Alpha-Lipoic Acid and Ginkgo Biloba Ameliorate Testicular Dysfunctions Induced by Silver Nanoparticles in Rats

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Abstract

Silver nanoparticles (AgNPs) are more commonly utilised in medicine, however they have negative effects on the majority of organs, including the reproductive system. AgNPs were reported to be able to reach the testes due to their small size, which allows them to pass through blood testicular barriers. The goal of this study was to see if LA (alpha lipoic acid) or GB (ginkgo biloba) might protect adult rat testes after intraperitoneal injection of AgNPs. Forty male healthy adult Wister albino rats were randomly assigned to four groups (10 rats each); control, AgNPs-intoxicated group intraperitoneally injected AgNPs 50 mg/kg b.w, 3 times a week, LA + AgNPs group intoxicated with AgNPs and orally gavaged with 100 mg LA/kg b.w, and GB + AgNPs group injected with AgNPs and orally given GB extract 120 mg/kg b.w were continued for 30 consecutive days. Biochemical changes in testicular tissue (testosterone, ACP, and Prostatic acid phosphatase), oxidative indices in testicles tissues, mRNA expression of pro-apoptotic (BAX) and anti-apoptotic (BCL-2) biomarkers, histological, and immunohistochemical changes were studied. Significant decrease in serum testosterone level and elevation in ACP and PACP enzyme activity in AgNPs treated group than in the control. In addition, lowering in tGSH, GSH GR, GPx and elevation MDA and GSSG were observed in AgNPs treated group than control. Decreasing in mRNA expression thioredoxin-1 (Txn-1), transforming growth factor-1β (TGF-1β), anti-apoptic (BCL-2) and elevation the expression of proapoptotic biomarkers (BAX) in the testis homogenates of rats exposed to silver nanoparticles. Strong positive action to BAX and lowering the action of Ki-67 antibody were observed. Because of their antioxidant, anti-inflammatory, and anti-apoptotic properties, co-treatment with LA or GB may be beneficial in reducing the harmful effects of AgNPs on the testicles.

1. Introduction

Surface cleansing agents, washing machines, toys and textiles, air and water filters, food canning, and antimicrobial coatings all contain silver nanoparticles (AgNPs) (Tolaymat et al., 2010). AgNPs' antibacterial activity has led to their use as a sterilising agent in medical devices such as contraceptives, wound dressings, surgical equipment, and medical catheters (Wijnhoven et al., 2009). AgNPs were also used in the administration of medications for tumour and retinal treatment (Kalishwaralal et al., 2010). Because of the vast range of AgNPs uses, the amount of AgNPs released into the environment is increasing, as is the vulnerability of living species to AgNPs exposure via various routes such as inhalation, ingestion, cutaneous, and injection (Marambio-Jones and Hoek, 2010). AgNPs can pass through the blood-testicular barrier and the cell membrane (Schrand et al., 2010). The negative effects of AgNPs on the male reproductive system at various doses, include a decrease in spermatocytes, spermatids, and spermatozoa counts in rats (Miresmaeili et al., 2013). Moreover, (Baki et al., 2014a) reported the disruption of male sexual hormones, with deleterious consequences for sexual organ maturation and sperm characteristics in rats exposed to varied dosages of AgNPs. The endocrine; follicle stimulating hormone (FSH) and luteinizing hormone (LH) and paracrine hormonal pathways; testosterone, which affect germ cell proliferation and effective spermatogenesis and are implicated in apoptosis when their levels are low, are required for optimal testicular activities (Sofikitis et al., 2008). Despite the fact that AgNPs caused testicular toxicity, the putative molecular processes underpinning this
remained unknown. The proposed hypothesis includes three levels: disruption in endocrine hormones, decreased spermatogonia and primary spermatocyte proliferation, and direct influence on germ and Leydig cells via induction of oxidative stress and apoptosis, all of which are intertwined in AgNPs-induced testicular dysfunctions. Many attempts have been made to mitigate the negative effects of AgNPs on testicular function, including the use of natural compounds.

The natural product generated by mitochondria, alpha lipoic acid (ALA), had a role in enzymatic mitochondrial bioenergetics as a cofactor and possessed substantial antioxidative action via many pathways (Sadek et al., 2018). Biewenga et al. (1997) reported that ALA and its reduced counterpart dihydrolipoic acid have antioxidant properties through scavenging activity, antioxidant regeneration, metal chelation, and repair mechanisms. Furthermore, ALA showed an anti-inflammatory effect, lowering pro-inflammatory cytokines such as IL-6 and IL-1 and modulating NF-kB (Dinicola et al., 2017). In rats with surgical varicocele, ALA was found to improve sperm parameters and minimise lipid peroxidation and DNA damage (Shaygannia et al., 2018). Consequently, (Lebda et al., 2014) revealed ALA protects rats from acrylamide-induced testicular damage by increasing blood testosterone, testicular antioxidative enzymes, and decreased glutathione levels while lowering MDA levels.

Flavonoids and terpenoids such as quercetin, kaempferide, rutin, and ginkgolides are the primary ingredients of Ginkgo biloba tree leaves extract (GBE) (Watanabe et al., 2001), showing free radical scavenging activity (Li et al., 2003). GBE’s antioxidative and antiapoptotic properties make it a good candidate for treating and preventing oxidative damage in many tissues (Akgül et al., 2008). The goal of this study was to see if ALA and GBE could protect rats from AgNPs-induced testicular injury by looking at endocrine hormonal balance, cell proliferative activity, oxidative stress, and apoptotic pathways.

2. Methods

2.1. Chemicals and reagents

AgNPs fine powder was purchased from Sigma Aldrich (St. Louis, MO, USA). The particles were suspended in deionized water by vigorous vortexing and sonication prior to use to ensure the prevention of particle aggregation and characterized previously for their size and shape (Lebda et al., 2018b). LA was obtained from EVA Pharma Co. (Cairo, Egypt). *Ginkgo biloba L*. extract code# GK501 standardized to 24% Ginkgo flavonoids was purchased from Pharmaton SA (Lugano, Switzerland). ELISA testosterone kit was purchased from Cayman Chemical Co, USA. Other bio-diagnostic kits were obtained from Biodiagnostic Co, Giza, Egypt.

2.2. Animals, housing conditions, and experimental protocol

Forty male Wistar Albino rats weighing 180–200 g were purchased from Animal Breeding Unit, Medical Research Institute, Alexandria University. The animals were kept in metal cages under environmental-controlled conditions with optimum temperature, humidity, and dark/light cycle and free access to rat chow and drinking water. The international ethical guidelines for the care and use of laboratory animals
were performed to handle the animals and the experimental procedures were approved by the Experimental Animal Use and Ethics Committee at the Faculty of Veterinary Medicine, Alexandria University, Egypt. The rats were randomly assigned to four groups (10 rats each); control, AgNPs-intoxicated group intraperitoneally injected AgNPs 50 mg/kg b.w, 3 times a week, LA + AgNPs group intoxicated with AgNPs and orally gavaged with 100 mg LA/kg b.w (Pari and Murugavel, 2004), and GB + AgNPs group injected with AgNPs and orally given GB extract 120 mg/kg b.w (Huang et al., 2017). All treatments were continued for 30 consecutive days. The dose of AgNPs was selected based on the study of Tiwari et al., (2011). Twenty-four hours after the last doses, the rats were anesthetized using ketamine/xylazine (7.5–10 mg/kg, 1 mg/kg i.p). The blood was collected from the inner canthus, and the sera were separated for estimation of testosterone, total acid phosphatase (ACP) and prostatic acid phosphatase (pACP) activities according to the manufacturers' guidelines. The rats were then euthanized and the whole testicles were immediately dissected, rinsed with chilled normal saline 0.9% and divided longitudinal into two halves; one was used for histopathological and immunohistochemical analyses and the other half was used for estimation of oxidative indices, and transcriptome analyses.

2.3. Lipid peroxidation and antioxidant profile

The testicular tissue (about 500 mg) was homogenized using Teflon and pestle homogenizer in ice-cold 0.1 M phosphate buffer saline pH 7.4. The supernatant was separated after centrifuging the crude homogenate at 14,000 rpm for 10 min at 4°C. Lipid peroxide was measured after the reaction with thiobarbituric acid and expressed as nmol malondialdehyde (MDA) per tissue weight (Ohkawa et al., 1979). Glutathione peroxidase (GPX) activity was analysed according to Paglia and Valentine (1967), which based on the reaction of hydrogen peroxide ($H_2O_2$) in the presence of NADPH, GSH, and glutathione reductase. The absorbance measured at 340 nm and the result was expressed as IU per tissue weight. The enzymatic method described by (Griffith, 1980) was used to measure the total glutathione content where it depends on the oxidation of GSH by 5, 5’-dithiobis-(2-nitrobenzoic acid) (DTNB) to yield GSSG and 5-thio-2-nitrobenzoic acid (TNB). Oxidized GSSG is reduced enzymatically by the action of glutathione reductase and NADPH to regenerate GSH which reacts again. The rate of TNB formation is monitored at 412 nm and is proportional to the sum of GSH and GSSG present in the sample. The GSSG content is determined by the same assay as total glutathione, but where the reduced glutathione is bound by 2-vinylpyridine.

2.4. RNA extraction and qRT-PCR

About 100 mg testicular tissues were rinsed in sterilized phosphate buffer saline and homogenized in liquid nitrogen using Teflon and pestle homogenizer then the homogenates were stored at −80°C till RNA isolation. Total RNA was isolated using the RNeasy Mini Kit (Qiagen GmbH, Germany) according to the manufacturer instructions. cDNA was synthesized from the purified RNA using QuantiTect Reverse Transcription Kit (Qiagen). The reaction mixture included RNA and master mix were placed at 42°C then inactivated at 95°C. The qRT-PCR for the target genes were performed using QuantiTect SYBR Green PCR Master Mix (Qiagen Rotor-Gene Q). The primer sequences of all target and reference genes and the PCR
conditions were recorded in Table 1. The fold change of mRNA expression was calculated after recording the Ct values for reference and target genes using the $2^{-\Delta\Delta C_t}$ method.

### 2.5. Immunohistochemical assessment

The standard horseradish-peroxidase immunohistochemistry technique was applied to positive charged slides of paraffin testicular tissue sections. Several 4-µm thick sections of the testicular tissues were deparaffinized in xylene, rehydrated in descending grades of ethanol, and pre-treated with 3% H$_2$O$_2$ to block endogenous peroxidase activity. Antigen retrieval was accomplished by placing slides in a microwave for 10 min in 10 mM sodium citrate buffer (pH 6.0). Slides were incubated with the specific primary antibody; monoclonal rat anti-ki67 (Abcam, Cat: ab156956), and polyclonal rabbit anti-Bax (Abcam, Cat: ab53154) diluted in 1% BSA/PBS pH 7.4 at 1:100 then rinsed with PBS and the sections were incubated with biotin-conjugated goat anti-rat IgG antiserum (Abcam, Cat: 182018) for 60 min and then rinsed with PBS followed by Streptavidin-peroxidase conjugate (Histofine Kit, Nichirei Corp) incubation for 30 min. The sections were visualized using 3,3′-diaminobenzidine tetra-hydrochloride substrate chromogen solution then counterstained with hematoxylin stain and examined under light microscope.

### 2.6. Histopathologic examination

Testicular samples of 5 rats per group were collected and immediately fixed in 10% buffered formalin for at least 24 h. Tissue specimens were washed, dehydrated by serial dilutions of alcohol, cleared in xylene, and embedded in paraffin at 60°C in a hot air oven. Paraffin sections of 4–5 microns in thickness were prepared and stained with hematoxylin and eosin (HE) and examined under the light microscope.

### 2.7. Statistical analyses

The obtained values are expressed as the mean ± standard error (SE). Using the SPSS statistical package v22.0 for Windows (IBM, Armonk, NY, USA), one-way ANOVA followed by post hoc multiple comparisons Duncan's test were used to analyze obtained data. The significance level was set at p ≤ 0.05.

### 3. Results

#### 3.1. Reproductive hormonal changes

The adverse impacts of AgNPs on the reproductive hormonal status of male rats and the protective potency of LA and GB are shown in Table (2). Comparatively with controls, serum concentrations of testosterone showed a significant (p ≤ 0.05) reduction in AgNPs-treated rats, which had been associated with a significant (p ≤ 0.05) enhancement of total and prostatic ACP levels. However, these hormonal disturbances were significantly (p ≤ 0.05) attenuated following LA or GB co-treatment, compared with AgNPs-treated groups. The attenuation was more noticeable with LA, particularly in relation to testosterone (2.81 ± 0.12 IU/L) and total ACP (13.82 ± 1.28 IU/L) levels, (Group LA + AgNPs) vs GB + AgNPs-treated rats.
3.2. Testicular oxidant/antioxidant status

The effect of AgNPs on the testicular oxidant/antioxidant capacity of rats and the ameliorative effect of LA and GB are shown in Table (3). The testicular oxidants – MDA and GSSG concentrations were found to be significantly ($p \leq 0.05$) elevated, and antioxidants – total GSH, reduced GSH, GR and GPx activities were significantly ($p \leq 0.05$) lowered in the AgNPs-intoxicated rats. Meanwhile, compared to AgNPs group, LA and GB cotreatment were significantly ($p \leq 0.05$) reduced MDA and GSSG levels, and increased total GSH, reduced GSH content and GR and GPx activities in the testicular tissues. LA and GB administration reduced AgNPs-induced testicular oxidative stress in rat, without ensuring complete protection in relation to control ones. Moreover, LA cotreatment was generally offered better antioxidant properties than GB do.

3.3. Testicular antioxidant and apoptosis-related genes expression

Regarding the effects of AgNPs on the antioxidant (Txn-1, TGF-1$\beta$) and apoptosis (Bax, Bcl-2)–related mRNA gene transcripts, it has been observed that AgNPs down-regulated ($p \leq 0.05$) the relative mRNA expression of antioxidant Txn-1 ($\approx 68\%$) and TGF-1$\beta$ ($\approx 27\%$) genes, and anti-apoptotic Bcl-2 gene ($\approx 78\%$). However, it up-regulated ($p \leq 0.05$) the relative mRNA expression of pro-apoptotic Bax gene ($\approx 401\%$) in the testicles of rats (Table 4), compared to controls. Markedly, co-administration of either LA or GB were attenuated the mRNA expression the Txn-1 ($\approx 202\%, 186\%$) and TGF-1$\beta$ ($\approx 189\%, 147\%$) genes, and Bax ($\approx 199\%, 291\%$) genes. However, the mRNA expression of testicular Bcl-2 gene was upregulated ($\approx 1.18\%, p \leq 0.05$) following LA, and downregulated ($\approx 22\%, p \leq 0.05$) following GB co-administration, compared to controls (Table 4).

3.4. Histopathological analysis

Testicular tissue of the control healthy rats had normal histoarchitecture that composed of uniform, well-organized seminiferous tubules with complete spermatogenesis and normal interstitial connective tissue (Fig. 1a). Testicular sections of silver-nanoparticles-treated group showed degenerative changes of the seminiferous tubules as shrunken, disorganized seminiferous tubules with irregular basement membrane and vacuolar degeneration of spermatogonia cells (Fig. 1b). Some seminiferous tubules showed coagulative necrosis and depletion of germinal epithelium with hyalinization of the luminal contents, exfoliating of degenerated germinal epithelial cells and interstitial edema that was represented by faint eosinophilic material (Fig. 1c). The lumina of some seminiferous tubules contained giant cell formations (Fig. 1d). The microscopic pictures in silver-nanoparticles + lipoic acid treated group were interstitial edema and few tubules contained sloughed germinal epithelium (Fig. 1e) while the noticeable lesions in silver-nanoparticles + gink trated group the majority of seminiferous tubules had normal germinal epithelium and marked improvement of spermatogenesis with elongated spermatids and spermatozoa. Some tubules showed few vacuolated germinal epitheliums(Fig. 1f).
Hematoxiline and Eosin stained sections of the control Caput epididymis revealed normal histological architecture with normal sperm density (Fig. 2a). Epididymal sections of silver-nanoparticles-treated group showed vacuolation of some caput epididymal epithelium (Fig. 2b) beside sloughed germ cells in its lumina (Fig. 2c) and congestion of interstitial blood vessel with perivascular inflammatory cell infiltrations (Fig. 2d). Silver-nanoparticles + lipoic acid treated group and silver-nanoparticles + gink treated group showed normal histological structure with normal sperm density (Fig. 2e and f).

The control Couda epididymis sections had normal histological architecture with normal sperm density (Fig. 3a). The detectable histopathological pictures in silver-nanoparticles-treated group were vacuolation of some Couda epididymal epithelium (Fig. 3b), sloughed germ cells in its lumina with hyalinized of the luminal contents of some epididymal ductules (Fig. 3c) and low sperm density in the most of epididymal ductules beside congestion of interstitial blood vessel with perivascular inflammatory cell infiltrations (Fig. 3d). Silver-nanoparticles treated with lipoic acid and Gink showed normal histological structure with normal sperm density (Fig. 3e and f).

### 3.5. Immunohistochemistry

As appeared in figures (4 and 5) respectively, Silver-nanoparticles induced negative staining of KI-67 and positive staining for Bax. On the other hands, administration of lipoic acid and ginka induced positive staining of KI-67 and negative staining of Bax.

### 4. Discussion

Green chemistry is being developed to make environmentally acceptable nanomaterials such as AgNPs, which have a lot of attention because of their excellent qualities. Many studies, however, have discovered their potential harm on various organs (Roda et al., 2017, Brohi et al., 2017). Intraperitoneal injection was chosen above the other regularly utilised routes for AgNPs administration in this experimental study. In terms of pharmacokinetics, intraperitoneal injections are identical to oral injections. The intraperitoneal route, on the other hand, offers the advantage of delivering drugs into the bloodstream faster than the oral route (Elkhawass et al., 2015, Fathi et al., 2018).

The current study found that repeated intraperitoneal injections of AgNPs to rats result in changes in serum male sex hormone and enzymes. In comparison to the control and another treated group, the AgNPs-treated group saw a substantial reduction in serum testosterone levels. AgNPs block cholesterol transport into the inner mitochondrial membrane by lowering steroidogenic acute regulatory protein (STAR) expression, which eventually stops the conversion of cholesterol to pregnenolone levels (Baki et al., 2014b). Furthermore, decreased TGF-1 expression results in substantially insufficient male serum and intratesticular testosterone, as well as serum androstenedione. Because serum LH and serum FSH were lowered, and exogenous LH replacement with human chorionic gonadotropin (hCG) caused serum testosterone to decrease, the testosterone hormone shortage was secondary to disturbed pituitary gonadotropin release (Ingman and Robertson, 2007).
The concentration of serum prostatic acid phosphatase (Prostatic ACP) and the rise of serum acid phosphatase (ACP) produced in the liver, spleen, and prostate gland in the AgNPs group. This increase is a sign of benign prostatic hyperplasia (BPH) and the early stages of prostate cancer (Goto et al., 2009, Lee and Finn, 2012). Using of LA with AgNPs group improved the serum male sex hormones than AgNPs group. These result was correlated with Othman et al. (2012) who revealed that lipoic acid kept cholesterol and sex hormone-binding globulin (SHBG) levels the same as in control rats. These results could be attributable to LA's antioxidant activity, which improves the signal transduction pathways required for optimal hypothalamus-testicular axis function, resulting in normal testosterone release and sperm generation. Also, the treatment with GB can enhances testosterone synthesis and secretion of Leydig cells (Wu et al., 2008a). The prostatic acid phosphatase level is reduced when the general body condition and testicles improved in using GB or LA due to their antioxidant activity.

Durán et al. (2016) concluded that the toxicity of AgNPs can be explained by three mechanisms: (1) free silver ions are taken up by cells, decreasing ATP synthesis and DNA replication; (2) the creation of reactive oxygen species (ROS) is enhanced on the surface of AgNPs and Ag ions; and (3) AgNPs directly damage cell membrane. Total GSH, GSH, GR, and GPX levels were all lower in the AgNPs-treated group. GSH depletion and increased ROS formation intracellular may cause damage to cellular components, followed by an increase in lipid peroxidation, resulting in an increase in MDA (Piao et al., 2011). AgNPs reduced antioxidants like GSH and antioxidant enzymes like glutathione peroxidase and superoxide dismutase because they exhibited a strong attraction for thiol groups, which reacted with sulfur-containing proteins like GSH (El Mahdy et al., 2015). The increase in GSSG reductase activity is proportional to the amount of GSSG present. GSH concentration drops during acute oxidative stress, which is coupled with an increase in GSSG concentration, resulting in GSH/GSSG cycle turnover. (Jones, 2002).

Antioxidants are known to reduce oxidative radical-induced reaction (Lebda et al., 2018a, Sadek et al., 2016, Sadek, 2014, Sadek et al., 2019, Sadek et al., 2018, Sadek et al., 2017). Because of its stronger antioxidant qualities and restorative potential on oxidative stress-related damage, cotreatment with LA increased antioxidant indices. The ability of LA to function as a metal chelator and reduce free oxygen radicals while increasing the regeneration capacity of oxidised versions of other endogenous antioxidant agents is what gives it its antioxidant characteristics (El-Sayed et al., 2017).

It has anti-inflammatory, anti-neoplastic, and anti-proliferative properties as well. The antioxidant activity of GB was related to its components of terpenoids and flavonoids, which operate as broadspectrum free radical scavengers and lower lipid peroxidation, which may explain why cotreatment with GB improved antioxidant indices (Yeh et al., 2009, Mohamed and Abd El-Moneim, 2017). By blocking and terminating radical chain reactions and suppressing ROS and lipid peroxidation reactions, flavonoid glycosides can either eliminate free radicals to reduce the consumption of SOD and GSH-Px or promote the production of SOD and GSH-Px to scavenge free radicals such as superoxide anion and hydrogen peroxide as a result, the antioxidant testicular enzymes SOD and catalase were restored, and the concentration of testicular MDA was reduced, as described in our findings (Wu et al., 2008a, Amin et al., 2012).
When ROS generation surpasses the capacity of the antioxidant defence, which is connected to lipid peroxidation, an imbalance in oxidative stress and antioxidant capacity ensues (Quinteros et al., 2018) and induction of apoptosis. (Kim et al., 2011). Thioredoxin is found in a variety of biological systems. It prolongs life and guards against oxidative stress in various organs and cell types (Mitsui et al., 2002).

Thioredoxin-1 (Trx-1) is one of the most essential cellular antioxidant systems, lowering oxidised proteins through thiol-disulfide exchange processes and eventually redox-sensitive signal transduction (Lu and Holmgren, 2014, Lillig and Holmgren, 2007) and protects cells from apoptosis (Lu and Holmgren, 2012). To prevent stress and cytokine-induced apoptosis, the reduced/dithiol form of Trxs binds to apoptotic signal-regulating kinase 1 (ASK1) and suppresses its activity. Due to ROS, thioredoxin-1 expression was found to be lower in the silver nanoparticles treated group compared to the control and other groups, indicating that it dissociates from Ask1 and stimulates apoptosis. Trx interacting protein (TXNIP), which binds to Trx and removes Trx from ASK1, also contributes to the apoptotic process (Jun and Arne 2012).

AgNP-induced p53 activation has also been observed in mouse and human cells (Ahamed et al., 2008, Gopinath et al., 2010). P53 is activated by a variety of cell death events that can activate gene expression or permeabilize mitochondria, causing apoptosis. P53 builds up in the nucleus and regulates the production of the proapoptotic protein Bax (Wu et al., 2008b). P53 can enter mitochondria, interact with antiapoptotic Bcl-2 proteins, neutralise them, and trigger cell death (Li et al., 2015). The proapoptotic Bax and antiapoptotic Bcl-2 molecules are two key players in cell death, and the ratio of Bax/Bcl-2 is the numerator that determines whether or not cells will die. The AgNP-treated rats had higher Bax expression, as well as a strong positive immunological response and lower Bcl-2 expression, resulting in a higher Bax/Bcl-2 ratio, as seen in humans treated with AgNPs (Gopinath et al., 2010, Piao et al., 2011).

By upregulating the expression of two genes that encode the anti-apoptotic proteins Bcl-2 and Xiap via a process that appears to include NF-B, cotreatment with LA substantially prevented apoptosis of testicular cells (Antonio et al., 2011). Ginkgo biloba also, has antiapoptotic effects through the protection of mitochondrial membrane integrity, possibly by its flavonoid constituents (Takao, 2000) this agree with (Guan et al., 2014) who found that the apoptotic index was decreased with the antioxidant and anti-inflammatory effects of the Ginkgo biloba treatment on the organs.

In fact, histological and immunohistochemical examinations revealed that AgNPs have a harmful effect. Damage to testicular tissue was observed in the AgNPs-treated group, which could be attributed to silver nanoparticles crossing the blood-testis barrier (BTB) and accumulating in the testicles, as previously reported in multiple studies (Asare et al., 2012) due to particle size of it ((Amin et al., 2015). The toxicity is related to the nano-surface. silver's In the environment and biological systems, it is easily oxidised by O2 and other molecules, resulting in the release of Ag, a recognised hazardous ion. Nano-silver has been demonstrated to infiltrate and internalise cells. As a result, nano-silver is frequently used as an Ag source within cells (McShan et al., 2014). In a concentration- and time-dependent way, AgNPs are more cytotoxic, producing apoptosis, necrosis, and reduced proliferation (Asare et al., 2012). In comparison to the control group, AgNPs treated groups had lower Ki-67 antibody expression. The reduction in Ki67 expression as a
AgNPs can bind to membrane proteins and activate signalling pathways, resulting in cell proliferation inhibition (Asharani et al., 2008). AgNPs can also enter the cell via diffusion or endocytosis, causing mitochondrial malfunction and the production of reactive oxygen species (ROS), which causes damage to proteins and nucleic acids inside the cell, as well as cell proliferation inhibition (Lim et al., 2012).

Histopathological analysis of testicular sections from the silver nanoparticles-treated group revealed degenerative alterations in the seminiferous tubules, such as smaller, disordered seminiferous tubules with uneven basement membranes, which could lead to significant testis functional impairment (Liu et al., 2013). The loss of germinal epithelium could also be attributed to a significant disruption of the Sertoli-germ cell connection. The sloughing of the germ cells from the seminiferous epithelium could have been caused by a breakdown in this physical contact (Erkanlı Şentürk et al., 2012). Hyalinization of luminal contents, exfoliation of degenerated germinal epithelial cells, and interstitial edema in seminiferous tubules with giant cell formations were also seen. The earliest physical symptom of testicular injury is vacuolation degeneration of spermatogonia cells, also known as coagulative necrosis. It is thought that vacuolation causes spermatogenic cells to detach from Sertoli cells, which is the first step toward cell death or apoptosis (Asare et al., 2012).

The epididymal sections of the silver-nanoparticles-treated group showed vacuolation of some caput epididymal epithelium alongside sloughed germ cells in its lumina and congestion of interstitial blood vessel with perivascular inflammatory cell infiltrations, indicating that continued exposure to toxic chemicals may lead to some histologic changes. The vacuolation of some Couda epididymal epithelium, sloughed germ cells in its lumina, hyalinization of the luminal contents of some epididymal ductulus, and low sperm density in the majority of epididymal ductulus were all observed in this study, along with congestion of interstitial blood vessels and perivascular inflammatory cell infiltrations. The blood–testis barrier must remain intact in order to maintain reproductive potential. Exposure to environmental toxins can compromise this barrier, causing the production of reactive oxygen species (ROS) in the testes, resulting in oxidative DNA damage and infertility. Because LA is a fat- and water-soluble antioxidant, it is found in cellular membranes (Prahalathan et al., 2006). LA protects against nanoparticle-induced oxidative disruption of the blood–testis barrier and testicular histological alterations, according to our findings.

5. Conclusion

AgNPs had a deleterious impact on male reproductive processes in rat testis, according to recent findings (biochemical, oxidative stress, apoptosis, immunohistochemical and pathological examination). As a result, it's possible to conclude that AgNPs are very hazardous to reproductive function and may affect animal fertility. Hormonal disturbances, apoptosis, mRNA expression abnormalities, and pathological alterations could all be caused by mechanisms linked to oxidative stress after rats were exposed to
AgNPs. Furthermore, LA and GB are reported to be a recent and effective preventative treatment for AgNP-induced reproductive damage in male rats.

**Declarations**

**Ethical Approval**

This review was written according to the guidelines of ethical committee of faculty of Veterinary Medicine, Damanhour University, Egypt

**Consent to Participate**

This review was written by single author

**Consent to Publish**

This review was written by single author and agrees to publish it

**Authors Contributions**

Hossam G. TOHAMY do the histopathology, share in manuscript drafting

Mohamed A. LEBDA share in biochemical and molecular analysis, share in manuscript drafting

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No any competing interests to be disclosed

**Availability of data and materials**

All data and materials were present in the review article
References

1. AHAMED, M., KARNS, M., GOODSON, M., ROWE, J., HUSSAIN, S. M., SCHLAGER, J. J. & HONG, Y. 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. *Toxicology and applied pharmacology*, 233, 404-410.

2. AKGÜL, T., AYYILDIZ, A., NUHOGLU, B., KARAGÜZEL, E., OGÜS, E., YAGMURDUR, H., USTÜN, H. & GERMİYANOGLU, C. 2008. Ginkgo biloba (Egb 761) usage attenuates testicular injury induced by testicular ischemia/reperfusion in rats. *International urology and nephrology*, 40, 685-90.

3. AMIN, A., ABRAHAM, C., HAMZA, A. A., ABDALLA, Z. A., AL-SHAMSI, S. B., HARETHI, S. S. & DAoud, S. 2012. A standardized extract of Ginkgo biloba neutralizes cisplatin-mediated reproductive toxicity in rats. *Journal of Biomedicine and Biotechnology*, 2012.

4. AMIN, Y. M., HAWAS, A. M., EL-BATAL, A. & ELSAYED, S. H. H. E. 2015. Evaluation of acute and subchronic toxicity of silver nanoparticles in normal and irradiated animals. *British Journal of Pharmacology and Toxicology*, 6, 22-38.

5. ANTONIO, A. M., GILLESPIE, R. A. & DRUSE–MANTEUFFEL, M. J. 2011. Effects of lipoic acid on antiapoptotic genes in control and ethanol-treated fetal rhombencephalic neurons. *Brain Research*, 1383, 13-21.

6. ASARE, N., INSTANES, C., SANDBERG, W. J., REFSNES, M., SCHWARZE, P., KRUSZEWSKI, M. & BRUNBORG, G. 2012. Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. *Toxicology*, 291, 65-72.

7. ASHARANI, P. V., WU, Y. L. & GONG, Z. 2008. Toxicity of silver nanoparticles in zebra fishmodels. *Nanotechnology*, 19, 255102.

8. BAKI, M. E., AMRAII, E., YOUSEFI, V., SPENANI, H. R., FAZILATI, M., FALLAH, A. A., MIRESMAILI, S. M., MIRESMAILI, S. M., POURENTEZARI, M., TALEBI, A. R., ANVARI, M., MANGOLI, E., TALEBI, A. R., ANVARI, M. & MANGOLI, E. 2014a. Effects of silver nano-particles on sperm parameters, number of Leydig cells and sex hormones in rats. *Iran. J. Reprod. Med. Iranian Journal of Reproductive Medicine*, 12, 139-144.

9. BAKI, M. E., MIRESMAILI, S. M., POURENTEZARI, M., AMRAII, E., YOUSEFI, V., SPENANI, H. R., TALEBI, A. R., ANVARI, M., FAZILATI, M. & FALLAH, A. A. 2014b. Effects of silver nano-particles on sperm parameters, number of Leydig cells and sex hormones in rats. *Iranian journal of reproductive medicine*, 12, 139.

10. BIEWENGA, G. P., HAENEN, G. R. M. M. & BAST, A. 1997. The pharmacology of the antioxidant lipoic acid. *General Pharmacology*, 315-331.

11. BROHI, R. D., WANG, L., TALPUR, H. S., WU, D., KHAN, F. A., BHATTARAI, D., REHMAN, Z.-U., FARMANULLAH, F. & HUO, L.-J. 2017. Toxicity of nanoparticles on the reproductive system in animal models: a review. *Frontiers in pharmacology*, 8, 606.

12. DINICOLA, S., BIZZARRI, M., PROIETTI, S., CUCINA, A. & FUSO, A. 2017. Alpha-lipoic acid downregulates IL-1b and IL-6 by DNA hypermethylation in SK-N-BE neuroblastoma cells. *Antioxidants*
13. DURÁN, N., DURÁN, M., DE JESÚS, M. B., SEABRA, A. B., FÁVARO, W. J. & NAKAZATO, G. 2016. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomedicine: Nanotechnology, Biology and Medicine, 12, 789-799.

14. EL-SAYED, E. S. M., MANSOUR, A. M. & EL-SAWY, W. S. 2017. Alpha lipoic acid prevents doxorubicin-induced nephrotoxicity by mitigation of oxidative stress, inflammation, and apoptosis in rats. Journal of biochemical and molecular toxicology, 31, e21940.

15. EL MAHDY, M. M., ELDIN, T. A. S., ALY, H. S., MOHAMMED, F. F. & SHAALAN, M. I. 2015. Evaluation of hepatotoxic and genotoxic potential of silver nanoparticles in albino rats. Experimental and toxicologic pathology, 67, 21-29.

16. ELKHAWASS, E. A., MOHALLAL, M. E. & SOLIMAN, M. F. 2015. Acute toxicity of different sizes of silver nanoparticles intraperitonally injected in Balb/C mice using two toxicological methods. Int J Pharm Pharm Sci, 7, 94-99.

17. ERKANLı ŞENTÜRK, G., ERSOY CANILLIOĞLU, Y., UMAY, C., DEMIRALP-EKSIOLU, E. & ERCAN, F. 2012. Distribution of Zonula Occludens-1 and Occludin and alterations of testicular morphology after in utero radiation and postnatal hyperthermia in rats. International journal of experimental pathology, 93, 438-449.

18. FATHI, N., HOSEINIPANAH, S., ALIZADEH, Z., ASSARI, M. J., MOGHIMBEIGI, A., MORTAZAVI, M., HOSSEINI, M. & BAHMANZADEH, M. 2018. The effect of silver nanoparticles on the reproductive system of adult male rats: A morphological, histological and DNA integrity study. Advances in clinical and experimental medicine : official organ Wroclaw Medical University, 28.

19. GOPINATH, P., GOGOI, S. K., SANPUI, P., PAUL, A., CHATTOPADHYAY, A. & GHOSH, S. S. 2010. Signaling gene cascade in silver nanoparticle induced apoptosis. Colloids and Surfaces B: Biointerfaces, 77, 240-245.

20. GOTO, T., KAWANO, H., AKIYAMA, T., SHINODA, Y., OKUMA, T., KOBAYASHI, H., NEMOTO, T. & FUNATA, N. 2009. Serum acid phosphatase can be a useful tumour marker for giant cell tumour of bone. Archives of orthopaedic and trauma surgery, 129, 1641.

21. GRIFFITH, O. W. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analytical biochemistry, 106, 207-12.

22. GUAN, H., QIAN, D., REN, H., ZHANG, W., NIE, H., SHANG, E. & DUAN, J. 2014. Interactions of pharmacokinetic profile of different parts from Ginkgo biloba extract in rats. Journal of Ethnopharmacology, 155, 758-768.

23. HUANG, W. L., MA, Y. X., FAN, Y. B., LAI, S. M., LIU, H. Q., LIU, J., LUO, L., LI, G. Y. & TIAN, S. M. 2017. Extract of Ginkgo biloba promotes neuronal regeneration in the hippocampus after exposure to acrylamide. Neural Regen Res, 12, 1287-1293.

24. INGMAN, W. V. & ROBERTSON, S. A. 2007. Transforming growth factor-β1 null mutation causes infertility in male mice associated with testosterone deficiency and sexual dysfunction. Endocrinology, 148, 4032-4043.
25. JONES, D. P. 2002. Redox potential of GSH/GSSG couple: Assay and biological significance. In: SIES, H. & PACKER, L. (eds.) Methods in Enzymology. Academic Press.

26. JUN, L. & ARNE, H. 2012. Thioredoxin System in Cell Death Progression. Antioxidants & Redox Signaling, 17, 1738-1747.

27. KALISHWARALAL, K., BARATHMANIKANTH, S., PANDIAN, S. R., DEEPAK, V. & GURUNATHAN, S. 2010. Silver nano - A trove for retinal therapies. JOURNAL OF CONTROLLED RELEASE, 145, 76-90.

28. KIM, S.-W., KIM, S.-S., LEE, S.-M., KWON, B.-B., CHOI, J.-H., HYUN, J.-W. & KIM, S.-M. 2011. Characterization of the effects of silver nanoparticles on liver cell using HR-MAS NMR spectroscopy. Bulletin of the Korean Chemical Society, 32, 2021-2026.

29. LEBDA, M., GAD, S. & GAAFAR, H. 2014. Effects of lipoic Acid on acrylamide induced testicular damage. Materia socio-medica, 26, 208-12.

30. LEBDA, M. A., SADEK, K. M., ABOUZED, T. K., TOHAMY, H. G. & EL-SAYED, Y. S. 2018a. Melatonin mitigates thioacetamide-induced hepatic fibrosis via antioxidant activity and modulation of proinflammatory cytokines and fibrogenic genes. Life Sci, 192, 136-143.

31. LEBDA, M. A., SADEK, K. M., TOHAMY, H. G., ABOUZED, T. K., SHUKRY, M., UMEZAWA, M. & EL-SAYED, Y. S. 2018b. Potential role of a-lipoic acid and Ginkgo biloba against silver nanoparticles-induced neuronal apoptosis and blood-brain barrier impairments in rats. Life Sci. Life Sciences, 212, 251-260.

32. LEE, J. & FINN, C. E. 2012. Lingonberry (Vaccinium vitis-idaea L.) grown in the Pacific Northwest of North America: Anthocyanin and free amino acid composition. Journal of functional foods, 4, 213-218.

33. LI, B., GAO, Y., RANKIN, G. O., ROJANASAKUL, Y., CUTLER, S. J., TU, Y. & CHEN, Y. C. 2015. Chaetoglobosin K induces apoptosis and G2 cell cycle arrest through p53-dependent pathway in cisplatin-resistant ovarian cancer cells. Cancer Lett, 356, 418-33.

34. LI, W., TROVERO, F., CORDIER, J., WANG, Y., DRIEU, K. & PAPADOPOULOS, V. 2003. Prenatal exposure of rats to Ginkgo biloba extract (EGb 761) increases neuronal survival/growth and alters gene expression in the developing fetal hippocampus. Developmental Brain Research Developmental Brain Research, 144, 169-180.

35. LILLIG, C. H. & HOLMGREN, A. 2007. Thioredoxin and related molecules–from biology to health and disease. Antioxidants & redox signaling, 9, 25-47.

36. LIM, D., ROH, J. Y. & EOM, H. J. 2012. Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode Caenorhabditis elegans. Environ Toxicol Chem, 31, 585-592.

37. LIU, X. L., CHEN, X. Y., WANG, Z. C., SHEN, T. & ZHAO, H. 2013. Effects of exposure to bisphenol A during pregnancy and lactation on the testicular morphology and caspase-3 protein expression of ICR pups. Biomedical reports, 1, 420-424.

38. LU, J. & HOLMGREN, A. 2012. Thioredoxin system in cell death progression. Antioxidants & redox signaling, 17, 1738-1747.
39. LU, J. & HOLMGREN, A. 2014. The thioredoxin antioxidant system. *Free Radical Biology and Medicine, 66*, 75-87.

40. MARAMBIO-JONES, C. & HOEK, E. M. 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research, 12*, 1531-1551.

41. MCSHAN, D., RAY, P. C. & YU, H. 2014. Molecular toxicity mechanism of nanosilver. *Journal of food and drug analysis, 22*, 116-127.

42. MIRESMAEILI, S. M., NIKAHAD, N., MIRESMAEILI, S. M., HALVAEI, I., FESAHAH, F., FALLAH, A., FESAHAH, F. & TAHERINEJAD, M. 2013. Evaluating the role of silver nanoparticles on acrosomal reaction and spermatogenic cells in rat. *Iran. J. Reprod. Med. Iranian Journal of Reproductive Medicine, 11*, 423-430.

43. MITSUI, A., HAMURO, J., NAKAMURA, H., KONDO, N., HIRABAYASHI, Y., ISHIZAKI-KOIZUMI, S., HIRAKAWA, T., INOUE, T. & YODOI, J. 2002. Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. *Antioxidants and Redox Signaling, 4*, 693-696.

44. MOHAMED, N. E.-S. & ABD EL-MONEIM, A. E. 2017. Ginkgo biloba extract alleviates oxidative stress and some neurotransmitters changes induced by aluminum chloride in rats. *Nutrition, 35*, 93-99.

45. OHKAWA, H., OHISHI, N. & YAGI, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry, 95*, 351-8.

46. OTHMAN, A. I., EL-MISSIRY, M. A., KORIEM, K. M. & EL-SAYED, A. A. 2012. Alfa-lipoic acid protects testosterone secretion pathway and sperm quality against 4-tert-octylphenol induced reproductive toxicity. *Ecotoxicol Environ Saf, 81*, 76-83.

47. PAGLIA, D. E. & VALENTINE, W. N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med, 70*, 158-69.

48. PARI, L. & MURUGAVE, P. 2004. Protective effect of alpha-lipoic acid against chloroquine-induced hepatotoxicity in rats. *J Appl Toxicol, 24*, 21-6.

49. PIAO, M. J., KANG, K. A., LEE, I. K., KIM, H. S., KIM, S., CHOI, J. Y., CHOI, J. & HYUN, J. W. 2011. Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. *Toxicology letters, 201*, 92-100.

50. PRAHALATHAN, C., SELVAKUMAR, E. & VARALAKSHMI, P. 2006. Lipoic acid modulates adriamycin-induced testicular toxicity. *Reproductive Toxicology, 21*, 54-59.

51. QUINTEROS, M. A., VIVIANA, C. A., ONNAINTY, R., MARY, V. S., THEUMER, M. G., GRANERO, G. E., PARAJE, M. G. & PÁEZ, P. L. 2018. Biosynthesized silver nanoparticles: Decoding their mechanism of action in Staphylococcus aureus and Escherichia coli. *The international journal of biochemistry & cell biology, 104*, 87-93.

52. RODA, E., BARNI, S., MILZANI, A., DALLE-DONNE, I., COLOMBO, G. & COCCINI, T. 2017. Single silver nanoparticle instillation induced early and persisting moderate cortical damage in rat kidneys. *International journal of molecular sciences, 18*, 2115.
53. SADEK, K., ABOUZED, T. & NASR, S. 2016. Lycopene modulates cholinergic dysfunction, Bcl-2/Bax balance, and antioxidant enzymes gene transcripts in monosodium glutamate (E621) induced neurotoxicity in a rat model. *Can J Physiol Pharmacol*, 94, 394-401.

54. SADEK, K. M. 2014. Chemotherapeutic efficacy of an ethanolic Moringa oleifera leaf extract against chromium-induced testicular toxicity in rats. *Andrologia*, 46, 1047-54.

55. SADEK, K. M., LEBDA, M. A. & ABOUZED, T. K. 2019. The possible neuroprotective effects of melatonin in aluminum chloride-induced neurotoxicity via antioxidant pathway and Nrf2 signaling apart from metal chelation. *Environ Sci Pollut Res Int*.

56. SADEK, K. M., LEBDA, M. A., ABOUZED, T. K., NASR, S. M. & EL-SAYED, Y. 2018. The molecular and biochemical insight view of lycopene in ameliorating tramadol-induced liver toxicity in a rat model: implication of oxidative stress, apoptosis, and MAPK signaling pathways. *Environ Sci Pollut Res Int*, 25, 33119-33130.

57. SADEK, K. M., LEBDA, M. A., NASR, S. M. & SHOUKRY, M. 2017. Spirulina platensis prevents hyperglycemia in rats by modulating gluconeogenesis and apoptosis via modification of oxidative stress and MAPK-pathways. *Biomedicine & Pharmacotherapy*, 92, 1085-1094.

58. SCHRAND, A. M., RAHMAN, M. F., HUSSAIN, S. M., SCHLAGER, J. J., SMITH, D. A. & SYED, A. F. 2010. Metal-based nanoparticles and their toxicity assessment. *WILEY INTERDISCIPLINARY REVIEWS NANOmedicine and Nanobiotechnology*, 2, 544-568.

59. SHAYGANINIA, E., TAVALAEE, M., AKHAVANFARID, G. R., RAHIMI, M., DATTILO, M. & NASR-ESFAHANI, M. H. 2018. Alpha-Lipoic Acid improves the testicular dysfunction in rats induced by varicocele. *AND Andrologia*, 50.

60. SOFIKITIS, N., GIOTITSAS, N., TSOUNAPI, P., BALTOGIANNIS, D., GIANNAKIS, D. & PARDALIDIS, N. 2008. Hormonal regulation of spermatogenesis and spermiation. *J. Steroid Biochem. Mol. Biol. Journal of Steroid Biochemistry and Molecular Biology*, 109, 323-330.

61. TOLAYMAT, T. M., SCHEKEL, K. G., LUXTON, T. P., EL BADAWY, A. M., SUIDAN, M. & GENAIDY, A. 2010. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. *Sci. Total Environ. Science of the Total Environment*, 408, 999-1006.

62. WATANABE, C. M., WOLFFRAM, S., ADER, P., RIMBACH, G., PACKER, L., MAGUIRE, J. J., SCHULTZ, P. G. & GOHIL, K. 2001. The in vivo neuromodulatory effects of the herbal medicine ginkgo biloba. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 6577-80.

63. WIJNHOVEN, S. W. P., PEIJNENBURG, W. J. G. M., HERBERTS, C. A., HAGENS, W. I., OOMEN, A. G., HEUGENS, E. H. W., ROSZEK, B., BISSCHOPS, J., GOSENS, I., MEENT, D. V. D., DEKKERS, S., JONG, W. H. D., ZIJVERDEN, M. V., SIPS, A. & GEERTSMA, R. E. 2009. Nano-silver - a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology*, 3, 109.

64. WU, X. Y., WANG, W. Y., WANG, R. R., XIE, L., FANG, Z. X. & CHEN, G. R. 2008a. [Ginkgo biloba extract enhances testosterone synthesis of Leydig cells in type 2 diabetic rats]. *Zhonghua Nan Ke Xue*, 14, 371-6.
65. Wu, Y., Xing, D., Liu, L. & Gao, B. 2008b. Regulation of Bax activation and apoptotic response to UV irradiation by p53 transcription-dependent and-independent pathways. *Cancer letters*, 271, 231-239.

66. Yeh, Y. C., Liu, T. J., Wang, L. C., Lee, H. W., Ting, C. T., Lee, W. L., Hung, C. J., Wang, K. Y., Lai, H. C. & Lai, H. C. 2009. A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. *British journal of pharmacology*, 156, 48-61.

**Tables**

Table 1 Primer sequences used for qRT-PCR

| Gene symbol | Gene description                  | Sequence (5'– 3') | GenBank Accession No. |
|-------------|-----------------------------------|-------------------|----------------------|
| Bax         | B-cell lymphoma 2 like protein 4   | F: CACCAGCTCTGAACAGATCATGA  
R: TCAGCCCATCTTCTTCCAGATGGT | NM_009741.5         |
| Bcl-2       | B-cell lymphoma 2                 | F: CACCCCTGGCATCTTCTCCTT  
R: AGCGTCTTCAGAGACAGCCAG | NM_007527.3         |
| TGF-1β      | Transforming growth factor-1β     | F: TCACTTTTGTGTGATGC  
R: TTCTGTCTCTCAAGTCCCCC | NM_021578.2         |
| Txn-1       | Thioredoxin-1                     | F: TTTCTGAAGTAGACGTGGATGAC  
R: AGAGAACTCCCCAACCTTTTGAC | NM_053800.3         |
| Actb*       | β-actin                           | F: TGTTGTCCCTGTATGCCCTCT  
R: TAATGTCACGCACGATTTCC | NM_031144.3         |

* Housekeeping gene

Table 2 Effect of α-lipoic acid and *Ginkgo biloba* L extract on serum testicular biomarkers (testosterone, prostatic ACP, and total ACP) and in rats exposed to silver nanoparticles
Table 3: Effect of α-lipoic acid and *Ginkgo biloba* L extract on the lipid peroxidation biomarker (MDA) and antioxidant molecules (total GSH, GSH, GSSG, GR and GPX) in the testis homogenates of rats exposed to silver nanoparticles.

| Groups      | MDA (nmol/g tissue) | Total GSH (nmol/g tissue) | GSH (IU/g tissue) | GSSG (IU/g tissue) | GR (IU/g tissue) | GPX (IU/g tissue) |
|-------------|---------------------|---------------------------|-------------------|--------------------|------------------|------------------|
| Control     | 166.3±2.7^d         | 3.93±0.8^a                | 3.61±0.07^a       | 0.29±0.005^d       | 81.34±2.37^a     | 171.5±2.73^a     |
| AgNPs       | 269.7±5.4^a         | 1.95±0.6^d                | 1.47±0.07^d       | 0.45±0.01^a        | 39.28±1.74^d     | 123.3±3.41^d     |
| LA+AgNPs    | 221.3±3.9^c         | 3.38±0.4^b                | 3.01±0.13^b       | 0.34±0.003^c       | 66.62±2.28^b     | 152.7±2.83^b     |
| GB+AgNPs    | 237.8±7.3^b         | 2.78±0.4^c                | 2.37±0.04^c       | 0.38±0.005^b       | 61.37±2.62^c     | 141.9±1.97^c     |

Different letters within the same column indicate significantly different mean values (p ≤ 0.05). MDA, malondialdehyde; GSH, reduced glutathione; GSSG, oxidized glutathione; GPX, glutathione peroxidase; GR, glutathione reductase; AgNPs, silver nanoparticles; LA, α-lipoic acid; GB, *Ginkgo biloba* L.

Table 4: Effect of α-lipoic acid and *Ginkgo biloba* L extract on thirodexin-1 (Txn-1), transforming growth factor-1β (TGF-1β) proapoptotic biomarkers (BAX) and anti-apoptic (BCL-2) in the testis homogenates of rats exposed to silver nanoparticles.

| Groups      | Testosterone (IU/L) | Prostatic ACP (IU/L) | Total ACP (IU/L) |
|-------------|---------------------|----------------------|------------------|
| Control     | 3.30±0.13^a         | 3.54±0.18^c          | 11.76±1.61^d     |
| AgNPs       | 1.38±0.09^d         | 6.26±0.26^a          | 18.36±1.63^a     |
| LA+AgNPs    | 2.81±0.12^b         | 4.38±0.29^b          | 13.82±1.28^c     |
| GB+AgNPs    | 2.28±0.11^c         | 4.84±0.17^b          | 15.52±1.21^b     |

Different letters within the same column indicate significantly different mean values (p ≤ 0.05). ACP, Total acid phosphatase; pACP, prostatic acid phosphatase; AgNPs, silver nanoparticles; LA, α-lipoic acid; GB, *Ginkgo biloba* L.
### Groups

|        | Txn-1   | TGF-1β  | Bax    | Bcl-2   |
|--------|---------|---------|--------|---------|
| Control| 1.00 ± 0.007c | 1.00 ± 0.007c | 1.00 ± 0.007d | 1.00 ± 0.007b |
| AgNPs  | 0.32 ± 0.036d | 0.73 ± 0.051d | 4.01 ± 0.13a | 0.22 ± 0.17c |
| LA+AgNPs| 2.02 ± 0.21a | 1.89 ± 0.071a | 1.99 ± 0.09c | 1.18 ± 0.38a |
| GB+AgNPs| 1.86 ± 0.044b | 1.47 ± 0.095b | 2.91 ± 0.06b | 0.88 ± 0.18b |

Different letters within the same column indicate significantly different mean values (p ≤ 0.05). Txn-1, thioredoxin-1; TGF-1β, tissue growth factor-1β; Bax, B-cell lymphoma 2 like protein 4; Bcl-2, B-cell lymphoma 2; AgNPs, silver nanoparticles; LA, α-lipoic acid; GB, *Ginkgo biloba* L.

### Area % of immunoreactions

|         | control | AgNPs  | Lipoic + AgNPs | Ginkgo + AgNPs |
|---------|---------|--------|----------------|----------------|
| Ki-67   | 28.4 ± 0.25 a | 9.08 ± 0.28 d | 20.04 ± 0.22 b | 17.6 ± 0.21 c  |
| Bax     | 1.04 ± 0.9 d  | 10.5 ± 0.27 a | 3.04 ± 0.16 c  | 5.5 ± 0.25 b   |

### Figures
Figure 1

Photomicrograph of rat testis stained with HE (X.200). Control group showing normal histoarchitecture of seminiferous tubules. (b,c,d) silver-nanoparticles-treated group showing vacuolation of the germinal epithelium of seminiferous tubules (black arrows), depletion of germinal cells and hyalinization of the luminal contents (A), sloughing of the germinal epithelium in the lumen of seminiferous tubules (blue arrow), interstitial edema (asterisks) and finally giant cell formations (red arrow) in the lumen of
seminiferous tubules. (e) silver-nanoparticles + lipoic acid treated group showing interstitial edema (asterisks) and few tubules contained sloughed germinal epithelium (blue arrow). (f) silver-nanoparticles + gink trated group the majority of seminiferous tubules had normal germinal epithelium with few vacuolated germinal epitheliums (black arrow).

Figure 2
Photomicrograph of rat Caput epididymis stained with HE (X.200). Control group showing normal histological structure with normal sperm density. (b, c, d) silver-nanoparticles-treated group showing vacuolation of the epithelium of some caput epididymal ductules (black arrows) contained sloughed germ cells in its lumina (black arrow) beside congestion of interstitial blood vessel with perivascular inflammatory cell infiltrations (red arrow). (e, f) silver-nanoparticles + lipoic acid treated group and silver-nanoparticles + ginko treated group showing normal histological structure with normal sperm density.

Figure 3
Photomicrograph of rat Cauda epididymis stained with HE (X.200). Control group showing normal histological structure with normal sperm density. (b, c, d) silver-nanoparticles-treated group showing vacuolation of the epithelium of some caudaepididymal ductules (black arrows) contained sloughed germ cells (black arrow) with hyalinized of the luminal contents(A) beside congestion of interstitial blood vessel with perivascular(red arrow) and interstitial inflammatory cell infiltrations (black arrow) . (e, f) silver-nanoparticles + lipoic acid treated group and silver-nanoparticles +Ginka treated group showing normal histological structure with normal sperm density.

Figure 4

Immunohistochemical stained sections of rat testis with anti-Ki-67 antibody protein expression (×400). (a) the control testis showing strong positive immunoreaction in the nuclei of spermatogonia and primary spermatocytes (b) silver-nanoparticles-treated group showing negative immunoreaction in the nuclei of spermatogonia and primary spermatocytes (c) silver-nanoparticles + lipoic acid -treated group showing positive immunoreaction in some spermatogonia and primary spermatocytes (d) silver-nanoparticles + Ginka -treated group showing positive immunoreaction in few numbers of spermatogonia and primary spermatocytes. Arrow= positive immunoreaction, arrowheads= negative immunoreaction.
Figure 5

Immunohistochemical stained sections of rat testis with Bax protein (X400) (a) the control testis showing negative bax staining of germ cells and Leydig cells (b) silver-nanoparticles-treated group showing negative immunoreaction of germ cells and positive immunoreaction by heavy Bax staining in the cytoplasm of Leydig cells (longarrows) (c) silver-nanoparticles + lipoic acid -treated group showing negative immunoreaction of germ cells and weak immunoreaction in the cytoplasm of Leydig cells (arrowheads) (d) silver-nanoparticles + Ginka -treated group showing negative immunoreaction of germ cells and moderate immunoreaction in the cytoplasm of Leydig cells (shot arrows)