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Evaluation of reproductive capacity in adult male rats exposed to silver nanoparticles and the ameliorative role of lycopene

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Abstract

The present study was aimed to assess reproductive capacity of male albino rats exposed to silver nanoparticles (AgNPs) and the possible ameliorating role of lycopene (LYC). Twenty eight male albino rats were divided as follows: group I, received distilled water; group II received 100 mg/kg BW AgNPs; group III received 5 mg/kg BW LYC and group IV received both AgNPs+LYC at their same dosage. All doses were administrated orally and daily for 60 days. The results showed that AgNPs exposure decreased body weight gain as well as testicular and epididymal weight, reduced sperm count and induced sperm malformations. Histological findings revealed histo-morphometric change to the seminiferous tubules in AgNPs group. Biochemical investigation showed that AgNPs increased MDA level and decreased SOD, GSH and CAT levels in testicular tissue. Also it increased testicular ALP level and decreased testicular SDH level. Immuno-histochemical staining showed an increased caspase-3 and TNF-α expression in AgNPs group. Meanwhile, lycopene co-treated rats showed improvements in the body weight, testicular and epididymal weight status, sperm characteristics, histological architecture, oxidative, inflammatory and apoptotic biomarkers.

Keywords: Reproductive toxicity, Silver nanoparticles, Lycopene, male albino rats.

1. Introduction

Infertility has become a worldwide dilemma that raises concerns to the scientific communities. Assessment of reproductive toxicity is crucial to regulate this health hazard issue [1].Many toxicants can affect reproductive organs and subsequently impair gametogenesis process. Recently, a special attention has been paid to the nanoparticles-induced toxicity for several reasons, their wide applications in consumer products, and their ability to enter biological systems and pass specialized barriers such as hemato-testicular barrier and blood-brain barrier [2] and their ability to interfere with the defense mechanisms in reproductive cells, finally destructing the exposed tissue. Silver nanoparticles (AgNPs) are mostly applied

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nanoparticles in many aspects such as industry and medicine owing to their bactericidal, viricidal and fungicidal properties [3]. Their wide application raises their environmental release and subsequent exposure [4]. Several previous studies reported that AgNPs induce testicular toxicity represented by seminiferous tubules morphometric changes, reduced sperm concentration and oxidative stress to the testicular tissue [5, 6, 7]. Furthermore, AgNPs have been shown to trigger inflammatory response leading to apoptosis [8, 9]. Concerning AgNPs elimination, Antsiferova et al. [10] studied AgNPs accumulation in various tissues and observed that AgNPs accumulation was more prominent in the testicular tissues.

On the contrary, it is well known that antioxidants exhibit free radicals scavenging properties. Lycopene, a carotenoid known for its anti-oxidative and anti-inflammatory capability has been proven to alleviate testicular toxicity induced by many environmental insults, including cisplatin [11], titanium dioxide nanoparticles [12], benzo[a]pyrene [13] and diethyl nitrosamine [14]. Lycopene is considered to have the highest antioxidant potential among carotenoid pigments [15].

Therefore, this study was aimed to investigate the toxic effect of AgNPs on male reproductive parameters and whether lycopene can attenuate these toxic effects.

2. Materials and Methods:

2.1. Animals and treatment

Twenty-eight Wister strain male albino rats weighing 120± 20 g from the Central Animal House of the Medical Research Centre, Faculty of Medicine, Ain Shams University were used. Standard conditions of temperature (23-25ºC) with 12 h light/12 h dark were kept during the whole experimental period. Rats were categorized into four groups, each 7 rats in a cage, and allowed to access standard laboratory animal food and water. Rats' handling was in accordance to laboratory animal care and use guidelines at The Medical Research Centre, Ain Shams University.

The four rat groups were treated as follows: group I received distilled water (control group), group II received silver nanoparticles (Sigma-Aldrich Chemicals, St. Louis, US) 100 mg/kg bodyweight [50] (AgNPs group), group III received lycopene (The Vitamin Shoppe, New Jersey, US) 5 mg/kg bodyweight [58] (LYC group), and group IV received LYC and AgNPs of their same dosage (AgNPs+LYC group). All doses were administered daily by oral gavage for 60 days. Body weight measures were recorded weekly to evaluate bodyweight gain changes between groups.

2.2. Sample collection

After two months, rats were dissected under anesthesia of diethyl ether. Right testis and epididymis were measured separately. Left cauda epididymis from each rat were minced for sperm collection to evaluate sperm count and morphological changes. Right and left testes were either stored at -80ºC for later biochemical analysis or fixed in 10% formalin for histological sectioning.

2.3. Sperm concentration and morphology

Left cauda epididymis from each rat were placed in a sterile watch glass containing physiological saline, minced and incubated to allow sperms to leave the epididymal tubules. Ten µl of sperm suspension of each rat was placed in Neubauer chamber of Haemocytometer to evaluate the sperm count using light microscope (20× objective) [59]. The sperms' head, neck and tail morphological abnormalities were detected using eosin-stained sperm smears under light microscope (40× objective) according to the method of Bearden and Fuquay [16].

2.4. Histological evaluation

Left testis from each rat were fixed in formalin (10%), dehydrated in alcohol of ascending grades, embedded in paraffin wax to obtain tissue blocks, sectioned at 5 µm thickness, stained with HE stain and examined by light microscope [17]. HE-stained testicular sections were subjected to morphometry evaluation of seminiferous tubules (STs) diameter and thickness of STs germinal epithelium thickness in ten microscopic fields of each rat at magnification of 100 using computer-aided microscopy, based on "ImageJ program" image analysis system and statistically analyzed.

2.5. Biochemical assays

A known weight of right testis was homogenized in phosphate buffer solution and centrifuged (4000 rpm) for 15 minutes then the supernatant was collected for assaying oxidative stress biomarkers. Levels of malondialdehyde (MDA), activities of superoxide dismutase (SOD) and catalase (CAT), and reduced glutathione (GSH) content were assayed [18-21, respectively]. Activity of testicular alkaline phosphatase (ALP) was assayed according to Belfield and Goldberg [22] and succinate dehydrogenase (SDH) activity was assayed according to the instructions of manufacture of colorimetric kit from Abcam (ab228560).

2.6. Immuno-histochemical investigation

Paraffin-embedded testicular sections of thickness of 5 µm were subjected to immuno-detection of caspase-3 and tumor necrosis factor-α (TNF-α). The sections were dewaxed, microwave oven-heated with citrate buffer for antigen retrieval and incubated with methanol and hydrogen peroxide to inhibit endogenous peroxidase activity. The sections were then incubated with horse serum for blocking of non-specific antibody binding and incubated with either anti-caspase3 rabbit polyclonal primary antibody (Boster Bio, PA1302-1) for caspase 3 detection or anti-TNF alpha mouse monoclonal primary antibody (Abcam, ab220210) for TNF-α detection. The sections were then incubated with HRP-conjugated anti-rabbit secondary antibody (Boster Bio, SV0002-1) and exposed to diaminobenzidine (Abcam, ab64238) for chromogenic detection. Then, sections were rinsed with phosphate buffered saline (PBS), counterstained with hematoxylin and visualized under light microscope [23].

2.7. Statistical analysis

The obtained data were represented as mean ± standard error (SE) using statistical package for social science software (SPSS, Chicago, IL, version 20). The mean difference significance between the studied groups was obtained using one-way analysis of variance (ANOVA test) followed by post hoc test LSD test for pairwise comparison of the means of the different studied groups [60], with significance level set at $p<0.05$.

3. Results

3.1. Effect of AgNPs exposure on bodyweight gain and testis and epididymis weight:

After two months of AgNPs daily exposure, the treated rats showed a significant decline in bodyweight gain as compared with their controls (control group and LYC group). Despite that, LYC co-treated rat group displayed almost normal weight gain as compared to AgNPs group (Table 1).

In addition, an observed decrease in testis and epididymis weight was shown in AgNPs group as compared to its controls, while LYC co-treated group showed a significant increase ($p<0.05$) in testis and epididymis weight as compared to AgNPs group (Table 1).

Table (1): Final bodyweight, bodyweight gain, weight of right testis and right epididymis (g) of Control, LYC, AgNPs and AgNPs+LYC groups.

Groups / Parameters	Control	LYC	AgNPs	AgNPs+LYC
Final bodyweight (g)	182.14±3.06	180.71±4.31	129.57±4.91[a]	172.86±5.65[b]
Bodyweight gain (g)	63.57±2.16	65.34±2.86	14.29±3.02[a]	50.72±2.79[b]
Right testis weight (g)	1.25±0.03	1.30±0.08	0.81±0.11[a]	1.18±0.04[b]
Right epididymis weight (g)	0.39±0.03	0.42±0.04	0.27±0.03[a]	0.34±0.02[b]

Values are represented as mean ±SE.
Significance level ($p < 0.05$).
[a] Significantly different from control group.
[b] Significantly different from AgNPs group.

3.2. Effect of AgNPs exposure on sperm concentration and morphology

The present study revealed that AgNPs exposure affect sperm concentration in the nanoparticles treated group evidenced by such decrease represented in (Table 2) as compared to their respective controls, while LYC appeared to ameliorate the reduction in sperm concentration.

Table (2):Epididymal sperm count of Control, LYC, AgNPs and AgNPs+LYC groups.

Groups / Parameters	Control	LYC	AgNPs	AgNPs+LYC
Sperm count ($\times 10^6$) sperm/ml	42.14±2.82	46.29±3.53	22.00±1.85[a]	32.86±1.94[b]

Values are represented as mean ±SE.
Significance level ($p < 0.05$)
[a] Significantly different from control group.
[b] Significantly different from AgNPs group.

Moreover, sperm abnormalities were detected in AgNPs intoxicated rats' group, represented as detached head, detached tail, coarse tail, looped neck and looped tail (Figure 1), meanwhile LYC co-treated group showed normal morphology of sperm structures.

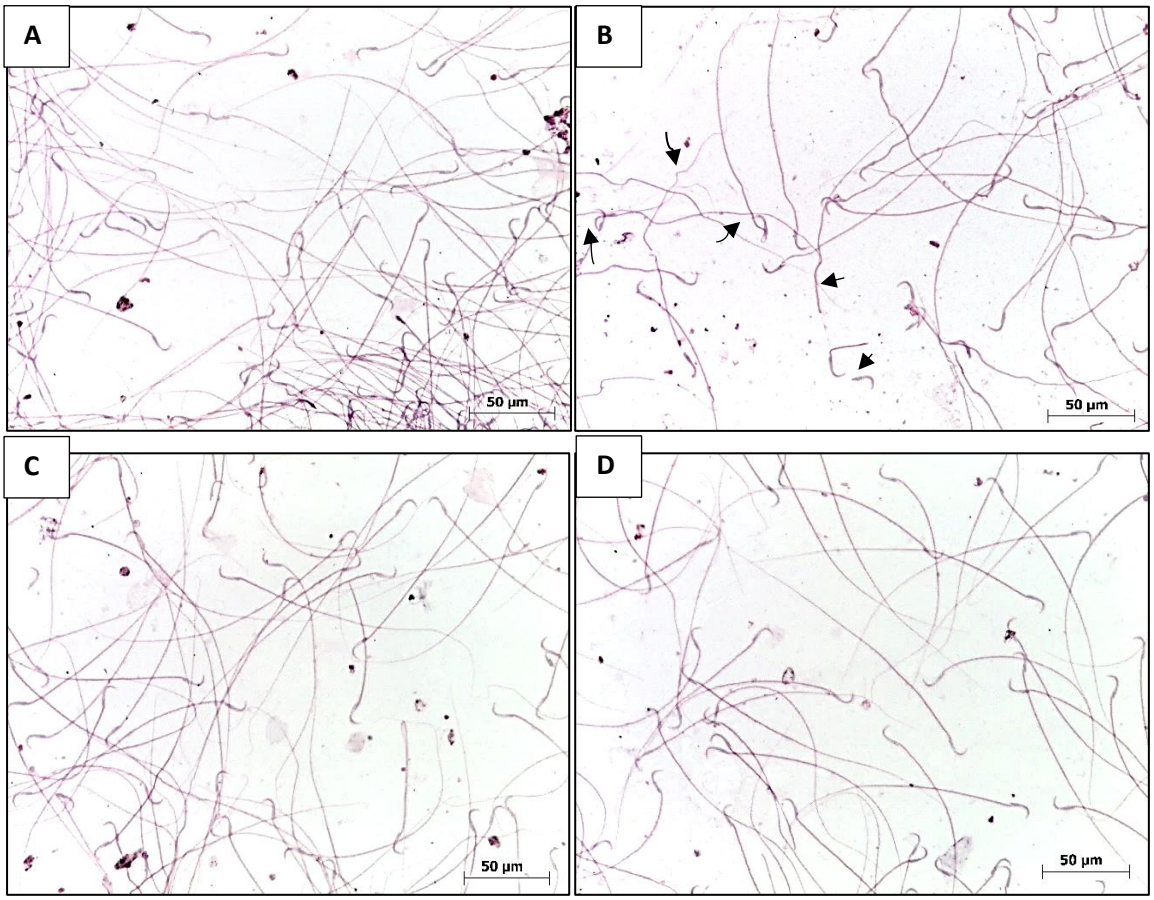

Fig.1: Eosin-stained sperm smears showed normal appearance of sperm structure in control (A) and LYC (C) groups, sperm abnormalities such as detached head and tail (straight arrows) and looped neck and bent tail (curved arrows) were detected in AgNPs group (B), while LYC co-treated group (D) showed normal appearance of sperm morphology.

3.3. Effect of AgNPs on biochemical parameters

3.3.1. Oxidative stress parameters

Testicular homogenate showed a significant elevation ($p<0.05$) of malondialdehyde (MDA) in AgNPs treated group as compared to the controls, meanwhile, SOD, CAT and GSH were significantly decreased (Table 3). On the other hand, testicular homogenate from LYC co-treated group showed a significant decline in MDA level accompanied by a significant increase ($p<0.05$) in the activities of SOD, and CAT and levels of GSH (Table 3).

3.3.2. Testicular enzymes

As shown in table (3), testicular homogenate showed a marked elevation of ALP and a marked decline in SDH activity levels in AgNPs group ($p<0.05$) as compared to controls, in contrast, LYC in co-treated group restored the unbalanced enzymes level to normalcy.

Table (3): Levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), alkaline phosphatase (ALP) and succinate dehydrogenase (SDH) in testis homogenate of Control, LYC, AgNPs and AgNPs+LYC groups.

Groups / Parameters	Control	LYC	AgNPs	AgNPs+LYC
MDA (nmol/mg)	0.48±0.04	0.40±0.04	6.77±0.43[a]	1.89±0.29[b]
SOD (mmol/mg)	58.07±2.37	49.35±2.60	27.67±2.07[a]	42.36±2.14[b]
CAT (mmol/mg)	1.77±0.24	1.87±0.21	0.61±0.06[a]	1.22±0.19[b]
GSH (mmol/mg)	4.50±0.62	5.81±0.43	1.64±0.28[a]	2.82±0.30[b]
ALP (U/mg)	28.78±1.56	25.14±2.24	43.46±2.66[a]	33.95±1.56[b]
SDH (U/mg)	38.30±1.73	40.29±1.82	22.55±0.91[a]	30.00±2.28[b]

Values are represented as mean ±SE.
Significance level ($p < 0.05$).
[a] Significantly different from control group.
[b] Significantly different from AgNPs group.

3.4. Effect of AgNPs exposure on testicular histology and histometry

Hematoxylin and Eosin (HE) stained testicular sections from AgNPs group exhibited a degenerative appearance in seminiferous tubules (STs) appeared as: vacuolar formation, germinal epithelium exfoliation, STs atrophy, congested interstitial spaces and loss of luminal spermatozoa. Testicular sections from LYC co-treated group showed an inhibitory effect of LYC against AgNPs-induced histological alterations as it appeared nearly normal (Figure 2).

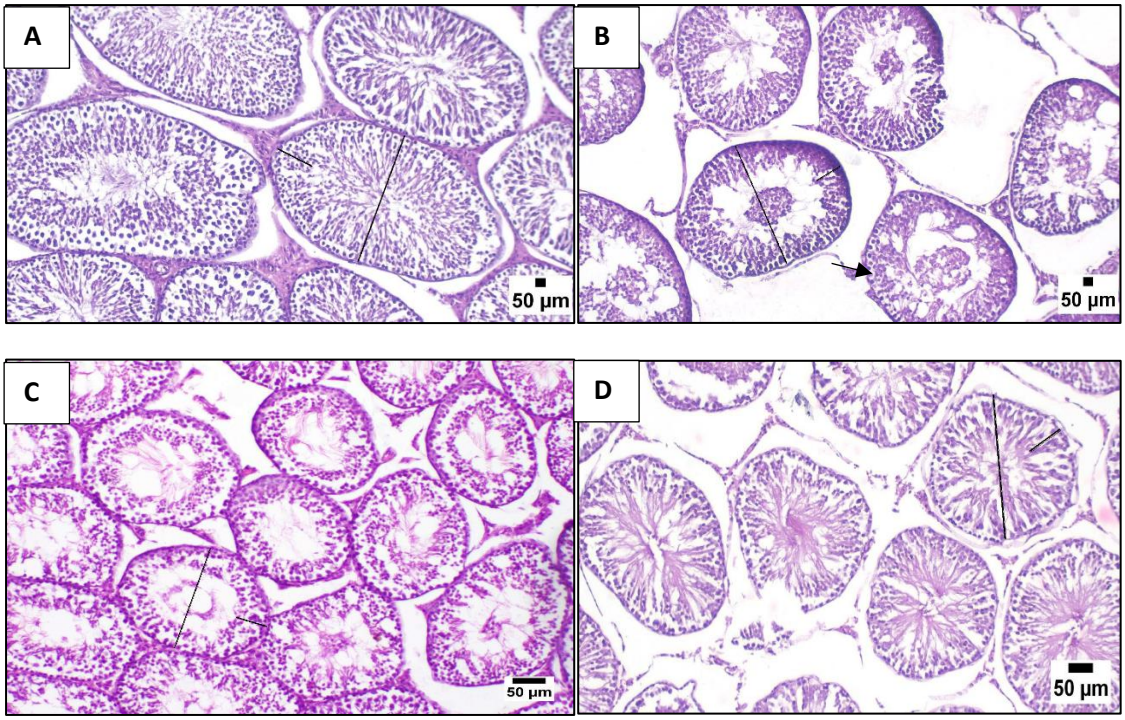

Fig.2: H&E testicular cross sections of control (A), AgNPs (B), LYC (C) and AgNPs+LYC (D) groups. Sections (A) and (C) show normal cellular architecture of seminiferous tubules (STs), while section (B) shows irregular arrangement of germinal cells, vacuolar degeneration (black arrow) of STs and interstitial edema. However, LYC co-treatment (section D) partially restored the normal histological structure of the testis.

Morphometric evaluation of STs have shown a significant decrease of STs diameter and germinal epithelium thickness ($p<0.05$) in AgNPs group compared to controls, while LYC was found to normalize the STs diameter and germinal cell thickness in LYC co-treated group as shown in table (4).

Table (4): Seminiferous tubules (STs) diameter and germinal epithelium thickness of Control, LYC, AgNPs and AgNPs+LYC groups.

Group / Parameter	Control	LYC	AgNPs	AgNPs+LYC
STs diameter (µm)	240.6±3.29	252.5±5.24	201.4±4.56[a]	232.5±3.35[b]
Germinal cell thickness (µm)	67.7±0.98	69.9±1.93	45±1.84[a]	65.7±1.20[b]

Values are represented as mean ±SE.
Significance level ($p < 0.05$).
[a] Significantly different from control group.
[b] Significantly different from AgNPs group.

3.4. Effect of AgNPs on inflammation and apoptosis

As shown in figure (3), immuno-histochemical detection of TNF-α revealed a strong positive reaction in AgNPs treated group as compared to the controls, while LYC co-treated group show mild expression of TNF-α as compared to AgNPs treated group.

In figure (4), caspase-3 immunostaining revealed marked apoptotic activity in AgNPs administrated group compared to controls, however; LYC co-treated group showed mild reactivity for caspase-3 compared to AgNPs treated group.

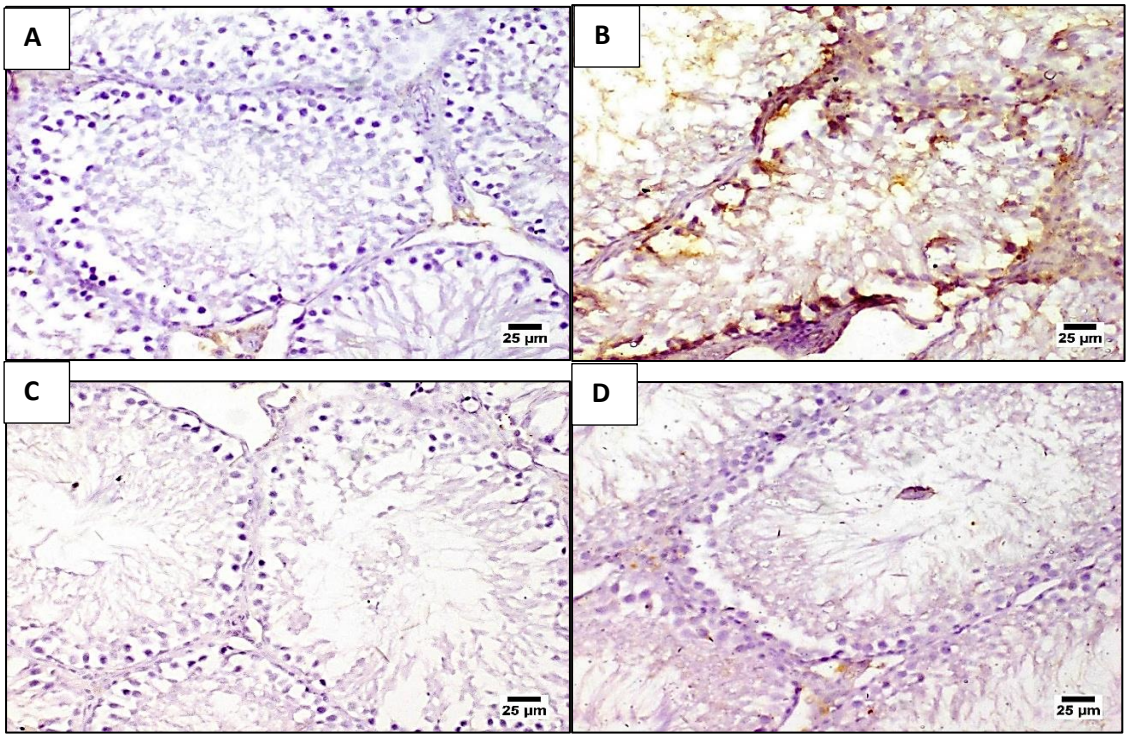

Fig. 3: Immunostaining for TNF-α expression in the testis of control (A), AgNPs (B), LYC (C) and AgNPs+LYC (D) treated groups. Sections (A) and (C) show low expression of TNF-α, while AgNPs treated group in section (B) shows strongly positive expression of TNF-α (brown stain). Meanwhile, section (D) from LYC co-treated group shows mild TNF-α expression.

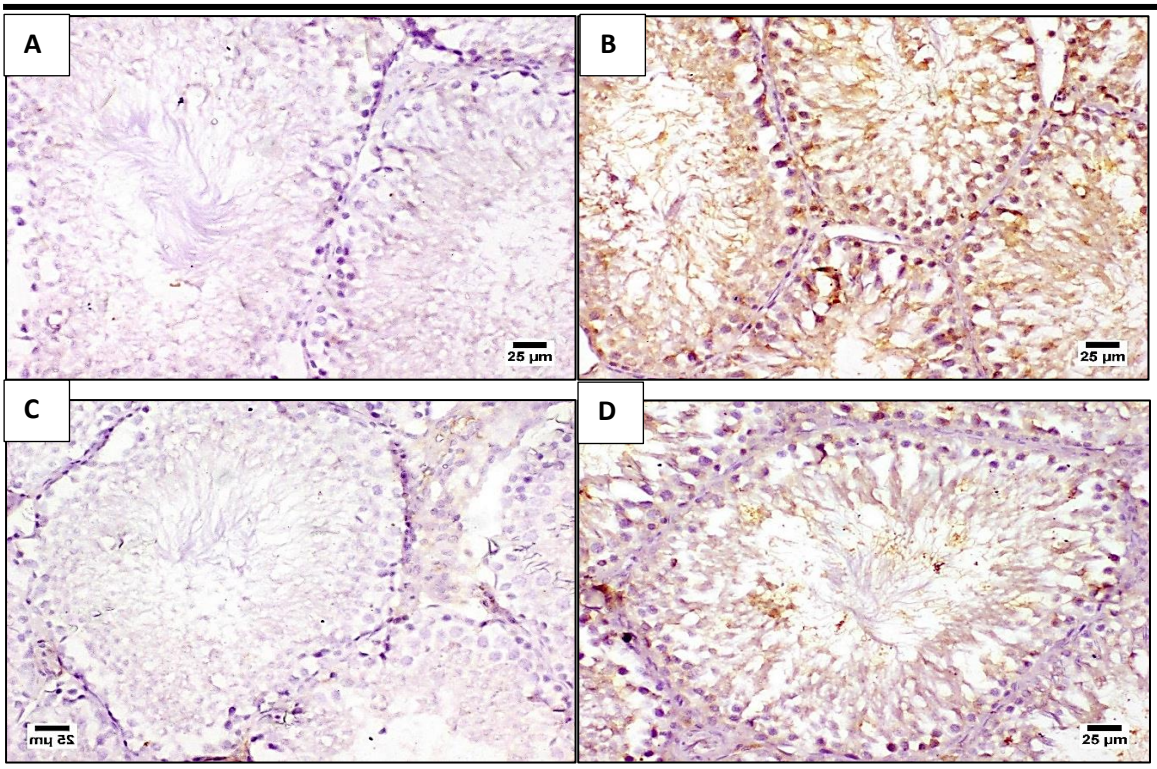

Fig. 4: Immunostaining for caspase-3 activity in the testis of control (A), AgNPs (B), LYC (C) and AgNPs+LYC (D) treated groups. Sections (A) and (C) reveal mild to no caspase-3 activity while section (B) shows high caspase-3 activity (brown stain). Section (D) shows moderate activity of caspase-3 in LYC co-treated group.

4. Discussion

The current study revealed a significant decline in rats' body weight gain upon AgNPs oral administration, this result may be related to the oxidative damage induced by the nanoparticles that led to altered physiological processes and subsequent loss of body weight gain [24, 25]. Meanwhile, lycopene co-treatment prevented the reduction in body weight gain probably due to its counteract action of alleviating the oxidative damage induced by AgNPs. In the present study, AgNPs exposure significantly decreased the testes and epididymis weight. The depression of testicular weight is probably due to spermatogenesis defect which result in germ cells loss as evidenced by the histological and morphometric evaluation of the testis. The loss of epididymal weight was most likely due to the decline in spermatozoa production, as evidenced by low epididymal sperm count as shown in AgNPs group. On the contrary, lycopene co-treated rats showed no changes in testicular and epididymis weight compared to the control group. This result can be explained as follows, lycopene as an antioxidant, prevented the oxidative damage and consecutive organ atrophy.

Sperm indices are key indicators of spermatogenesis impairment. The present work demonstrated that AgNPs could reduce sperm concentration and induce sperm morphological alterations. The same observation was reported in previous studies [6, 26, 27]. According to Ryu *et al.* [28], reactive oxygen species (ROS) are able to destroy sperm cell membrane through lipid peroxidation, since spermatozoa have very high content of unsaturated fatty acids that make them vulnerable to free radicals attack. Interestingly, in this study lycopene co-treatment was found to restore sperm count and morphology to almost normalcy. The protective effect of lycopene may be related to the antioxidant properties and free radical scavenging effect [29].

Histological investigation in the current study showed degenerative and morphometric changes to the STs of AgNPs exposed rats. Such changes appeared as atrophy of STs characterized by irregular basement membrane, exfoliated and vacuolated germinal epithelium, reduced sperm density in STs lumen, tissue edema, increased interstitial tissue width and presence of congested blood vessels. The STs diameter and their germinal epithelium thickness were significantly ($p<0.05$) smaller in AgNPs treated groups. Allying with this result, Ray and Nath [30] reported that damaged cellular architecture of STs is due to the ability of AgNPs to pass blood-testis barrier (BTB) and accumulate into STs exerting their adverse effects. However, lycopene co-administration was found to attenuate such degenerative alterations. Elsayed *et al.* [11] returned this ameliorative effect of lycopene to its ability to stabilize cellular membranes integrity and/or reinforce the regeneration of damaged cells.

To further elucidate the possible mechanisms related to AgNPs induced toxicity, biochemical studies were worthy to be done. In the present study, AgNPs treated group showed significant increase in the lipid peroxidation marker (MDA) level, while the cellular antioxidant enzyme system (SOD, CAT and GSH) levels were significantly decreased. Previous studies reported the same findings [31, 32, 33], indicating that one of the main mechanisms associated with AgNPs toxicity is ROS generation that leads to oxidative damage of the cell. According to Nie *et al*. [34], silver ions (Ag+) are responsible for AgNPs toxicity, which are released from the nanoparticles upon cell entry, inducing ROS production. Despite, AgNPs themselves may exhibit toxic potential depending on the type of their stabilizing coat [35]. Consistently, the increased cellular uptake of antioxidant enzymes to overcome the increased ROS production contributes to their observed marked decrease. Also, Tohamy *et al*. [36] reported lowering in GSH level and elevation in MDA level after AgNPs intraperitoneal injection. On contrast, lycopene co-treatment in this study have shown a decrease in MDA level and an increase in SOD, CAT, GSH compared to AgNPs treated group. The ameliorating effect of lycopene is

owing to its ROS and free radicals quenching ability [37].The present result is supported by previous reports mentioned that lycopene had an attenuating effect against the induced oxidative stress in the testicular tissue [12, 38].

Regarding evaluation of the testicular enzymes in the present study, AgNPs-treated group showed an elevation in ALP level and a decline in SDH level in testicular homogenate, while lycopene co-treated group showed a restored normal enzymatic concentration levels. Few reports from the literature review have been introduced considering testicular ALP. Tice and Barrnett [39] stated that, ALP has a vital role in material transport from Sertoli cells to the different germinal cells, germinal epithelium proliferation and differentiation and testicular metabolism. According to Agu *et al.* [40], overexpression of ALP occurs when tissues are impaired. Sharma and Kumar [41] explained that the unutilized ALP increases when spermatogenesis impairment occurs as evidenced in the present result. Kaur *et al.* [42] mentioned that testicular degeneration causes increased activity of ALP that indicates lytic activity. Restoration of ALP levels achieved by lycopene co-treatment in this study is probably due to its anti-oxidative efficiency exerted on the spermatogenic cells, protecting the cells from oxidative damage induced by AgNPs and utilizing the phosphatase enzyme in the spermatogenesis process. In a previous study on testicular tissue, Tripathy *et al.* [43] observed the effect of lycopene to decrease ALP expression level and attributed the protective role of lycopene to its probability to bind with ALP directly so as to decrease its enzymatic activity or stabilize the lysosomes so as to diminish ALP release to the cytosol. Supporting to this finding, Wadie *et al*. [44] found that lycopene decreased ALP activity in hepatic injured rat models, due to its membrane-stabilizing activity that prevent its leakage [45,63].

SDH is a marker enzyme of the testicular mitochondria [46] that reflects the mitochondrial functionality. The obtained result of decreased SDH activity in AgNPs group is consistent to mitochondrial dysfunction induced by ROS generation, and indicates that the energy is not adequate for spermatogenesis and subsequent reduced sperm count [47]. AgNPs was found to lower SDH level and generate mitochondrial cytotoxicity [48, 61]. Similarly, Lu *et al*. [32] reported that AgNPs reduce the activity of respiratory chain complexes including SDH in zebrafish model.

The present study revealed an increase in SDH activity in lycopene co-treatment group as compared to AgNPs group. This attenuation achieved by the free radical scavenging effect of lycopene that prevent mitochondrial damage and attenuate testicular energy metabolism [62]. Aly *et al.* [49] reported similar findings and suggested that the mitochondrial protection

of lycopene is due to the lipophilic nature that facilitate its passing through biological membranes.

Immuno-histochemical investigation in this study showed that AgNPs induce apoptosis evidenced by an increase of caspase-3 expression, and induce inflammation through excessive production of TNF-α in AgNPs group. Altwaijry *et al.* [50] observed an increase of caspase-3 due to AgNPs exposure and suggested that they can cause apoptosis by damaging DNA structure.

Wang *et al.* [51] explained that AgNPs could cause mitochondrial mediated apoptosis since generated intracellular ROS induced by AgNPs decrease the mitochondrial membrane potential that trigger apoptosis. However, lycopene co-treatment was shown to decrease the expression of caspase-3 that reveals an inhibition of apoptosis. Similar observation was also reported and attributed this effect to the lycopene antioxidant capability [11, 12, 38].

Oxidative stress is closely linked to inflammation by amplification loop [52]. According to Kim and Ryu [53]**,** nanoparticles being oxidants, lead to expression of cytokines and TNF-α which in turn contribute to ROS generation. Nosrati *et al.* [54] mentioned that upregulation of TNF-α is because of increased infiltrated leukocytes, which indicate inflammatory response following an oxidative stress event. Increased TNF-α expression upon AgNPs exposure was also reported in previous studies [55, 56]. However, lycopene co-treatment showed a decrease in TNF- α expressions that reveals an inhibition of inflammatory response and subsequently inhibition of extrinsic induced-apoptosis. Jiang *et al*. [57] found that lycopene normalize TNF-α level in prostatic cancer owing to its role in suppressing inflammatory response. Also, Wadie *et al.* [44] noticed TNF-α lowering effect of lycopene in hepatic tissue toxicity.

5. Conclusion

It can be concluded that AgNPs exert adverse effects on male reproductive system expressed as decrease in testicular and epididymal weight, reduced sperm count, induce oxidative stress in the spermatogenic cells, affect the testicular enzymes concentration, trigger inflammatory response and finally induce apoptosis, cumulatively leading to structural and functional testicular damage. However, uptake of antioxidants such as lycopene can successfully reduce the potential risks associated with AgNPs exposure.

References

[1] R. Scudiero, Molecular Research on Reproductive Toxicity, Int J Mol Sci. 24(4): (2023) 3538.

[2] C. Recordati, M. De Maglie, C. Cella, S. Argentiere, S. Paltrinieri, S. Bianchessi, M. Losa, F. Fiordaliso, A. Corbelli, G. Milite, F. Aureli, M. D'Amato, A. Raggi, F. Cubadda, S. Soldati, C. Lenardi, E. Scanziani, Repeated oral administration of low doses of silver in mice: tissue distribution and effects on central nervous system, Part FibreToxicol. 18(1): (2021) 23.

[3] A. Sousa, T.D. Bradshaw, D. Ribeiro, E. Fernandes, M. Freitas, Pro-inflammatory effects of silver nanoparticles in the intestine, Arch Toxicol. 96(6): (2022) 1551-1571.

[4] A.N. Skvortsov, E.Y. Ilyechova, L.V. Puchkova, Chemical background of silver nanoparticles interfering with mammalian copper metabolism, J Hazard Mater. 451: (2023) 131093.

[5] A.A.E. Mahgoob, E. Tousson, M.A. AbdEldaim, S. Ullah, A.G. Al-Sehemi, H. Algarni, I.E.T. El Sayed, Ameliorative role of chitosan nanoparticles against silver nanoparticle-induced reproductive toxicity in male albino rats, Environ SciPollut Res Int. 30(7): (2023) 17374-17383.

[6] A.M. Shehata, F.M.S. Salem, E.M. El-Saied, S.S. Abd El-Rahman, M.Y. Mahmoud, P. A. Noshy, Zinc Nanoparticles Ameliorate the Reproductive Toxicity Induced by Silver Nanoparticles in Male Rats, Int J Nanomedicine 16: (2021) 2555-2568.

[7] G.M. Abu-Taweel, H.M. Albetran, M.G. Al-Mutary, M. Ahmad, I.M. Low, Alleviation of silver nanoparticle-induced sexual behavior and testicular parameters dysfunction in male mice by yttrium oxide nanoparticles, Toxicol Rep. 8: (2021) 1121-1130.

[8] B. Salama, K.J. Alzahrani, K.S. Alghamdi, O. Al-Amer, K.E. Hassan, M.A. Elhefny, A.J.A. Albarakati, F. Alharthi, H.A. Althagafi, H. Al Sberi, H.K. Amin, M.S. Lokman, K.F. Alsharif, A. Albrakati, A.E. Abdel Moneim, R.B. Kassab, A.S. Fathalla, Silver Nanoparticles Enhance Oxidative Stress, Inflammation, and Apoptosis in Liver and Kidney Tissues: Potential Protective Role of Thymoquinone, Biol Trace Elem Res. 201(6): (2022) 2942-2954.

[9] F. Chen, C.Y. Zhao, J.F. Guan, X.C. Liu, X.F. Li, D.Z. Xie, C. Xu, High-Carbohydrate Diet Alleviates the Oxidative Stress, Inflammation and Apoptosis of *Megalobramaamblycephala* Following Dietary Exposure to Silver Nanoparticles, Antioxidants (Basel) 10(9): (2021) 1343.

[10] A.A. Antsiferova, M.Y. Kopaeva, V.N. Kochkin, P.K. Kashkarov, Kinetics of Silver Accumulation in Tissues of Laboratory Mice after Long-Term Oral Administration of Silver Nanoparticles, Nanomaterials (Basel) 11(12): (2021) 3204.

[11] A. Elsayed, A. Elkomy, M. Alkafafy, R. Elkammar, A. El-Shafey, A. Soliman, M. Aboubakr, Testicular toxicity of cisplatin in rats: ameliorative effect of lycopene and N-acetylcysteine, Environ SciPollut Res Int. 29(16): (2022) 24077-24084.

[12] X. Meng, L. Li, H. An, Y. Deng, C. Ling, T. Lu, G. Song, Y. Wang, Lycopene Alleviates Titanium Dioxide Nanoparticle-Induced Testicular Toxicity by Inhibiting Oxidative Stress and Apoptosis in Mice, Biol Trace Elem Res. 200(6): (2022) 2825-2837.

[13] A. Xu, J. Wang, H. Wang, Y. Sun, T. Hao, Protective effect of lycopene on testicular toxicity induced by Benzo[a]pyrene intake in rats, Toxicology 427: (2019) 152301.

[14] E. Kaya, S. Ozer Kaya, S. Yilmaz, A.O. Ceribasi, G. Turk, Evaluation of ameliorating effect of lycopene against testicular toxicity due to diethylnitrosamine using biochemical, spermatological and histopathological data, Andrologia 51(6): (2019) e13274.

[15] S. Przybylska, G. Tokarczyk, Lycopene in the Prevention of Cardiovascular Diseases, Int J Mol Sci. 23(4): (2022) 1957.

[16] H.J. Bearden, J.W. Fuquay, Applied animal reproduction, (1980), English book. Reston: Reston Publ. Co.

[17] G.L. Humason, 1979, Chap. 9 in (4th ed) Animal tissue techniques (San Francisco: W.H. Freerman and Company)

[18] H. Ohkawa, W. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem 95: (1979) 351–358.

[19] M. Nishikimi, N.A. Roa, K. Yogi, Biochem. Bioph. Res. Common 46: (1972) 849 – 854.

[20] H. Aebi ,Methods Enzymol 105: (1984) 121 – 126.

[21] E. Beutler, O. Duron, M.B. Kelly, J. Lab Clin. Med. 61: (1963) 882.

[22] A. Belfield, D.M. Goldberg , Enzyme 12: (1971) 561.

[23] F. Calabrese, E. Carturan, C. Chimenti, M. Pieroni, C. Agostini, A. Angelini, M. Crosato, M. Valente, G.M. Boffa, A. Frustaci, G. Thiene, Overexpression of tumor necrosis factor (TNF) α and TNFα receptor I in human viral myocarditis: clinicopathologic correlations, Mod Pathol. 17(9): (2004) 1108-1118.

[24] J.O. Olugbodi, O. David, E.N. Oketa, B. Lawal, B.J. Okoli, F. Mtunzi, Silver Nanoparticles Stimulates Spermatogenesis Impairments and Hematological Alterations in Testis and Epididymis of Male Rats, Molecules 25(5): (2020) 1063.

[25] A.A. Al-Doaiss, Q. Jarrar, M. Alshehri, B. Jarrar, *In vivo* study of silver nanomaterials' toxicity with respect to size, ToxicolInd Health 36(8): (2020) 540-557.

[26] N. Fathi, S.M. Hoseinipanah, Z. Alizadeh, M.J. Assari, A. Moghimbeigi, M. Mortazavi, M.H. Hosseini, M. Bahmanzadeh, The effect of silver nanoparticles on the reproductive system of adult male rats: A morphological, histological and DNA integrity study, AdvClinExp Med. 28(3): (2019) 299-305.

[27] A.H. Arisha, M.M. Ahmed, M.A. Kamel, Y.A. Attia, M.M.A. Hussein, Morin ameliorates the testicular apoptosis, oxidative stress, and impact on blood-testis barrier induced by photo-extracellularly synthesized silver nanoparticles, Environ SciPollut Res Int. 26(8): (2019) 28749-28762.

[28] D.Y. Ryu, K.U. Kim, W.S. Kwon, M.S. Rahman, A. Khatun, M.G. Pang, Peroxiredoxin activity is a major landmark of male fertility, Sci Rep 7: (2017) 17174–17174.

[29] N. Bahrami, S. Mehrzadi, M. Goudarzi, E. Mansouri, I. Fatemi, Lycopene abrogates di-(2-ethylhexyl) phthalate induced testicular injury by modulating oxidative, endocrine and inflammatory changes in mice, Life Sci 207: (2018) 265–271.

[30] A. Ray, D. Nath, Dose dependent intra-testicular accumulation of silver nanoparticles triggers morphometric changes in seminiferous tubules and Leydig cells and changes the structural integrity of spermatozoa chromatin, Theriogenology 192: (2022) 122-131.

[31] O.E. Shalaby, Y.H. Ahmed, A.M. Mekkawy, M.Y. Mahmoud, H.M.A. Khalil, G.A. Elbargeesy, Assessment of the neuroprotective effect of selenium-loaded chitosan

nanoparticles against silver nanoparticles-induced toxicity in rats, Neurotoxicology 95: (2023) 232-243.

[32] C. Lu, Y. Lv, G. Kou, Y. Liu, Y. Liu, Y. Chen, X. Wu, F. Yang, J. Luo, X. Yang, Silver nanoparticles induce developmental toxicity via oxidative stress and mitochondrial dysfunction in zebrafish (Danio rerio), Ecotoxicol Environ Saf. 243 (2022) 113993.

[33] S.M.A. Shariatzadeh, S.A. Miri, E. Cheraghi, The protective effect of Kombucha against silver nanoparticles-induced toxicity on testicular tissue in NMRI mice, Andrologia 53(3): (2021) e13982.

[34] P. Nie, Y. Zhao, H. Xu, Synthesis, applications, toxicity and toxicity mechanisms of silver nanoparticles: A review. Ecotoxicol Environ Saf. 253: (2023) 114636.

[35] L. Strużyńska, J. Skalska, Mechanisms Underlying Neurotoxicity of Silver Nanoparticles, Adv. Exp. Med. Biol. 1048: (2018) 227–250.

[36] H.G. Tohamy, M.A. Lebda, K.M. Sadek, M.S. Elfeky, Y.S. El-Sayed, D.H. Samak, H.S. Hamed, T.K. Abouzed, Biochemical, molecular and cytological impacts of alpha-lipoic acid and Ginkgo biloba in ameliorating testicular dysfunctions induced by silver nanoparticles in rats, Environ SciPollut Res Int. 29(25): (2022) 38198-38211.

[37] J. Costa-Rodrigues, O. Pinho, P.R.R. Monteiro, Can lycopene be considered an effective protection against cardiovascular disease? Food Chem 245: (2018) 1148–1153.

[38] H. Aly, Testicular toxicity of gentamicin in adult rats: Ameliorative effect of lycopene, Hum ExpToxicol. 38(11): (2019) 1302-1313.

[39] L.W. Tice, R.J. Barrnett, The fine structural localization of some testicular phosphatases, Anat Rec. 147: (1963) 43-63.

[40] P.C. Agu, P.M. Aja, E. EkponoUgbala, H.A. Ogwoni, E.M. Ezeh, P.C. Oscar-Amobi, A. AsukAtamgba, O.G. Ani, J.N. Awoke, F.E. Nwite, O.U. Ukachi, O.U. Orji, P.C. Nweke, E. EkponoUgbala, G.O. Ewa, I.O. Igwenyi, C.O. Egwu, E.U. Alum, D.C. Chukwu, A.C. Famurewa, *Cucumeropsismannii* seed oil (CMSO) attenuates alterations in testicular biochemistry and histology against Bisphenol a-induced toxicity in male Wister albino rats, Heliyon 8(3): (2022) e09162.

[41] G. Sharma, M. Kumar, Antioxidant and modulatory role of Chlorophytumborivilianum against arsenic induced testicular impairment, J Environ Sci (China) 24(12): (2012) 2159-65.

[42] R. Kaur, C.K. Dhanju, K. Kaur, Effects of dietary selenium on biochemical composition of rat testis, Indian J Exp Biol. 37(5): (1999) 509-11.

[43] A. Tripathy, F. Ara, P. Ghosh, D. Ghosh, Effect of lycopene on testicular androgenic and anti-oxidative enzymes in cyproterone acetate-induced male infertile Wistar strain albino rats: An in vitro study, Andrologia 52(3): (2020) e13494.

[44] W. Wadie, A.H. Mohamed, M.A. Masoud, H.A. Rizk, H.M. Sayed, Protective impact of lycopene on ethinylestradiol-induced cholestasis in rats, NaunynSchmiedebergs Arch Pharmacol. 394(3): (2021) 447-455.

[45] M. Anusha, M. Venkateswarlu, V. Prabhakaran, S.S. Taj, B.P. Kumari, D. Ranganayakulu, Hepatoprotective activity of aqueous extract of Portulacaoleracea in combination with lycopene in rats, Indian J Pharmacol. 43(5): (2011) 563-7.

[46] H.A. El-Beshbishy, H.A. Aly, M. El-Shafey, Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats, ToxicolInd Health. 29(10): (2013) 875-87.

[47] Y.Z. Zhu, H. Sun, Y. Fu, J. Wang, M. Song, M. Li, Y.F. Li, L.G. Miao, Effects of sub-chronic aluminum chloride on spermatogenesis and testicular enzymatic activity in male rats, Life Sci. 102(1): (2014) 36-40.

[48] A. Loghman, I.S. Haghdoodst, A.D. Naghi, M. Pejman, Histopathological and apoptotic effect of nanosilver in liver of broiler chickens, Afr J Biotechnol. 11: (2012) 6207–6211.

[49] H.A. Aly, H.A. El-Beshbishy, Z.M. Banjar, Mitochondrial dysfunction induced impairment of spermatogenesis in LPS-treated rats: modulatory role of lycopene. Eur J Pharmacol. 677(1-3): (2012) 31-8.

[50] N. Altwaijry, T.A. El-Masry, B. Alotaibi, E. Tousson, A. Saleh, Therapeutic effects of rocket seeds (Eruca sativa L.) against testicular toxicity and oxidative stress caused by silver nanoparticles injection in rats, Environ Toxicol. 35(9): (2020) 952-960.

[51] R. Wang, B. Song, J. Wu, Y. Zhang, A. Chen, L. Shao, Potential adverse effects of nanoparticles on the reproductive system, Int J Nanomedicine 13: (2018) 8487-8506.

[52] R. Ebabe Elle, S. Gaillet, J. Vidé, C. Romain, C. Lauret, N. Rugani, J.P. Cristol, J.M. Rouanet, Dietary exposure to silver nanoparticles in Sprague-Dawley rats: effects on oxidative stress and inflammation, Food ChemToxicol. 60: (2013) 297-301.

[53] S. Kim, D.Y. Ryu, Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues, J ApplToxicol. 33(2): (2013) 78-89, doi: 10.1002/jat.2792.

[54] H. Nosrati, M. Hamzepoor, M. Sohrabi, M. Saidijam, M.J. Assari, N. Shabab, Z. GholamiMahmoudian, Z. Alizadeh, The potential renal toxicity of silver nanoparticles after repeated oral exposure and its underlying mechanisms, BMC Nephrol. 22(1): (2021) 228.

[55] L. Kobos, S. Alqahtani, L. Xia, V. Coltellino, R. Kishman, D. McIlrath, C. Perez-Torres, J. Shannahan, Comparison of silver nanoparticle-induced inflammatory responses between healthy and metabolic syndrome mouse models, J Toxicol Environ Health A. 83(7): (2020) 249-268.

[56] S. Nayek, A.K. Lund, G.F. Verbeck, Inhalation exposure to silver nanoparticles induces hepatic inflammation and oxidative stress, associated with altered renin-angiotensin system signaling, in Wistar rats, Environ Toxicol. 37(3): (2022) 457-467.

[57] L.N. Jiang, Y.B. Liu, B.H. Li, Lycopene exerts anti-inflammatory effect to inhibit prostate cancer progression, Asian J Androl. 21(1): (2018) 80–5.

[58] Y. Zhao, L.G. Cui, M. Talukder, J.G. Cui, H. Zhang, J.L. Li, Lycopene prevents DEHP-induced testicular endoplasmic reticulum stress *via* regulating nuclear xenobiotic receptors and unfolded protein response in mice, Food Funct. 12(24): (2021) 12256-12264.

[59] J. Zhang, F. Zheng, C. Liang, Y. Zhu, Y. Shi, Y. Han, J. Wang, Sulfur dioxide inhalation lowers sperm quality and alters testicular histology via increasing expression of CREM and ACT proteins in rat testes, Environ Toxicol Pharmacol. 47: (2016) 47-52.

[60] H.A. El Kattawy, E.R. Abozaid, D.M. Abdullah, Humanin Ameliorates Late-onset Hypogonadism in Aged Male Rats, Curr Mol Pharmacol. 15(7): (2022) 996-1008.

[61] A. Choudhary, S. Singh, V. Ravichandiran, Toxicity, preparation methods and applications of silver nanoparticles: an update, Toxicol Mech Methods 32(9): (2022) 650-661.

[62] W. Huang, Z. Cao, Y. Cui, S. Huo, B. Shao, M. Song, P. Cheng, Y. Li, Lycopene ameliorates aflatoxin B_1-induced testicular lesion by attenuating oxidative stress and mitochondrial damage with Nrf2 activation in mice, Ecotoxicol Environ Saf. 256: (2023) 114846.

[63] H. Chang, L. Li, Y. Deng, G. Song, Y. Wang, Protective effects of lycopene on TiO_2 nanoparticle-induced damage in the liver of mice, J Appl Toxicol. 43(6): (2023) 913-928.

الملخص العربى

تقييم الكفاءة التناسلية لذكور الجرذان المعرضة لجزيئات الفضة النانونية والدور المحسن لليكوبين

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تشهد الآونة الحالية تناقص ملحوظ في معدلات الخصوبة لدى الذكور، فقد أدى التطور التكنولوجي في علوم المادة والاستخدام الواسع لتطبيقات المواد النانونية إلي تقليل الكفاءة التناسلية لديهم. تعتبر جزيئات الفضة النانونية واحدة من أهم المواد النانونية التي تستخدم بصورة منتشرة نظرا لخصائصها المضادة للبكتيريا والفيروسات،مع ذلك فقد ثبتت آثارها العكسية علي القدرة الإنجابية للذكور. وعلي النقيض، تقوم مضادات الأكسدة بالحد من الآثار العكسية المستحثة بالعديد من السموم، فيعتبر مركب الليكوبين من أهم مضادات الأكسدة التي لها القدرة علي تحسين الصحة الإنجابيةوتعزيز إنتاج الخلايا التناسلية. لذلك، تمتهذه الدراسة لتقييم الأداء الإنجابي لذكور الجرذان المهقاء المعرضة لجزيئات الفضة النانونية بجرعة يومية١٠٠ مجم/كجم والدور المحسن لليكوبين بجرعة يومية ٥ مجم/كجم لمدة ٦٠ يوم. كشفت النتائج عن قدرة جزيئات الفضة النانونية علي تقليل الزيادة الطبيعية في وزن الجسم والأعضاء التناسلية مثل الخصي والبربخ وكذلك أعداد الحيوانات المنوية لدى ذكور الجرذان.أظهرت نتائج الفحص النسيجى آثارا مدمرة بالانيبيبات المنوية المعرضة لجزيئات الفضة تتمثل في ضمور الانيبيبات وتكوين فجوات بداخلها، ظهور وذمات بين الانيبيبات وبعضها واختفاء الحيوانات المنوية داخل تجويفها.أيضا، أظهرت نتائج التحليل الحيوي الكيميائي وكيمياء الأنسجة لعينات ونسيج الخصية عن ارتفاع مستدلات الجهد التأكسدى ومعامل الإلتهاب والموت المبرمج للخلايا وانخفاض مستويات الإنزيمات المضادة للأكسدة، بينما أدي التعرض المشترك لجزيئات الفضة النانونية ومركب الليكوبين إلي تحسين جميع الآثار العكسية المستحثة بجزيئات الفضة. لذا، توصي هذه الدراسة بالحد من التعرض لهذه الجزيئات وخاصة في العمر الإنجابي والإكثار من تناول مضادات الأكسدة خاصة الليكوبين.