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RESEARCH ARTICLE

Effect of *Moringa oleifera* leaves extract -SeNPs conjugate administration on testicular toxicity induced by melamine in rats

Mohamed F. Mansour[1], Ahmed H. Arisha[2*], Mohamed AlGamal[1], Ahmed A. Elsayed[3], Saydat Saad[1], Khlood M. El Bohi[4]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, 44511, Zagazig, Egypt

[2]Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44511, Zagazig, Egypt

[3]Department of Photochemistry, Industrial Chemical Division, National Research Center, Dokki, Giza, 12622, Egypt

[4]Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Zagazig University, 44511, Zagazig, Egypt

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Abstract

This work was designed to evaluate the role of selenium nanoparticles loaded on *Moringa oleifera* leaves extract (MOLE-SeNPs conjugate) on the testicular toxicity induced by melamine in rats. Forty male Albino rats of 10 weeks old were assigned to four groups; control group, group 2; SeNPs group (0.5 mg/kg BW orally) for 6 weeks, group 3; melamine (800 mg/kg BW orally) for 6 weeks and group 4; melamine followed by MOLE-SeNPs conjugate (200 mg/kg BW orally) for 6 weeks. Reproductive parameters; sex steroid hormonal level, molecular analysis of the transcriptional levels of steroidogenic enzymes and testicular histopathology were evaluated. In the melamine (MA) group, significant decrease in the semen quality, testicular weight, gonadosomatic index, FSH, LH, free testosterone and total testosterone were detected in comparison to the control group, however, estradiol (E2) level was significantly elevated (187.45%) in comparison to the control group. These parameters were significantly reversed in the MOLE-SeNPs conjugate group. Moreover, a significant decrease (45.21% and 23.75%) was detected in steroidogenic enzyme genes (CYP11A1 and HSD17B3) in the MA group than in the control one ($P < 0.05$). However, the transcriptional level of aromatase gene (CYP19A1) showed a significant increase (171.28%) in testicular tissue in comparison to the control group. The MO-SeNPs conjugate treatment increased the expression of CYP11A1 and HSD17B3 genes in testicular tissue but the transcriptional level of aromatase gene (CYP19A1) showed a significant decrease in comparison with the melamine group. The levels of testicular antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) showed a significant decrease in the MA group than the control group. These changes were meaningfully increased in the MO-SeNPs conjugate treated group in comparison to the melamine group. Histopathologically, the testicular tissue indicated improvement of injury in the concurrent MA+ MO-SeNPs conjugate group in comparison to MA group. In conclusion, MO-SeNPs conjugate can ameliorate the toxic impacts of melamine on testicular tissue in rats mainly via affecting the steroidogenic pathway.

Keywords: *Moringa oleifera* leaves, selenium nanoparticles, melamine, testicular toxicity, steroidogenesis, and semen picture.

Introduction

Spermatogenesis and steroidogenesis are important processes that take place in the testes which requires a high level of energy. Disruption in energy metabolism may lead to impairment in the cells of testis [1]. The polyunsaturated fatty acids (PUFA) are abundant in testicular tissue and in the plasma membrane of sperm to contribute in the fluidity of the sperm structure

***Corresponding author e-mail: (vetahmedhamed@zu.edu.eg), Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt.**

that is essential for capacitation correlates to fertilization. The PUFA are extremely liable to be damaged by reactive oxygen species (ROS) [2]. Sperm motility and viability declined by ROS [3]. *Moringa oleifera (MO)* is an edible plant generally named as a Drumstick tree [4]. The analgesic, anti-inflammatory anti-oxidant, anti-toxic, anti-cancer hepatoprotective, hypocholesterolemic, and anti-ulcer impacts in the gastrointestinal tract of extracts from many components of the plants were evidenced by several experiments in researches [5]. Many reports declared that *Moringa oleifera leaves (MOL)* can enhance the toxicity of the testis that is caused by chromium and cyclophosphamide [6, 7].

Selenium (Se) plays an essential role in various biological mechanisms. It has a role in the trapping of free oxygen particles and removing and remodeling of these particles into stable ones [8]. Selenium is the main component in seleno-enzymes, as glutathione peroxidase and thioredoxin reductase that reduce specific oxidized particles and conserve DNA and other ingredients from oxidative damage [9]. Several investigations have revealed that Se has an essential role in the fertility of males [10].

Selenium nanoparticles (SeNPs) has a great interest among researchers in response to its high bioavailability and low toxicity as well as nanometer molecules revealed novel properties, as large surface area, active centers, high level of catalytic and adsorbing capability [11]. Multiple investigations revealed that SeNPs are more efficient than selenite, selenomethionine, and methyl-selenocysteine in improving the seleno-enzymes, and downregulating its toxicity [12, 13].

Melamine (2,4,6-triamino-1,3,5-triazine), a chemical compound generally used in the synthesis of plastics, coatings, filters, glues, dishware, and also kitchenware [14]. Many reports declared that the administration of melamine separately or with cyanuric acid may lead to the toxicity of testis. Only one report investigated that melamine can increase the abnormality rate and destruction of DNA of sperms in mice [15].

The main aim of this study was to assess the ameliorative effects of *Moringa oleifera* leaves extract and selenium nanoparticles on the semen picture, male sex hormones, antioxidant status, steroidogenic gene expression and histopathological examination of the testis in melamine-induced testicular toxicity in rats.

Materials and Methods

Moringa oleifera and the aqueous extraction procedure

Moringa oleifera leaves *extract* that were extracted according to Okechukwu *et al*. [16] was obtained from the Egyptian Scientific Society of Moringa National Research Centre, Dokki, Giza. Melamine (99.5 % purity) from Alpha Chemica (Mumbai, India) was diluted with distilled water to form a working stock solution to be used further in this study. According to Adams [17], the GC-MS analysis of *Moringa oleifera* ethanol extract was performed on an 1310 TRACE GC Ultra Gas Chromatographs (Thermo Fisher Scientific Inc., Waltham, MA, USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) Regional Center for Mycology and Biotechnology, Al Azhar University Campus, Nasr city, Cairo, Egypt. The separated ingredient of the extract has been fixed with the coordinatinon of their mass spectra published by the National Institute of Standards and Technology (NIST) statistics.

Synthesis of Moringa oleifera extract in-situ Selenium Nanoparticles (SeNPs)

Dry *Moringa oleifera* leaves (20 g) have been ground by a mortar. The resulting powder was suspended in distilled water (100 mL) was heated at 60°C under stirring for 1 h; the formed suspension has been filtered. The *Moringa oleifera* extract (filtrate, 10 mL) was diluted in deionized water (90 mL) in a conical flask. Selenious acid (H_2SeO_3, 0.013 g, 0.01 mmol) in 10 mL deionized water was added to this extract, with continuous stirring and heating at 60°C for 1h; forming in situ after that 200 µL of 40 mM ascorbic acid was added as a catalyst the ruby red SeNPs were suspended. The formation of selenium nanoparticles was confirmed and characterized by UV Spectrophotometer and transmission electron microscope (TEM). Every 1 cm^3 of the conjugate (MO-SeNPs) contains 130 µ of selenium nanoparticles with 0.1 mL of Moringa Oleifera.

Animal grouping and treatment

Forty mature Albino male rats, 10 weeks old, weighing about 140 - 160 g were used as experimental animals in this work. They were purchased from Laboratory Animal Farm at Zagazig University. The animals were allowed to acclimatize for 2 weeks. The rats were housed in cages made from stainless steel at standard temperature ranged from 21 – 25ºC and humidity ranged from (50 – 60 %) within a 12 h light- dark cycle.

The protocol of this study was accepted by the ethics committee at Zagazig University (acceptance number ZU-IACUC / 2/ F/ 163/ 2019). Albino rats were allocated within 4 equal groups (10 rats), group 1 (control group): in which, each rat received distilled water (1 mL/daily) for 6 weeks, group 2: SeNPs control group in which each rat received a single oral dose of 0.5 mg/kg BW for 6 weeks [18], group 3: each rat received 800 mg /kg BW. orally,once daily for 6 weeks of melamine [19]. Group 4: each rat was given one oral dose of 800 mg /kg BWdaily for 6 weeks of melamine 1 h before (MO-SeNPs) conjugate (0.2 mg /kg BW. orally, daily for 6 weeks). The experiment continued for 6 weeks.

Sampling

When the experiment ended, blood samples were collected [20] and centrifuged then separated and stored at −20^{o}C. Testes were cut out and weighed then rinsed by cold saline. Testes samples were divided into three parts one was stored in -80 ^{o}C for molecular investigation, another part was homogenized to measure the first line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The last part was kept for histopathological evaluation by using 10 % neutral buffered formalin.

Body and testicular (relative & absolute) weight estimation

Body weight was calculated weekly for 6 weeks. Testes were immediately harvested then weighed after decapitation (absolute). The relative weight of the gonadosomatic index (GSI) of the testis was determined as follow:

$$GSI = \frac{\text{Gonad weight}}{\text{Bodyweight}} \times 100$$

[20]

Evaluation of sperm characteristics

The epididymal caudal part was removed and evacuated into a clean Petri dish with normal saline, this suspension treated nearly as in semen [21-24]. The solutions and equipment were sterilized at 37 °C. Briefly; one drop of the suspension was examined under high power to evaluate the motility of the sperm. Another drop stained with eosin nigrosine (EN) to determine the live/dead sperms ratio (viability ratio). Briefly, semen was mixed with saline 1: 4 and a small amount of formalin (40 %). Hemocytometer was used to count the spermatozoa in this mixture [25]. The stained smears were examined for estimating the percentage of sperm deformities after examining at 100 spermatozoa [26].

$$\text{sperm cell count} = \text{total number of spermatozoa} \times 4 \times 2500 \times \text{dilution factor}$$

Biochemical parameters:

Superoxide dismutase (SOD), CAT and GPx have been estimated in testis homogenate as previously described [27-31]. Shortly, a considerable amount of the homogenate was centrifuged at 10,000 rpm at 4^{o}C for 30 min. The supernatant was used for evaluation of CAT, SOD, GPx activity by kits purchased from Bio diagnostic Company, Dokki, Giza, Egypt.

Hormonal evaluation

The hormonal level of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), total and Free testosterone were evaluated by rat ELISA kits applied from MyBioSource (San Diego, CA, USA) [32].

Quantitative real-time PCR analysis

The real-time analysis of the steroidogenic enzyme genes (CYP11A1 and HSD17B3) and aromatase gene (CYP19A1) expression in the testicular tissue was performed as reported previously [21, 23, 24]. For extraction of total RNA, Trizol reagent Invitrogen (Thermo Fisher Scientific; Waltham, MA, United States), and HiSenScript™ RH (-) cDNA Synthesis Kit (iNtRON Biotechnology Co., South Korea) for cDNA synthesis were used according to the manufacturer instruction. The amplification reaction contained 4µL of the 5 x HOT FIRE Pol Eva Green qPCR Mix Plus (Solis Bio Dyne, Tartu, Estonia) , 0.5 of both forward and reverse

primers, 1µL of cDNA and 14µL of RNase/DNase free water. The used primers targeting CYP11A1 gene (F 5′–AAG TAT CCG TGA TGT GGG 3′), (R 5′–TCA TAC AGT GTC GCC TTT TCT 3′), CYP17A1 gene (F 5′–TGG CTT TCC TGG TGC ACA ATC 3′), (R 5′–TGA AAG TTG GTG TTC GGC TGA AG 3′), HSD17B3 gene (F5′AGT GTG TGA GGT TCT CCC GGT ACC T 3′), (R5′TAC AAC ATT GAG TCCA TGT CTG GCC AG 3′), CYP19A1 gene (F5′-GCT GAG AGA CGT GGA GAC CTG 3′) , (R5′CTC TGT CAC CAA CAA CAG TGT GG 3′), and GAPDH (F5′–GGC ACA GTC AAG GCT GAG AAT G 3′) (R 5′–ATG GTG GTG AAG ACG CCA GTA 3′). The cycling condition contains an initial denaturation at 95 °C/ 12 min, then 40 cycles of denaturation for 20 sec / 95 °C, annealing for 30 sec / 60 °C, and extension at 72 °C / 30 sec as previously reported [21, 23, 24]. The target genes expression level was normalized to the housekeeping gene *Gapdh*. The relative gene expression fold changes were calculated based on the equation of the $2^{-\Delta\Delta CT}$ method [33].

Histopathological examination

The testes were fixed in paraffin, then were washed in 70% alcohol, dried via a series of ethanol, then used xylene for removing the ethanol, and then dipped into paraffin. The blocks were divided and tint with hematoxylin and eosin (H & E). Then the examination of these blocks was done by light microscopy [21, 24, 34, 35].

Statistical analysis

One-way analysis of variance (ANOVA), followed by a post hoc Bonferroni test was used and data were presented as mean ± SE using GraphPad prism 8 (GraphPad Software Inc., San Diego, 189 CA, USA). A *P* value of < 0.05 was used to indicate statistical significance.

Results

Oral administration of SeNPs did not significantly alter the sperm motility and count in comparison to the control group. Both parameters were significantly declined in the melamine group than the control one ($P < 0.05$). The co-exposed group (MA + MO-SeNPs conjugate) showed a significant elevation in the sperm motility and count than in melamine alone (Table 1). The sperm abnormalities were significantly increased in the melamine treated group, but not SeNPs administered group, compared to the control one ($P < 0.05$). However, the co-exposed group (MA + MO-SeNPs conjugate) significantly revealed a decrement in these abnormalities in comparison to melamine administrated group ($P < 0.05$) (Table 1).

On the other hand, testicular weight and gonadosomatic index were significantly diminished in the melamine group, but not SeNPs administered group, compared to control one ($P < 0.05$). This decrease was significantly increased in the MO-SeNPs conjugate group in comparison to the melamine administrated group ($P < 0.05$) (Table 2). The SeNPs did not significantly alterations in comparison to the control group.

Table 1: Effect of orally administered melamine or MA+MO-SeNPs conjugate on the sperm picture

	Control	SeNPs	Melamine	Co-treated
Sperm motility (%)	85.33 ± 2.60^{a}	88.66 ± 1.33^{a}	51.67 ± 7.26^{b}	81.67 ± 6.01^{a}
Sperm count (sperm cell concentration /ml x 125 x 10^4)	86.33 ± 5.04^{a}	89.66 ± 4.91^{a}	44.00 ± 3.61^{c}	78.00 ± 4.58^{b}
Sperm abnormalities (%)	11.67 ± 2.40^{c}	10.66 ± 2.60^{c}	38.33 ± 5.21^{a}	24.67 ± 3.76^{b}

Values are mean ± SE. Means bearing different superscripts were significantly different at $P < 0.05$.

Table 2: Effect of orally administered melamine or MA+MO-SeNPs conjugate on the testicular weight and gonadosomatic index

	Testicular weight (g)	Gonadosomatic index
Control group	1.44 ± 0.07^{a}	$0.50 \pm .0.01^{a}$
Nano Se group	1.45 ± 0.06^{a}	$0.54 \pm .0.04^{a}$
Melamine (ma) group	1.28 ± 0.08^{b}	0.43 ± 0.03^{b}
Ma+mo-SeNPs group	1.45 ± 0.01^{a}	0.49 ± 0.05^{a}

. Values are mean ± SE. Means bearing different superscripts were significantly different at $P < 0.05$.

Regarding hormonal measurements, FSH, LH, free testosterone and total testosterone were significantly declined in melamine administrated group, but not SeNPs administered group, compared to control one ($P < 0.05$). These decreased levels were significantly increased in the MO-SeNPs conjugate group in comparison to the melamine administrated group ($P < 0.05$). Moreover, our results indicated that estradiol level was significantly increased in the melamine treated group while significantly decreased with SeNPs administration, compared to the control one ($P < 0.05$). However, administration of MO-SeNPs significantly declined this level to normal level ($P < 0.05$) in group 4 (Figure 1).

The transcriptional level of steroidogenic hormones including CYP11A1 and HSD17B3 genes showed a significant downregulation in melamine administrated group, but not SeNPs administered group, compared to the control group ($P < 0.05$). These decrement levels were significantly increased in concurrent MA + MO-SeNPs conjugate group in comparison to melamine administrated group ($P < 0.05$). There is a significant increase in the expression level of aromatase gene (CYP19A1) in the melamine group, but not SeNPs administered group, compared to the control group ($P < 0.05$). This increase level was significantly decreased in the concurrent MA + MO-SeNPs conjugate group in comparison to the melamine administrated group ($P < 0.05$) (Figure 1).

Regarding the antioxidant status in the testis, the levels of SOD, catalase and glutathione peroxidase antioxidant enzymes in the testis showed significant downregulation in the melamine administrated group than the control one ($P < 0.05$). These decreases levels were significantly increased in the MO-SeNPs conjugate group in comparison to the melamine administrated group ($P < 0.05$) (Figure 2).

Normal histological appearance of the seminiferous tubules of rat testes tissue of the control group with spermatozoa seen within the tubular lumen (Figure 3A, 3B) was observed. Testes of melamine administrated group revealed atrophy in the seminiferous tubule, disorganization and vacuolization of germinal epithelium, and loss of spermatogenic cells (Figure 3C). Figure 3D showed oedema in intratubular space with mild vacuolization of germinal epithelium.

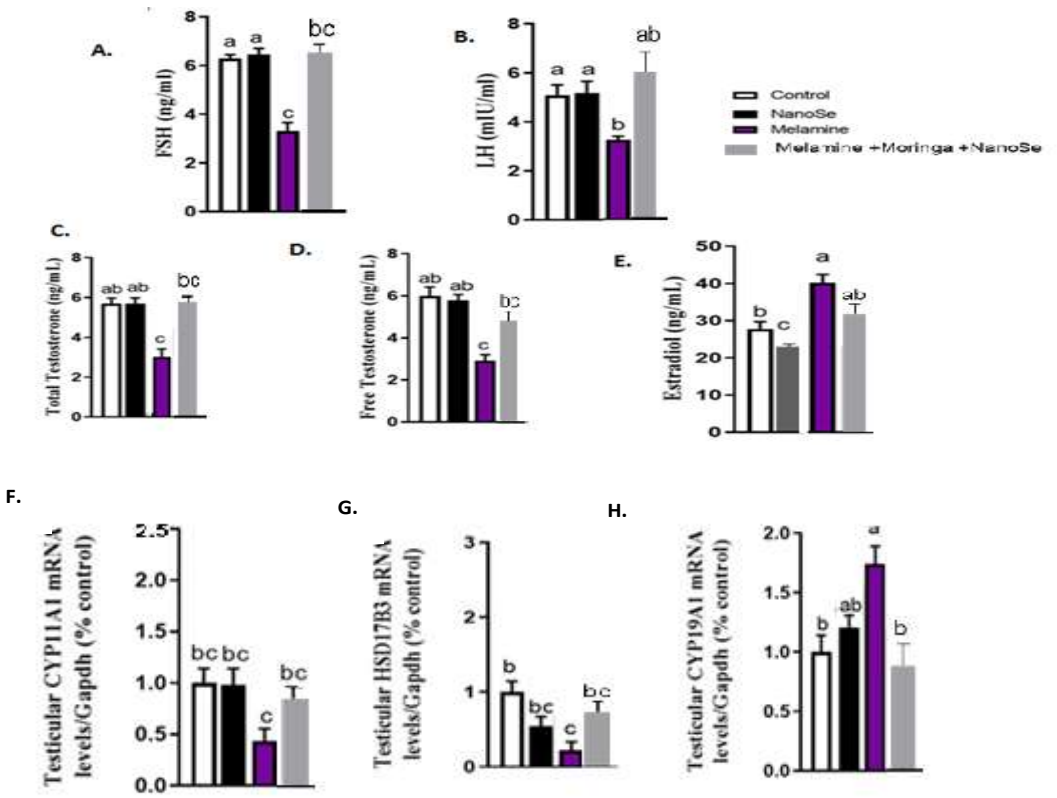

Figure 1: Effect of orally administered melamine or MA+MO-SeNPs conjugate on the serum levels of gonadotropins (FSH and LH), sex steroids (Total and free Testosterone as well as estradiol) and testicular mRNA expression of CYP11A1, HSD17B3 and CYP19A1 (A-H). Values are mean ± SE. Means bearing different superscripts were significantly different at $P < 0.05$.

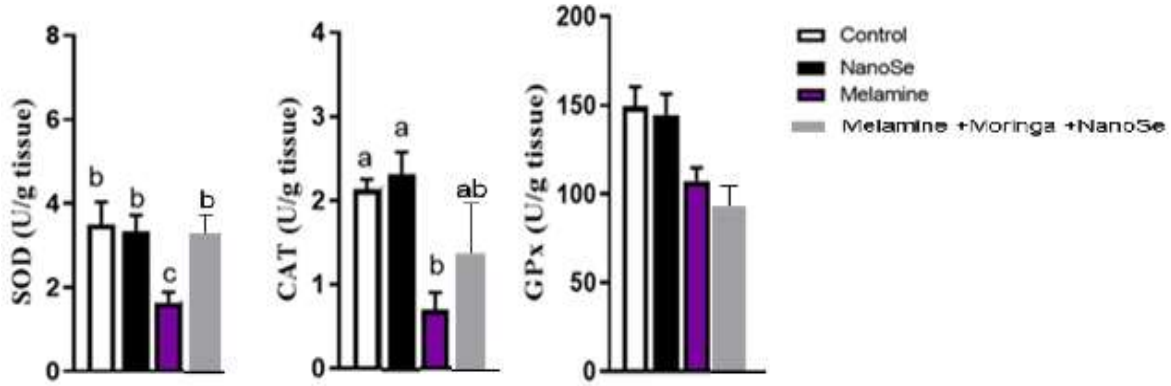

Figure 2: Effect of orally administered melamine or MA+MO-SeNPs conjugate on testicular antioxidant defense superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) **. Values are mean ± SE. Means with different superscripts were significantly different at $P < 0.05$.**

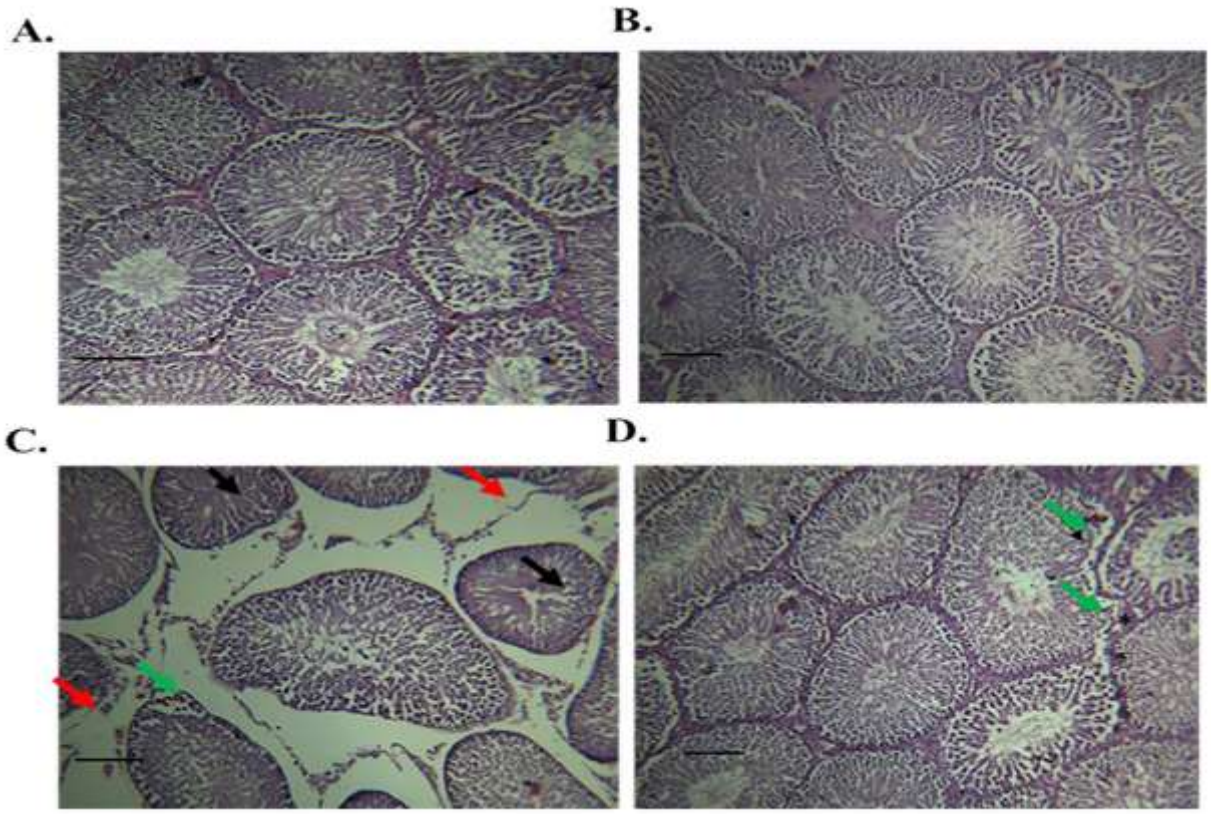

Figure 3: Photomicrograph of testicular cross section stained with hematoxylin and eosin of adult male rat (A-D). A. Control group, B. Nano Se group, C. Melamine (ma) group and Ma+mo-SeNPs group (H&E, X100, bar = 100µm). **Sever tubular atrophy (black arrow) and edema (red arrow) and vacuolated cytoplasm (green arrow).**

Discussion

In this study, we hypothesized that *Moringa oleifera* leaves extract and selenium nanoparticles have ameliorative effects on the semen picture, male sex hormones, antioxidant status, steroidogenic gene expression and histopathological examination of the testis in melamine-induced testicular toxicity in rats. The results of this study indicated that sperm motility and count were significantly decreased in the melamine group than the control one and significantly elevated in the co-exposed (MA + MO-SeNPs conjugate) group, also the obtained results indicated that the sperm abnormalities were significantly elevated in the melamine treated group than the control one. However, in the concurrent MA + MO-SeNPs conjugate group the sperm abnormalities are significantly decreased in comparison to the melamine treated group.

Melamine exposure causes reproductive toxicity in males; however, the mechanism remains unclear [36]. *Moringa oleifera* contains flavonoid pigments such as kaempferol that revealed potent antioxidant power as vitamins C, E, A [37]. Moreover, Moringa leaves administration has shown anti-inflammatory, anti-oxidant, anti-hypertensive, anti-tumor, cholesterol lowering, renal, anti-diabetic, [38] and hepatoprotective activities [39].

The results of this study are consistent with those of Sadek [6], who reported that administration of chromium and *moringa oleifera* extract significantly improved the testicular toxicity effects on the sperm motility and concentration. The secure limit of Se concentrations is low, which restricts the use of Se [40]. Also, Se administration has been suggested to positively impact on the sperm parameters [41].

The results of this study could be attributed to the antioxidants effect of SeNPs. Moreover, these results are consistent with the studies

which have shown that SeNPs have potent anti-oxidant impacts, bio-efficacy and enhance the toxicity than ordinary Se [11]. Besides, SeNPs reveal more potent impacts on the reproductive system by improving the spermatogenesis and sperm characters [42]. SeNPs administrated orally also enhance the characters of spermatozoa and spermatogenesis (the integrity of DNA and motility) over oxidative damage caused by Cisplatin [43].

Semen quality is the main indicator for male fertility, as sperm count, motility and abnormality [44]. The results of this study agree with those reported by Priyadarshani *et al* . [45] who remarked a significant elevation in the testicular weight after treatment with *Moringa oleifera* leaf powder. In the same study, they declared that in Se deficiency there was a decrease in sperm concentration and motility. In the group administrated overdose of Se, indicated the equidistant, midpieces of the tail present in the cytoplasm was shown with vacuolization in the cytoplasm this means that the motility and fertilizing capability of spermatozoa are agreed.

Different trials investigated the role of Se supplementation in sperm efficacy. A recent meta-analysis of several clinical trials found the efficacy of Se supplementation in raising sperm concentration, motility, and morphology [46]. Olson *et al* .[47] also implied that a decrease in male fertility through Se deficiency due to sperm abnormalities. Feed deficient in Se (0.02 ppm) showed a decline in the number of the spermatogenic cell lines in mice such as the decrease in the number of matured sperm and spermatid [48]. Moreover, Scott *et al*. [49] revealed that sperm motility was upregulated in men with low fertility after following the administration of Se for the subsequent 3 months.

Recently, in another animal species (buffalo bull) the semen was improved in quality parameters and membrane integrity after supplementation of Se in freezing and thawing semen [50]. The sperm abnormalities were more confined to the head than midpiece and tail. The frequency of chromosomal defects in spermatocytes was seen between Se deficient and control groups [51]. Afolabi *et al.* [52] reported that methanolic extract of MOL enhanced the spermatozoon and biochemical elements in cryptorchidism diseased rats.

The results of this study indicated that testicular weight and gonadosomatic index were significantly decreased in the melamine group than the control one. Although the testicular weight in the co-exposed (MA + MO-SeNPs conjugate) group is increased insignificantly than the melamine one, and the gonadosomatic index is significantly increased in MA/MO-SeNPs conjugate group. These results agree with those reported by Priyadarshani *et al.* [45], in which testicular weight of diabetic mice was significantly increased after treatment with *Moringa oleifera* leaf powder.

The results of this study indicated that FSH, LH, Total testosterone, free testosterone were significantly decreased in the melamine group than the control one, and then increased significantly in the co-exposed (MA/MO-SeNPs conjugate) group, also our study results indicated that estradiol significantly increased in melamine treated group than control one. However, in (MA/MO-SeNPs conjugate) group, estradiol is significantly declined in compare to melamine group.

The results may be attributed to the antioxidant effect of *Moringa*, and SeNPs. These results are agreed with those reported by Dafaalla *et al.* [53] in which ethanol extract of Moringa oleifera leaves administration increased the level of serum testosterone, LH and FSH in male Wister rats. Such treatment in another study was significantly increased the levels of the three sex hormones to the normal values in comparison with the diabetic group. It is cleared that *MO* can improve the sexual activity in mice due to certain conditions i.e. adding of different herbal extract species in various doses and times and in sexual condition of male animals [54]. In another report the low level of testosterone in testicles causing a decrease in the serum level, thus improve the serum LH level via negative feedback. LH revealed an impact on the LH receptor in testicular interstitial cells to induce the secretion of testosterone, so its level was then enhanced in the serum [55].

Huang *et al*. [55] has reported that blood testosterone levels have a positive relation with levels of Se. Also, dietary Se administration induces the testosterone and semen `s quality in many animal species [56]. Shi *et al*. [57] declared that the improved levels of testosterone in both testis and serum related to Se administration. *In vitro* experiment revealed that Se could also enhance the testosterone formation in Leydig cells by stimulation of the extracellular-signal-regulated kinase (ERK) pathway [58]. The testicular activity is also self-regulated via negative feedback, so the testicular endocrine activity can be ensured at a constant level [59].

The results of this work indicated that the melamine treated group showed a significant decline in the testicular mRNA expression level of CYP11A1 and HSD17B3 genes than the control group and then the mRNA expressions are increased significantly in the co-exposed MA/MO-SeNPs conjugate group. Also, the obtained results indicated that the melamine treated group showed a significant elevation in mRNA expression level of the aromatase gene CYP19A1 gene in melamine group than the control group and then the mRNA expression decreased significantly in the co-exposed (MA/MO-SeNPs conjugate) group. Gan *et al*. [60] clarified that SeNPs could induce the reproductive viability in rats exposed to nickel (Ni). Oral SeNPs administration, for 2 weeks, adequately improved the Ni-induced testicular damage, DNA perturbations, and testosterone biosynthesis through activation of phosphorylation of ERK1/2, p38, and JNK-MAPK pathways. The mRNA and expression levels of StAR, CYP11A1, and 17β-HSD were also significantly induced by SeNPs administration. The expression of StAR, 3β-HSD and CYP11A1 was decreased by increasing the administration of Se in diet however, the expression of the androgenic receptor protein in testes of resulting progeny was improved [58]. Shi *et al*. [58] declared that Se can upregulate the expression of 3β-HSD that thought to have a relation with fertility and gametogenesis in males.

Our results revealed a significant decline in both SOD and CAT in MA group than the control group. Furthermore, both were significantly increased in the co-exposed MA/MO-SeNPs conjugate group. However, GPx has no significant difference among tested groups. In another investigation [61], zearalenone-induced testicular tissue and sperm damage was improved by Se yeast supplementation. Sperm concentration and motility were significantly improved in Se supplementation. The MDA levels were significantly decreased, however the GPx and SOD were significantly improved in Se supplementation than other groups. La Vignera *et al*. [62] studied the role of Se administration in the management of male infertility as the results obtained showed elevation in glutathione, catalase and SOD levels and reduce MDA, thus likely improving the sperm quality.

SeNPs act as a scavenger of ROS in sperm cells. SeNPs added to semen extender improved the post-thawing characters and oxidative status of rooster semen [63]. Shalini *et al*. [64] revealed that GPX activity and MDA levels were also decreased and elevated, respectively in a Se-deficient diet. Multiple studies revealed an elevation in detoxification and antioxidant enzymes due to the supplementation of Moringa oleifera tissue extracts [65, 66]. Supplementation with Se enhanced the semen and sperm quality characters in Holstein bulls. Also, TAOC and MDA were significantly enhanced in Se treated group other than the control [67]. SeNPs is act as anti-oxidative and anti-inflammatory and anti-apoptotic agent [67-69] which . significantly improve the testicular damage via declining the oxidative stress and apoptosis in streptozotocin induced diabetic rats [70]. The SeNPs revealed to be efficient in improving the GSH-Px, SOD, CAT and sperm characters more than other selenium forms [42,58].

In another study, catalase, SOD, and glutathione peroxidase activities and MDA levels were used as indicators of tissue antioxidant capacity. Furthermore, treated mice also showed greater sperm quality and *in vitro* fertilization outcomes in comparison with controls [71]. The results of our study indicated that the melamine group showing degeneration in the seminiferous tubule, and vacuolization compared with the control one,

but these findings ameliorated to be better and showing edema in intertubular space and mild vacuolization of germinal epithelium in MA/MO-SeNPs conjugate group.

Moreover, Zhang *et al*. [72] clarified that SeNPs debilitated $NiSO_4$ induced abnormal morphological changes by improvement seminiferous tubules structure, proving the main role of SeNPs against the harmful impacts of Ni toxicity.

These results are agreed with Huang *et al*. [55], who suggested that melamine showed reproductive toxicity against male mice as has an impact on the normal formation and maturation of sperms, damaged the structure of testis. Testes of MA/MOLE treated group showing mild edema in intertubular space and moderately number of spermatozoa seen within the tubular lumen and mild vacuolization of germinal epithelium. This finding has been confirmed with normal histological characters of seminiferous tubules of rat in Moringa treated group that showed spermatozoa in the tubular lumen.

Crissman *et al*. [73] demonstrate that histopathological examination showed that testicular tissue of rats in the melamine group has degenerative changes in the seminiferous tubule and vacuolization of germinal epithelium and damage of spermatogenic cells. This microscopic examination is the main way to determine the impact of chemical exposure on the tissue.

Conclusion

Based on the obtained results, it is concluded that co-exposed MA + MO-SeNPs conjugate succeeded to ameliorate the toxic effect of melamine on testicular tissue in rats. Improvement of semen picture, antioxidant status, histopathological findings and steroidogenic gene expression even with MA involvement were confirmed.

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

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الملخص العربي

التأثير العلاجي لجزيئات السيلينيوم النانويه المحملة على مستخلص أوراق المورينجا أوليفيرا على سمية الخصية التي يسببها الميلامين في الفئران

محمد فؤاد احمد [1],احمد حامد عريشه[2*],محمد الجمل[1], احمد السيد[3],سيدات سعد[1],خلود البوهي[4]
[١]قسم الكمياء الحيوية كليه الطب البيطري جامعة الزقازيق
[٢]قسم الفسيولوجيا كلية الطب البيطري جامعة الزقازيق
[٣]شعبه الكمياء الضوئية فرع الكمياء الصناعية المركز القومي للبحوث
[٤]قسم الطب الشرعي والسموم كليه الطب البيطري جامعة الزقازيق

تهدف هذه الدراسة إلى دراسة التأثير العلاجي لجزيئات السيلينيوم النانوية المحملة على مستخلص أوراق المورينجا أوليفيرا (MOLE-SeNPs conjugate)على سمية الخصية التي يسببها الميلامين في الفئران البيضاء. تتألف المجموعات من اربعين من ذكور الفئران البيضاء تبلغ ١٠ اسابيع من العمر, تم تقسيمهم إلى أربع مجموعات متساويه كل منها تتكون من١٠ فئران مجموعة ١ المكونه تسمي المجموعة الضابطة حيث تلقى كل فأرعن طريق الفم١ ملي ماء مقطرا ، و مجموعة ٢ تم إعطائها جزيئات السيلينيوم النانوية SeNPs حيث تلقى كل فأر ٠.٥ مجم / كجم عن طريق الفم , ومجموعة ٣ المكونه من١٠ فئران تم إعطائها ميلامين (MA) حيث تلقى كل فأر ٨٠٠ مجم / كجم عن طريق الفم , ومجموعة ٤ تم إعطاء كل فأر ٨٠٠ ملغم / كجم من الميلامين عن طريق الفم قبل ساعة واحدة من إعطائه ٢٠٠ ملجم / كجم عن طريق الفم من جزيئات السيلينيوم النانوية المحملة على مستخلص أوراق المورينجا أوليفيرا . واستمرت التجربة لمدة ٦ أسابيع. تم فحص مجموعه من الوظائف التناسلية و مستوي الهرمونات الجنسيه و التغير في التعبير الجزيئي لبعض جينات الإنزيمات الستيرويدية و التشريح المرضي في مجموعة الميلامين انخفضت جودة السائل المنوي بشكل ملحوظ ، وكذلك وزن الخصية ، و مؤشر الغدد التناسلية وكذلك هرمونات FSH ، LH ، هرمون تستوستيرون الحر و التستوستيرون الكلي بالمقارنة مع المجموعة الضابطة. بينما ازداد مستوى هرمون الإستراديول (187.45%) بشكل ملحوظ مقارنةً بالمجموعة الضابطة .وعلى النقيض تماما ازدادت قيم هذه البنود والهرمونات بشكل كبير في مجموعة جزيئات السيلينيوم النانوية المحملة على مستخلص أوراق المورينجا أوليفيرا. علاوةً على ذلك ، تم ملاحظة وجود انخفاضًا كبيرًا (23.75% 45.21%) في جينات الإنزيمات الستيرويدية CYP11A1 و HSD17B3 في مجموعة ٣مجموعة الميلامين مقارنة بالمجموعة الضابطة ($P < 0.05$). لكن المستوى النسخي لجين الأروماتيز (CYP19A1) ازداد زيادة ملحوظة (171.28%) في نسيج الخصية مقارنة بالمجموعة الضابطة. زاد العلاج بجزيئات السيلينيوم النانوية المحملة على مستخلص أوراق المورينجا أوليفيرا من التعبير عن الجينات التي انخفضت في أنسجة الخصية ، لكن المستوى النسخي لجين الأروماتيز (CYP19A1) أظهر انخفاضًا كبيرًا مقارنة بمجموعة الميلامين وأظهرت مستويات الإنزيمات المضاده للاكسده (فوق أكسيد الديسموتاز، كاتالاز، وبيروكسيديز الجلوتاثيون) في الخصية انخفاضًا كبيرًا في مجموعة ٣مجموعة الميلامين عن المجموعة الضابطة ، ثم زادت بشكل ملحوظ في مجموعة لجزيئات السيلينيوم النانويه المحملة على مستخلص أوراق المورينجا أوليفيرا مقارنة بمجموعة الميلامين. وبالفحص الباثولوجى للخصيه ، أشار الى التعافى والتحسن من الاصابة في المجموعة التى اخذت جزيئات السيلينيوم النانوية المحملة على مستخلص أوراق المورينجا أوليفيرا مقارنة بمجموعة الميلامين. فى الختام يمكن القول ان جزيئات السيلينيوم النانويه المحملة على مستخلص أوراق المورينجا أوليفيرا لها تأثيرمحسن ومعالج لسمية الخصية الناتجه من تعاطى الميلامين في ذكورالفئران عن طريق التأثير على مسار الستيرويد.