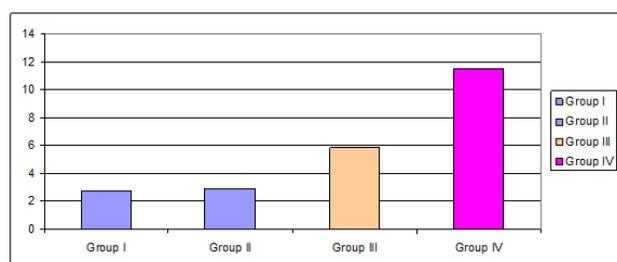
Histogram 1c: Mean serum testosterone sperm count and motility



Histogram 2b: Area% of caspase3 IE



Histogram 2c: Optical density of caspase3 IE



Histogram 2d: Count of Ki67 +ve nuclei

DISCUSSION

The present study investigated the biochemical, histological, histochemical, immunohistochemical and morphometric changes that may develop in the rat testis exposed to GM and the possible protective role of SeNPs. GM as a broad spectrum antibiotic used worldwide mainly by urologist but it affects male fertility that's why it raises the concern about studying the different protective modalities.

In the present study, GM influence testes and accessory sex organs weight as well sperm count and motility which showed sig reduction in GM group as compared to all other groups. This weight reduction might be due to decrease in the spermatogenic cell mass as well decreased seminiferous tubule size due to atrophy as a result of cell death. However, concomitant treatment with SeNPs showed slight improvement in the previous finding, that might be due to shorter duration of treatment. In agreement with these results, weight reduction was referred to direct cytotoxic effect of GM on accessory sex organs and their secretions^[2]. Same findings were reported by researcher who studied the effect of different aminoglycoside antibiotics such as gentamicin, neomycin and streptomycin on sperm count and motility^[25]. He added that GM sig decreased the epididymis, testes and seminal vesicles weights as well the sperm count and motility as a result of Leydig and spermatogenic cells destruction.

Oxidative stress is one of the most important causes of induction of different organs toxicity. In the current work, testicular homogenate content of GSH, CAT was sig decreased in GM group. While, MDA and H₂O₂ were sig increased than other groups which indicated the role of oxidative stress on testicular toxicity, therefore expecting these changes to be ameliorated by the antioxidant treatment as in the present work. That is in support, with a recent study which proved that free radicals have the potentiality to oxidize proteins, lipids and DNA^[26]. The authors added that the enzyme glutathione peroxidase (GPx) is a selenium dependent enzyme and its main function is H₂O₂ removal and it prevents formation of highly reactive hydroxyl (OH) radical.

It was explained that the spermatogenesis and Leydig cell steroidogenesis are vulnerable to oxidative stress due to very high rate of cell division and mitochondrial oxygen consumption in testicular tissue^[27]. In addition, it was stated that the testes contain many antioxidant enzymes and free

radical scavengers to ensure that both spermatogenic and steroidogenic functions of this organ not to be affected. Use of vitamin E supplement and selenium considerably improved sperm motility and viability. GPx use GSH as a source of electrons to reduce H₂O₂ to water. GPx was found in the mitochondria, nucleus and acrosomal domain of differentiating spermatozoa. Therefore animals fed on a selenium deficient diet exhibit a significant reduction of testicular GPx activity and an accompanying loss of germ cells from the germinal epithelium of the testes^[28].

Serum testosterone reduction found in the present study might be due to disturbed oxidant antioxidant state^[29]. This finding can relate decreased sperm count to gentamicin induced testosterone level reduction, being essential for normal spermatogenesis^[30]. Previously, it was suggested that necrosis of Leydig cell or diminished stimulation of the cells by interstitial cells stimulating hormone is the reason for testosterone reduction^[31].

In the present work, STAR and 3HSD genes in GM group were sig decreased as compared with the control group, both are prerequisites for testosterone production in Leydig cells of the mammalian testis. STAR protein regulates the transport of cholesterol to the inner mitochondrial membrane which is the rate-limiting step in steroid biosynthesis in various tissues^[15]. It is primarily expressed in Leydig cells in the testes, which make it essential for testosterone production and male fertility.

Histological and histochemical examination showed various morphological alterations in the seminiferous tubule, characterized by degeneration, reduced cell lining, decrease of spermatogenic cells numbers and BM thickening that might be as a direct effect of GM. Testicular testosterone concentration reduction might be the key factor in these degenerative changes^[30]. Another explanation for gentamicin induced testicular degeneration was assumed through the generation ROS as superoxide, H₂O₂ which is frequently used to produce oxidative and necrotic damages^[25].

Caspases are involved in proteins degradation and known to be participated in cleaving neighboring amino acids, once caspases are activated, there seems to be a sequence to cell death^[32]. The caspase-3 activates cytoplasmic endonucleases that degrade the nuclear materials and proteins then followed by DNA fragmentation^[33]. Area % of caspase 3 that have been measured in the current work revealed sig increase in GM group compared to the control which was obvious in interstitial cells than in the lining of seminiferous tubules. It has been implicated that caspase-3 activation is the principal factor in apoptotic cell death^[34]. Treatment with SeNPs sig reduced the area% of caspase3 IE that might be due to its antioxidant activity.

Ki67 is an antigen that is found during different cell cycle phases of proliferating cells and absent in cells that arrested in the G₀ phase^[35]. GM effect on testicular cells proliferation was done and assessed by means of Ki67 immunohistochemistry and highest count was measured in

SeNPs group indicating cellular proliferation and repair. It was postulated that the positive expression level of Ki-67 is a good response to spermatogenesis dysfunction^[35].

CONCLUSION

It can be concluded that GM induced morphological, morphometric and biochemical changes indicative of degeneration on the reproductive organs of the adult male rat particularly the testis. However, SeNPs elicited a sig protective role against GM-induced toxicity due to its antioxidant activity.

So the present study recommends that SeNPSs can be used concomitantly with the prescription of GM to reduce its adverse effect, and it can be proposed to be used for longer periods of time than that used in the present study to ensure more remarkable effect.

CONFLICTS OF INTEREST

There are no conflicts of interest.

LIST OF ABBREVIATION

GM: gentamicin.

SeNPs: selenium nanoparticles.

BW: Body weight.

Sig: significant.

CAT: catalase.

GSH: glutathione.

MDA: malonaldehyde.

BM: basement membrane.

IE: immunoexpression.

Se:selenium.

PBS: phosphate buffer saline.

TEM: transmission electron microscope.

IP: intraperitoneal.

qPCR: quantitative polymerase chain reaction.

FFPE: formalin fixed paraffin embedded.

+ve: Positive.

Ab: Antibody.

STAR:steriodgenic acute regulatory gene.

3HSD:3 hydroxy steroid dehydrogenase.

H₂O₂: hydrogen peroxide.

ANOVA: Analysis of variance.

C: Centigrade.

HandE: Haematoxylin and eosin.

PAS:Periodic acid Shiff.

SPSS: Statistical Package of Social Science.

OH: hydroxyl.

GPx: glutathione peroxidase.

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المخلص العربي

الدور المحتمل لجزيئات النانوسيلينيوم فى السمية الناجمة عن الجنتاميسين فى خصية الفئران: دراسة مورفولوجية وقياسية

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المقدمة: جنتاميسين هو مضاد حيوي يستخدم فى جميع أنحاء العالم. الآلية الدقيقة لسمية الخصية الناتجة عنه لا تزال غير معروفة. تهدف هذه الدراسة إلى دراسة الدور الوقائي المحتمل لجزيئات النانو سيلينيوم فى التغيرات النسيجية الناتجة والكيميائية الحيوية والقياسية فى خصية الفئران نتيجة استخدام الجنتاميسين.

الهدف من الدراسة: صممت الدراسة الحالية لتوضيح الدور المحتمل لجزيئات النانوسيلينيوم فى السمية الناجمة عن الجنتاميسين فى خصية الفئران.

المواد والطرق: تم تقسيم عشرون فأرا من فئران الألبينو إلى أربع مجموعات: مجموعتين مراقبتين، مجموعة الجنتاميسين ومجموعة الجنتاميسين مع النانوسيلينيوم. تم تحديد وزن الجسم، وزن الخصية، وزن البربخ، وزن الحويصلة المنوية، وزن البروستاتا ومستوى هرمون التستوستيرون. وفى نهاية التجربة تم أخذ عينات من الخصية وتم اعداد شرائح مجهرية وصبغها بالهيماتوكسولين والإيوسين ومن ثم القياسات المورفومترية والكيميائية الحيوية والإحصائية.

النتائج: الجنتاميسين يسبب انخفاض كبير فى وزن الخصيتين والأعضاء الجنسية التابعة مثل البربخ، الحويصلة المنوية، البروستاتا وعدد وحركة الحيوانات المنوية، ومستوى هرمون التستوستيرون. تسبب الدواء أيضا فى انخفاض نسبة الجلوتاثيون ونشاط انزيمات الأكسدة وانخفاض مساحة % من التعبير المناعي. عند حقن النانوسيلينيوم تم تحسين التغيرات المذكورة سابقا.

استنتاج: أدت إضافة النانوسيلينيوم إلى تحسين الآثار الضارة الناتجة عن استخدام الجنتاميسين فى خصية الفئران والتي قد تكون بسبب نشاطها المضاد للأكسدة.