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Possible Ameliorative Effect Of Platelet Rich Plasma On Testicular Toxicity Of Silver Nanoparticles In Adult Albino Rats

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Abstract

Silver nanoparticles (AgNPs) are being used increasingly in industrial and medical products owing to their unique properties. AgNPs can penetrate the testicular blood barrier and cause disorders in the male reproductive system. Platelet-rich plasma (PRP) contains many of growth factors that have regenerative and anti-inflammatory characteristics and have been used in different medical fields. In this work, we examined the toxicity of AgNPs and the possible ameliorative effect(s) of PRP on adult rat testes. Experimental study was conducted on 24 mature albino rat males that were allocated into four equal groups: G1: kept as control. G2: AgNPs-intoxicated group. G3: PRP–treated group. G4: recovery group. We collected samples for biochemical, immunohistochemical, histological, and genetic marker analysis. We found that AgNPs administration decreases glutathione (GSH), reproductive hormones, apoptotic marker; Bcl-2, germinal epithelial height, mRNA expression of claudin, and an increase in MDA, SOD, TNF-α, lipid profile, thickening of the tubules' basement membrane with various histopathological defects, and an increase in mRNA expression of connexion-43. However, these negative effects were enhanced after PRP treatment. In conclusion, AgNPs changed the male reproductive system's biochemical and structural composition and administration of PRP effectively alleviating these changes. So, the scientific community ought to devote care to establishing procedures for eliminating toxicity, promoting health awareness, and reducing exposure to such materials.

Keywords: Silver nanoparticle, Platelet rich plasma, Testicular toxicity, ROS, Connexion-43, Bcl-2.

1. Introduction

One of the most useful forms of heavy metals in nanotechnology applications is silver nanoparticles (AgNPs) [1]. They are extensively utilized in many industries as surface cleansing agents, washing machines, toys, water filters, food packaging, cosmetic products, antimicrobial coatings, and sterilizing agent in medical devices. This is because AgNPs have unique physical and chemical properties, small size, shape, particle morphology, and high surface area [2]. However, their possible risk can't be ignored due to their uncontrolled usage and emissions into the environment [3]. Exposure can occur occupationally in the workplace during the extraction, smelting, refining, and manufacturing of such materials and their application into products. Also, widespread consumer exposure can occur through various routes such as inhalation, ingestion, and direct skin contact. Subsequently, it can be absorbed and induce toxic effects [4].

The major biological impairments of AgNPs are due to their ability to penetrate the cells and accumulate in different organs, causing membrane injuries, mitochondrial destruction, DNA damage, and reactive oxygen species (ROS) formation [4,5]. These resulted in genes and protein damage and alteration of their transcription processes in several body tissues [6]. Experimental studies on animals showed that exposure to AgNPs were associated with decreased lung function and inflammatory response and histopathological changes in the liver and kidney [4]. AgNPs also induce reproductive toxicity because they can pass through the blood-testicular barrier (BTB) resulting in testicular inflammation and toxicity in rats [7, 3].

Platelet-rich plasma (PRP) is a fraction of plasma obtained from centrifugation of whole blood which has a 3-5 times higher concentration of platelets than the basal concentration [8]. Through their α-granules, the activated platelets have a therapeutic effect by releasing growth factors (GFs), cytokines, and chemokines, which are essential for immunomodulation and tissue restoration following minimal trauma or many pathological conditions [9]. The widespread utilization of PRP is attributed to its inexpensive and easy to be obtained, high compatibility, antimicrobial and antioxidative effects [10].

Based on the recommendation of the European Commission's Scientific Committee on Consumer Safety (SCCS) to gather information on AgNPs reproductive toxicity, the possible reproductive toxicity mechanism of

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AgNPs is of great interest [11]. Also, the possibility of the therapeutic effects of PRP on reproductive dysfunction induced by AgNPs has not been thoroughly explored yet. Consequently, the present research aimed to:

1- Evaluate the toxic impacts and the underlying mechanisms of AgNPs on adult rat testes.

2- Investigate the ability of PRP to reduce the adverse impacts of AgNPs on the structure and function of adult rats testes.

2. Materials and methods

2.1. Animals

Twenty four adult albino rat males that weighed between 150 and 200 g were obtained from the animal house of the Faculty of Science, Al-Azhar University for this study. They were maintained in a suitable environment (temp. (22±5°C) under a 12:12-hour cycle of light and dark) in cages made of stainless steel with a standard diet. They were kept for adaptation for one week. This study was authorized by the Research Ethics Committee at Al-Azhar University Faculty of Medicine for Girls, Egypt, and follows the National Health Institute's Guide for the Care and Use of Laboratory Animals [12].

2.2. Chemicals and reagents

2.2.1. *Silver nanoparticles (AgNPs)*

A colloidal solution of nanoparticles with a particle average size 45 ± 5 nm, spherical like-shapes, dark yellow in color at a concentration of 2000 ppm were purchased from NanoTech Egypt for Photo-Elctronics. The consistency of AgNPs was ensured through transmission electron microscopy (TEM) and UV/Visible spectral analysis (Fig. 1, 2). The XRD pattern confirm the crystalline nature of the synthesized Ag NPs was considerably observed at 2θ values of 38.21°, 43.38°, 64.17°, and 77.09°, which were attributed to (111), (200), (220) and (311) planes of pure face centered cubic (FCC) silver metal with a lattice value of 4.088 A° (Fig.3).

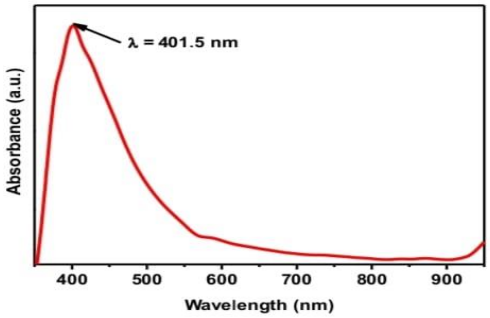

Fig.1. UV/ Visible absorption of prepared AgNPs at 401.5nm.

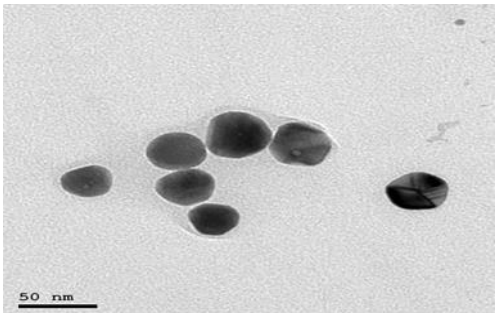

Fig.2. TEM images of synthesized AgNPs.

2.2.2. *Platelet-rich plasma (PRP)*

Ten healthy albino male rats that had been age-matched were employed as PRP donors. Whole blood has been drawn from a puncture of the median eye canthus vein and mixed at a 9/1 ratio with 3.2% sodium citrate. The whole blood was centrifuged at room temperature for 10 min. at 1000 r.p.m. for separation of the supernatant, the blood was centrifuged once more for 10 min. at 800 r.p.m. Then the lower third (sediment) has been gained as PRP. The mean platelet count in PRP has been assessed by Sysmex XT-1600i technology. The count of platelets has been 800×103 platelets/μL [13].

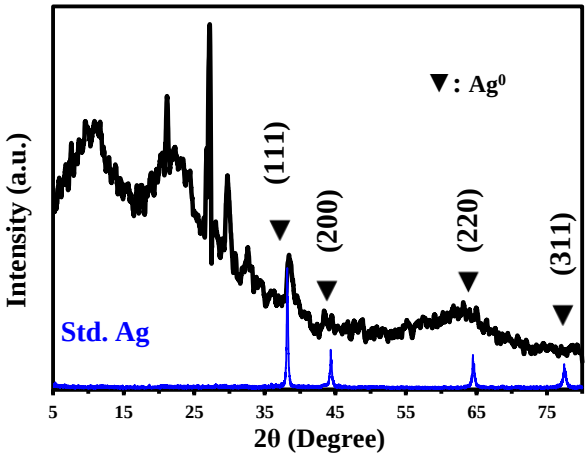

Fig.3. The XRD of the synthesized Ag NPs

2.2.2. *Platelet-rich plasma (PRP)*

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2.3. Experimental Animal Design

After a week of adaptation, the animals were separated into four groups, each with six rats. **Group (1)**: control (rats were fed normally and given distilled water). **Group (2):** (AgNPs-intoxicated group): Rats received AgNPs at 10 mg/kg.bw/day intraperitoneally for 28 days [14]. **Group (3):** (PRP-treated group) injected with AgNPs as group (2), then 24 hours following the last AgNPs injection dose, the rats were injected (i.p.) with platelet-rich plasma (PRP) at 0.5/mg/kg.bw. for another 3 weeks, twice a week (6 doses) [15]. **Group (4):** (recovery group): injected with AgNPs as group (2), then rats left without any treatment after the last dose of AgNPs injection for another 3 weeks.

2.4. Serum and tissue collection

We collected blood samples in clean, dry test tubes at the end of the experiment and centrifuged them to get the sera. That was stored at −20°C for lipid profiles and hormonal studies. After that, the abdomens of all animals

were opened by making a median incision while they were sacrificed under anesthesia. The testes were carefully dissected out. One testis was utilized for oxidative stress, TNF-α, and genetic marker studies, while the other was preserved for histochemical and immunohistochemical studies in 10% neutral buffered formalin.

2.4. Biochemical assay:

2.4.1. Testicular oxidative parameters assay

Malondialdehyde (MDA) contents, reduced glutathione (GSH) activity and superoxide dismutase (SOD) have been evaluated as oxidative stress parameters respectively, in accordance with [16, 17, 18].

2.4.2. Pro-inflammatory marker (TNF-α) assay

TNF- α was measured in the testicular tissue by the enzyme-linked immunosorbent assay Rat TNF alpha ELISA Kit (Cat.No. E0764Ra) following the manufacturer's instructions.

2.4.3. Lipid profile assay

The levels of serum cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) have been measured by a spectrophotometer [19, 20, 21,22].

2.4.4. Hormone assay

The levels of the reproductive hormones; testosterone, follicular stimulating hormone (FSH), and Luteinizing hormone (LH) have been assessed in the serum using commercially available rat ELISA kits (Cat.No. EA0023Ra, Cat.No. EA0015Ra, and Cat.No. EA0013Ra, respectively) and following the instructions provided by the manufacturer.

2.5. Histochemical assessment:

For routine histological investigation, fixed testes have been processed for paraffin sectioning and stained using hematoxylin and eosin (H&E). Periodic acid-Schiff (PAS) staining has also been employed to find mucopolysaccharides in the seminiferous tubules (STs) basement membrane [23].

2.6. Immunohistochemical assessment:

Testicular Bcl-2 (antiapoptotic protein) was detected according to CAS No. 85878, Sigma-Aldrich, Steinheim, Germany. Diaminobenzidine chromogen (DAB), along with Mayer's hematoxylin as a counterstain were used to complete the immunostaining. Microscopically, positive staining was confirmed by visually identifying brown-stained immunoreactive cells [24].

Table 1. PCR Primer sequence gene.

Gene	Sequence	Tm	Product size (bp)	Accession Number
CX-43	F: AGCAAGCTAGCGAGCAAAAC R: GAGTTCATGTCCAGCAGCAA	55	151	(NM_012567)
CLDN5	F: CGTGACGGCGCAGACGACTT R: TGCACTGAGCGCCGGTCAAG	57	650	(NM_031701.2)
GAPDH	F: TCAAGAAGGTGGTGAAGCAG R: AGGTGGAAGAATGGGAGTTG	55	1000	(NM_017008.4)

2.7. Morphometrical assessment

The histological measurement was performed at Al Azhar University's Faculty of Dentistry's Pathology Department, utilizing the image analyzer computer system (Leica Qwin 500; Leica, Cambridge, UK) and the results were expressed as mean ± SD.

2.8. Gene expression:

Total RNA has been extracted from homogenized tissue from the testicle using the miRNeasy® Mini Kit (Cat. No. 217004), then converted extracted RNA into double strand DNA for PCR reaction by reverse transcriptase according to Qiagen QuantiTect RT kit. An Applied Biosystems 7500 Instrument (Applied Biosystems, USA) was used to apply an optimized kit for quantitative, real-time PCR that contains Taq polymerase, quantitative, real-time PCR buffer, primers, SYBR® Green I dye, and nucleotides for estimating the relative expression of connexion-43 (CX-43) and claudin 5 (CLDN5). The double delta Ct analysis was used to calculate the relative gene expression [25]. As a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was applied. Table (1) displays the sequences of PCR primers.

2.9. Statistical Analysis

SPSS 20 has been employed to conduct statistical analysis. The data has been displayed in the form of the mean ± SD. The mean between the groups has been compared using ANOVA, followed by the post hoc test for multiple comparisons. It has been determined that a difference is statistically significant at $p \leq 0.05$.

Table 2. Testicular oxidative/antioxidant indices and pro-inflammatory markers (TNF-α) of the studied groups (presented as Mean ± SD).

	Control	AgNPs-intoxicated	PRP- treated	Recovery	P value
MDA (nmol/ gm tissue)	0.98 ± 0.11	3.7 ± 0.38^{a,c,d}	2.3 ± 0.43^{a,b,d}	3.0 ± 0 .18^{a,b,c}	0.00
GSH (mg/ gm tissue)	29.7± 1.1	16.3 ± 1.8^{a,c}	23.1 ± 2.3^{a,b,d}	16.8 ± 0.7^{a,c}	0.00
SOD (U/ gm tissue)	35.9 ± 5.9	67.8 ± 4.6 ^{a,c}	47.1 ± 5.0^{a,b,d}	50.3 ± 6.1^{a,c}	0.00
TNF-α (pg/ml)	38.9± 2.1	60.7±.5.8^{a,c,d}	45.9± 2.9^{a,b}	49.7 ± 1.8^{a,b}	0.00

a: significant differences compared to the control; b: significant differences compared to AgNPs; c: significant differences compared to PRP; d: significant differences compared to the recovery group.

Table 3. Lipid profile of the groups under study (presented as mean ± SD).

	Control	AgNPs-intoxicated	PRP- treated	Recovery	P value
Cholesterol (mg/ dl)	101.3 ± 8.6	125.7 ± 7.5[a,c]	109.7 ± 7.3[b]	116.2 ±5.7[a]	0.000
Triglycerides (mg/dl)	87.3 ± 2.2	114.9 ±13.5[a]	106.9 ± 5.5[a]	114.2 ± 6.4[a]	0.000
HDL (mg/dl)	32.3 ± 3.1	40.2 ± 2.4[a,d]	35.7 ± 4.9[a]	34.3 ± 0.96[b]	0.003
LDL (mg/dl)	43.0 ± 8.6	63.7 ± 9.0[a]	58.6 ±4.9[a]	60.3 ± 5.9[a]	0.000
v LDL (mg/dl)	19.8 ± 0.4	23.6 ± 1.3[a]	22.7 ±1.2[a]	22.2 ±1.5[a]	0.000

a: significant difference in comparison to the control; b: significant difference in comparison to AgNPs; c: significant difference in comparison to PRP; d: significant difference in comparison to the recovery group.

Table 4. Reproductive hormone profile of the groups under study (presented as mean ± SD).

	Control	AgNPs-intoxicated	PRP-treated	Recovery	P value
Testosterone (ng/ml)	4.1 ± 0.14	2.9 ± 0.18[a]	3.5 ± 0.65	3.2 ± 0.45[a]	0.002
F.S.H (mIU/ml)	3.0 ± 0 .14	2.04 ± 0.25[a,c]	2.6±.0.28[b]	2.2 ± 0.22[a]	0.000
L.H (mIU/ml)	3.3 ± 0.46	2.3 ± 0.57 [a,c]	3.1 ± 0.37[b]	2.4 ± .34[a]	0.002

a: significant differences compared to the control; b: significant differences compared to AgNPs; c: significant differences compared to PRP.

3. RESULTS

3.1. Testicular oxidant/antioxidant status

The AgNPs-intoxicated group showed significantly (p≤ 0.05) higher testicular MDA concentration and SOD activity, as well as significantly (p≤ 0.05) decreased GSH activity. Meanwhile, compared with the AgNPs-intoxicated group, the PRP-treated group significantly (p ≤ 0.05) lowered MDA level and SOD activity and raised GSH activity in the testis, with significant differences from those in the recovery group as shown in Table (2).

3.2. Pro-inflammatory marker (TNF-α)

The AgNPs-intoxicated group demonstrated a significant elevation in the testicular TNF-α levels in comparison with the control group. Contrarily, when comparing the AgNPs-intoxicated group, the recovery and PRP-treated groups revealed a significant decline in TNF-α level, with the lowest number in the PRP-treated group **(**Table 2).

3.3. Lipid profile parameters

Rats exposed to AgNPs had significantly higher serum levels of cholesterol, triglycerides, LDL, HDL, and vLDL than in the control group. On the other hand, PRP-treated and recovery groups show decline in these parameters with more decrease in PRP treated group (Table 3).

3.4. Reproductive hormonal changes

AgNPs' detrimental impacts on reproductive hormones and PRP's ameliorative effects in male rats are shown in (Table 4). Serum concentrations of testosterone, FSH, and LH displayed a significant (p≤ 0.05) decline in AgNPs-intoxicated rats compared to control group, with a significant (p≤ 0.05) improvement after PRP treatment and in the recovery group.

3.5. Results of light microscopic examination

Control adult male albino rat testis revealed normal morphology in hematoxylin and eosin-stained sections. Regular seminiferous tubules (STs) are lined with stratified germinal epithelium, composed of spermatogenic cells, including spermatogonia, primary spermatocytes, spermatids, spermatozoa, and supporting Sertoli cells. The interstitium is formed of clusters of Leydig cells (Fig. 4; A&B). Histopathological changes were detected in AgNPs-intoxicated group in the form of irregular-shaped, disorganized STs with few or no sperms. Interstitial space widening contained acidophilic hyaline material, and some Leydig cells appeared atrophied (Fig. 4; C, D&E). In PRP treated group, nearly normal architecture of STs and the interstitium (Fig. 4; F). In the recovery group, incomplete recovery was observed. Some STs resumed their normal general structure, while others were not (Fig. 4; G).

3.6 Histochemical and immunohistochemical results

Periodic acid Schiff reaction (PAS): A strong PAS+ve reaction in the STs' well-circumscribed, regular, thin basement membranes was observed in the control group PAS-stained sections (Fig. 5; A). Conversely, the AgNPs-intoxicated group (Fig. 5; B) and in the recovery group (Fig. 5; D) The STs' thick, irregular basement membranes and the thicker blood vessel wall within the interstitial tissue showed strong PAS+ve reactions. PRP-treated group showed PAS+ve staining, which was normally distributed, more or less than the control group (Fig. 5; C).

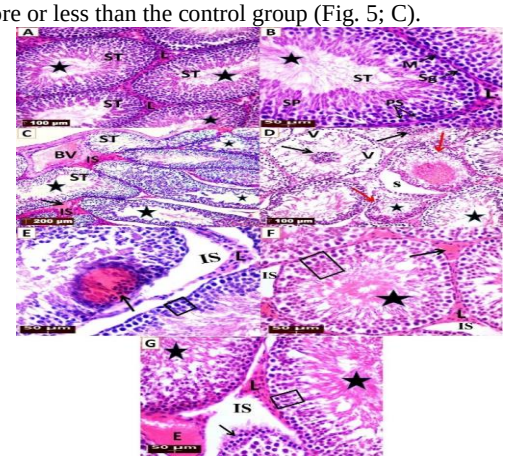

Fig.4. Photomicrographs of testicular sections from all groups. Control group (A&B): A; Showing normal pattern regular seminiferous tubules (ST), their lumina are full of sperms (stars) separated by narrow interstitial tissue containing clusters of Leydig cells (L). B; The ST is

surrounded with myoid cells (M), the spermatogenic cells that line the STs include spermatogonia (Sg), primary spermatocytes (PS), spermatids (SP), and spermatozoa (stars), as well as supporting Sertoli cells (white arrows). AgNPs- intoxicated group (C-E): C; STs are elongated with absence or few sperms in most of them (stars), interstitial space widening (IS) containing acidophilic hyaline substances (black arrow) and congested blood vessels (BV). D; disorganized and shrunken seminiferous tubules (red arrows), marked cellular loss (V), detachment of the germinal epithelium (S) from irregular basement membrane and their lumen reveal exfoliated cells (black arrows). E; STs lined with few spermatogenic cells having darkly stained nuclei and vacuolated cytoplasm (square), acidophilic hyaline area contains pyknotic nuclei (arrows), widening of the interstitium (IS) contains leydig cells with darkly stained nuclei (L) is also seen. PRP treated group: F; the histological pattern of STs appears nearly as that of the control group, a normally arranged germinal epithelium (rectangle), the lumen is full of sperms (star), little interstitial space widening in-between the tubules (IS), containing leydig cells (L) with little acidophilic hyaline material (black arrow). Recovery group: G; some STs with apparently normal arranged germinal epithelium (rectangle), spermatozoa in their lumina (star), other one appears shrunken disorganized (black arrow). The interstitial space is still wide (IS) with some leydig cells (L) and acidophilic hyaline material (E) (H&E stain, all X400, except (C) X100 & (A,D)X200).

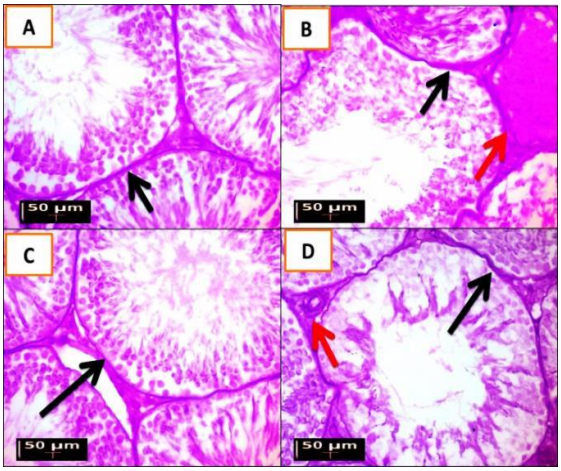

Fig.5. PAS-stained sections of testis of the control group: A; Showing normal pattern of strong PAS +ve reaction in the well-circumscribed thin, regular basement membrane of the STs (black arrow). AgNPs- intoxicated group: B; reveals strong positive reaction in the irregular thickened basement membrane (black arrow) and in the thickened wall of the blood vessel within the interstitium (red arrow). PRP treated group: C; showing nearly normal pattern of PAS +ve reaction of the basement membrane (black arrow). Recovery group: D; reveals strong PAS +ve reaction in the irregular thick basement membranes (black arrow) and in the wall of blood vessel within the interstitium (red arrow) (PAS reaction all x 400).

Immunohistochemical stained testicular sections of the control group for Bcl2 protein found positive cytoplasmic immunoreaction in most germinal epithelial cells and Leydig cells (Fig. 6; A). In the AgNPs-intoxicated group, there was few Bcl-2-positive cells (Fig.6; B). In the PRP-treated group, most cells showed nearly normal immunoreactions comparable to those in the control group (Fig. 6; C). Finally, the investigation of the recovery group displayed moderate cytoplasmic immunoreaction for Bcl-2.

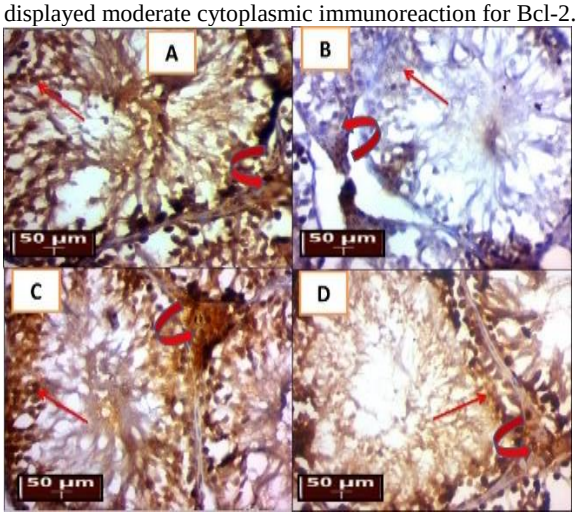

Fig.6. Immunohistochemical stained sections with Bcl-2 in the control group (A); Most of the germinal epithelial cells show strong positive cytoplasmic immunoreaction to Bcl-2 (red arrow) and also Leydig cells (curved arrow). (B); AgNPs- intoxicated group reveals weak positive Bcl-2 immunoreaction to in few spermatogenic cells (red arrow) and in the Leydig cells (curved arrow). (C); PRP treated group; showing strong positive cytoplasmic immunereaction to Bcl2 in germinal epithelium (red arrow) and in Leydig cells (curved arrow). (D); Recovery group; there are moderate positive Bcl-2 immunoreaction in the germinal epithelium (red arrow) and Leydig cells (curved arrow) (Immunoperoxidase for Bcl2 protein, all x 400).

3.7. Statistical analysis of morphometrical and genes expression parameters (Table 5).

3.7.1. Epithelium height: There was a significant reduction ($P \leq 0.05$) in the epithelial height of STs in the AgNPs-intoxicated group in comparison with the other groups, with a significant ($p \leq 0.05$) improvement after PRP treatment.

3.7.2. Basement membrane thickness of the seminiferous tubule: There was a significant increase ($P \leq 0.05$) in the AgNPs-intoxicated group when compared to the other groups. The PRP-treated group exhibited a significant ($p \leq 0.05$) enhancement.

3.7.3. The optical density of bcl-2 immunoreaction: The AgNPs- intoxicated group revealed a significant decline ($P \leq 0.05$) in the immunoreaction of bcl-2 in comparison with the control, with a significant ($p \leq 0.05$) enhancement after PRP treatment in comparison to the recovery group.

3.7.4. Genes expression: as shown in **Table (5)** AgNPs administration significantly ($P \leq 0.05$) increases in mRNA expression of connexion-43 but significantly ($P \leq 0.05$) decreased mRNA expression of the claudin 5 gene. This genetic change was significantly ($P \leq 0.05$) reversed better with PRP treated than recovery groups.

Table 5. Morphometric and genes expression parameters of the groups under study (presented as Mean ± SD).

	Control	AgNPs- intoxicated	PRP-treated	Recovery	**P value**
Epithelium height (µm)	118.7± 11.1	59.3± 5.9^{a,c,d}	107.8± 6.8^{b}	96.4± 13.2^{a,b}	0.000
Basement membrane diameter	2.28 ±1.1	12.6 ± 3.7^{a,b,c}	3.5 ± 1.8^{b}	5.2 ± 1.7^{a,b}	0.00
The optical density of bcl-2	90.8 ± 3.9	53.9 ± 8.4^{a,c}	70.6 ± 7.3^{a,b,d}	57.6 ± 4.9^{a,b}	0.000
Connexin-43 gene expression	5.8 ± 0.1	7.3 ± 0.37^{a,c,d}	2.4 ± 0.12 ^{a,b,d}	1.1 ±0.1 ^{a,b,c}	0.00
Claudin 5 gene expression	1.1 ± 0.46	0.65 ± 0.02 ^{a,c,d}	1.9 ± 0.017^{a,b,d}	0.85 ± 0.02^{a,b,c}	0.002

a: significant difference in comparison to the control; b: significant difference in comparison to AgNPs; c: significant difference in comparison to PRP; d: significant difference in comparison to the recovery group

4. DISCUSSION

The physicochemical characteristics of silver nanoparticles (AgNPs) make them beneficial in many sectors, increasing environmental contamination and human exposure. AgNPs become a potential threat to human health and living species. Thus, there is a need for further toxicological assessment [26].

In the current study, AgNPs intoxicated group displayed a significant change in oxidative stress markers in the form of a high MDA level. It is accompanied by a higher circulatory level of TNF-α which confirms the inflammatory status. In contrast, post-treatment with PRP showed a significant improvement in the mentioned parameters, with no enhancement in the recovery group.

According to our outcomes, Altwaijry et al. [27] stated that AgNPs generate ROS that may trigger inflammation, oxidation, cytotoxicity, genotoxicity, and apoptosis. In keeping with such outcomes, Faddah et al [28] discovered that NP-induced nephrotoxicity has been linked to an increase in TNF-α. Hou et al. [29] clarified that the immune system identifies nanoparticles as foreign molecules, which lead to the production of ROS and change the levels of cytokines.

The enhancing effects of PRP in the current results are validated by Hamdan et al. [30] and Rizal et al [31] who reported that PRP inhibits ROS and lowered TNF-α level by antioxidant and antiapoptotic action.

The present work documented dyslipidemia, including elevations of cholesterol, TG, HDL, LDL, and vLDL, which are strong atherogenic factors, with a slight lowering of their levels after PRP treatment. Sulaiman et al. [32] are in agreement with our finding. High cholesterol may be caused by reducing the use of steroidogenesis due to pituitary inhibition, or direct target tissue inhibition [33]. In addition, the pro-inflammatory cytokines productions by AgNPs were involved in the severity of the lipid disruption and may have a significant impact on the development of lipid metabolism [34]. Moreover, due to the hepatotoxic effects of nanoparticles, the liver is unable to control the metabolism of triglycerides and cholesterol in a healthy manner [35]. The enhancing effect of PRP on lipid metabolism might be referred to the presence of many growth factors and bioactive molecules as hepatocyte growth factor (HGF) which could be attributed to its regenerative, antioxidant and anti-inflammatory characteristics [36]

In the current study, the serum reproductive hormones were lowered in the AgNPs-intoxicated group in comparison with other groups. This finding agrees with Tohamy et al. [37], and Heydarnejad et al. [38]. This may be attributed to AgNPs effect on the pituitary-gonadal axis and consequently testicular endocrine system [39].Also, the elevated TNF-α level inhibits testosterone secretion by interference with the same axis [40]. Moreover, cerebral center, pituitary-testis axis, and testosterone synthesis are all directly affected by the reduction in arterial blood flow induced by hyperlipidemia, which leads in a decline in sexual function [41].

On the other hand, Mathias et al. [42] recorded no changes on the serum reproductive hormonal levels after treatments with AgNPs. Also, Lafuente et al. [43] observed that testosterone has significantly increased following intravenous injection of male mice with AgNPs. These discrepancies between the opposing results in the present study may be reflected in various parameters, including particle type, size, concentration, dose, route, and exposure duration. We demonstrated that PRP has beneficial impacts on AgNPs-induced oxidative stress and reproductive hormones production which are in accordance with, Dehghani et al. [44] and Rizal et al. [45].

H&E-stained sections of AgNPs-intoxicated rats showed marked separation of germinal epithelial cells with diminished thickness. This corresponds with those of El-Mesalmy et al. [46] , Cayli et al. [47] and Mohamed et al. [48]. The cytotoxicity of AgNPs owing to the cell membrane, mitochondrial, cytoskeletal damages and apoptosis of germinal epithelial cells. Basal lamina's irregularities may be due to the contraction of myoid cells or seminiferous tubule distortion. The exfoliated germ cells noted in this research agree with Creasy et al. [49] who explained this result to the loss of contact between the germinal epithelial cells with the cytoplasmic processes of the neighboring Sertoli cells. The wide interstitium matched the conclusions of Ahmed et al. [50 and Agarwal et al. [51]. The damaged interstitial tissue may be explained by AgNPs deposition that increase vascular permeability and significantly affect the testicular function. Atrophy observed in Leydig cells resulted in decline of testosterone levels in the serum. As a result, the structural changes in the STs found in the present study could be related to hormonal effects.

Light-microscopic-stained sections of the PRP-treated group showed a significant rise in the mean epithelial height compared to the AgNPs group. Also, we found an improvement in the testicular architecture attributed to PRP, which has a higher level of growth factors than whole blood and stimulates tissue repair [52]. Examination of the recovery group showed incomplete recovery, which agreed with the findings of El-Mesalmy et al. [46]. In addition, Amin et al. [53] displayed in their research slowly cleared AgNPs from organs, which have barriers like the brain and testis vice the other organs.

The results were confirmed by PAS-stained sections of AgNPs-intoxicated rats that revealed a strong PAS +ve

reaction in the STs' thick, irregular basement membrane due to severe testicular functional impairment caused severe alteration of the basement membrane structure [37] , [54]

In our results, Bcl-2 immunostaining in the STs cells was significantly reduced in the AgNPs- intoxicated group, which was confirmed statistically. These were in accordance with the finding of El-Mesalmy et al. [46], who mentioned that AgNPs triggered a significant decline in Bcl-2 in rats administered one mg of AgNPs for thirty days. In the germinal epithelial cells and Leydig cells, the PRP-treated group displayed a cytoplasmic immunoreaction for Bcl-2 that was almost normal. Griffeth et al. [55] ascribed the balance of spermatogenic cell proliferation and differentiation to the growth factor content of PRP, which plays an important role in spermatogenesis.

Blood-testicular barrier (BTB) contains gap junctions (GJ), tight junctions (TJs), demosomes, and ectoplasmics. To mainten of BTB integrity, GJ is necessary for communication between various junction types. It was discovered that tight junction proteins (TJPs) are necessary for the progression of spermatogenesis. Additionally, TJPs such as Connexin 43 and Claudin 5 are essential for preserving the BTB's homeostasis [56]. AgNPs in the current work induced a significant rise in connexin-43 (Cx43) and a significant reduction in claudin-5 gene expressions in testicular tissue compared to the control group. These results concur with those of Qin et al. [57], and Elblehi et al. [58]. Contrary to our results, Zhang et al. [59] found that AgNPs caused a significant decrease in connexin-43 gene expression in male somatic Leydig, Sertoli cells and spermatogonial stem cells.

PRP in the present work caused a significant decrease in connexin-43 gene expression and a significant rise in claudin-5 gene expression in testicular tissue compared to the AgNPs group. This is in agreement with Squecco et al. [60], Sassoli et al. [61], and Cheu et al.[62]. These could be attributed to reinforcing tissue repair via cell proliferation and differentiation that increasing the reconstruction of tight junctions [63].In addition to the anti-inflammatory and the antioxidant effects of PRP, it maintains cellular homeostasis by protecting against apoptosis and enhancing tissue vascularity [64].

5. Conclusions and recommendations

- AgNPs had a harmful effect on rat testes, according to the study results (biochemical, histological, immunohistochemical, and genetic markers). Therefore, it is feasible to conclude that AgNPs are extremely dangerous to reproductive function and can impair the fertility of animals.
- Hormonal disturbance, apoptosis, and anomalies of mRNA expression may all have been caused by oxidative stress mechanism following AgNP exposure in rats. So, the scientific community ought to devote care to establishing procedures for eliminating toxicity, promoting health awareness, and reducing exposure to such materials.
- Furthermore, PRP is reported to be effective in alleviating AgNPs-induced reproductive damage. This was attributable to the fact that platelet-rich plasma had an increased level of growth factors compared with whole blood.
- The authors recommend that further studies with a prolonged recovery period be conducted and compared to PRP therapy. Also, we recommend human studies to assess AgNPs exposure either occupational or environmental and its toxicological effects.

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This research received no funding.

7. Institutional Review Board Statement

The study was conducted in agreement with the ethical procedures and policies approved by the ethical committee of Faculty of Medicine for Girls, Al-Azhar University and follow the National Health Institute's Guide for the Care and Use of Laboratory Animals (National Health Institute, 1996).

8. Conflicts of Interest

The authors declare no conflict of interest.

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