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Ameliorative Role of Resveratrol in Silver Nanoparticle–Induced Oxidative Stress and Testicular Alterations in Male Mice

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ABSTRACT

Background & Objective: Silver nanoparticles (AgNPs) are among the most commonly used silver derivatives, and, due to their potential reproductive toxicity, may pose a potential hazard to fertility. This study aimed to evaluate the protective effect of resveratrol against AgNPs-induced testicular damage and oxidative stress in a male mice model.

Materials & Methods: In this experimental study, 24 adult male mice were randomly divided into four groups (n=6/group): control, AgNPs (200 mg/kg), resveratrol (20 mg/kg), and a combination of AgNPs and resveratrol. Treatments were administered daily by oral gavage for 35 days. At the end of the treatment period, serum malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), testosterone, testicular 8-OHdG levels, and histological changes including seminiferous tubule diameter, Leydig cell count, tissue structure, and spermatogenic status, were evaluated and analyzed among the groups.

Results: AgNPs significantly reduced serum testosterone levels and increased oxidative stress, as indicated by elevated SOD and 8-OHdG levels ($P< 0.001$). Resveratrol treatment significantly restored testosterone levels and reduced oxidative DNA damage, with values approaching those of the control group ($P< 0.01$). No significant changes were observed in TAC and MDA levels among the groups. AgNPs caused marked reductions in seminiferous tubule diameter, epithelial thickness, and counts of primary spermatocytes, spermatozoa, Sertoli, and Leydig cells ($P<0.001$). These adverse effects were significantly ameliorated by resveratrol, particularly in the resveratrol-only group ($P<0.01$).

Conclusion: AgNPs induce oxidative stress-mediated testicular damage and hormonal disruption in male mice. While resveratrol exerts a protective role by mitigating some of these effects, its impact was limited and did not fully reverse AgNP-induced toxicity.

Keywords: Silver nanoparticles, Resveratrol, Reproductive Toxicity, Testicular Damage, Mice

1. Introduction

For many years, silver has been used as an antimicrobacterial agent alone and in part with other agents. With the development of nanotechnology, silver nanoparticles (AgNPs) are widely usedin pharmaceutical applications, wound dressings, food products, electronics, cosmetics, and hygiene products. The extensive use of AgNPs has raised concerns for their potential sensitivity to humans and the environment (1-2). AgNPs have been shown to adversely affect multiple organs, including the skin, liver, lungs, brain, vascular system, and reproductive and urinary tracts following inhalation or exposure. AgNP toxicity is related to oxidative stress, which is an imbalance between radical production and the body's antioxidant system (3).

Spermatozoa are among the most sensitive cells to oxidative stress, especially reactive oxygen species (ROS) - induced stress, which may lead to lipid peroxidation, protein damage, apoptosis, and ultimately impaired cellular function (4-6). Resveratrol, a natural polyphenol present in grapes, peanuts, and berries, is a potent antioxidant that promotes antioxidant enzyme production such as catalase, superoxide dismutase, and glutathione peroxidase and reduces oxidative stress (7). Resveratrol also affects the control of inflammation, tumor growth,

cardiovascular disease, neurological disease, platelet aggregation, and metabolic disorders, and it has previously been reported to protect reproductive and other organs against various oxidative insults, including heavy metals and nanoparticles (8, 9).

However, despite extensive use of silver nanoparticles, data on their specific effects on testicular structure, spermatogenesis, and oxidative status are still limited, and the potential protective role of resveratrol against AgNP-induced testicular toxicity has not been comprehensively characterized. Therefore, the present study was designed to evaluate whether resveratrol, plant –derived with antioxidant properties can mitigate AgNP-induced oxidative stress, histopathological alterations, and hormonal changes in the testes of adult male mice.

2. Materials and Methods

2.1 Animals

Twenty-four adult male mice (8–10 weeks old, 40–50 g) were obtained from the animal house of Lorestan University of Medical Sciences, Lorestan, Iran. Animals were housed under standard laboratory conditions (12:12 h light–dark cycle, temperature 20–25°C, and relative humidity 55–60%) with ad libitum access to food and water. All experimental procedures were approved by the Institutional Ethics Committee and complied with international guidelines for the care and use of laboratory animals.

2.2 Experimental groups and study design

The animals were randomly divided into four equal groups (n = 6 per group): The animals were randomly assigned to four groups: (1) the control group, which received normal saline by oral gavage; (2) the AgNP group, which received silver nanoparticles at a dose of 200 mg/kg/day (10); (3) the resveratrol group, which received resveratrol at a dose of 20 mg/kg/day (11); and (4) the AgNP + resveratrol group, which received both AgNPs (200 mg/kg/day) and resveratrol (20 mg/kg/day).

All treatments were administered once daily by oral gavage for 35 consecutive days, which covers more than one complete spermatogenesis cycle in mice.

2.3 Silver nanoparticles (AgNPs)

Commercially available silver nanoparticles (Nano Metal Coating Co., Iran) with a nominal particle size of 20–40 nm were used. The choice of this size range was based on previous toxicological studies demonstrating that AgNPs of approximately 20–40 nm process effective tissue penetration and biological activity in vivo. The nanoparticles were provided as a suspension by the manufacturer, and their physicochemical specifications (size, purity, dispersion stability) were confirmed according to the supplier's certificate of analysis. AgNPs were administered at 200 mg/kg/day, a dose that has been widely used in earlier studies evaluating nanoparticle-induced reproductive toxicity.

2.4 Resveratrol treatment

Resveratrol (Raha Pharmaceutical Co., Iran) was administered at 20 mg/kg/day via gavage. This dose was selected based on previous reports showing significant antioxidant and cytoprotective effects without overt toxicity. Resveratrol was used as a free compound to allow independent evaluation of its protective effects against AgNP-induced oxidative and histological alterations. Resveratrol was dissolved each day fresh lyin an appropriate vehicle before administration.

2.5 Sample collection

At the end of the treatment period, mice were weighed and anesthetized using ketamine/xylazine. Blood samples were collected from the heart using a 2mL syringe without anticoagulants, and serum was separated by centrifugation at 3000 rpm for 10 minutes at -70°C. Testes were excised, weighed, and processed for biochemical assays and histopathological evaluation.

2.6 Biochemical measurements

The obtained serum samples were analyzed for biochemical markers, including malondialdehyde (MDA), total antioxidant capacity (TAC), and superoxide dismutase (SOD), using Navand Salamat biochemical kits via spectrophotometry, following the kit instructions. Testosterone levels were measured using an Ideal Diagnostic laboratory kit via the ELISA method. The 8-hydroxy-2′-deoxyguanosine (8-OHdG) factor in homogenized testicular tissue was assessed using the Zell Bio ELISA kit per the manufacturer's protocol.

2.7 Histological evaluation

The right testis and epididymis were weighed, fixed in 10% formaldehyde (pH7.4), sectioned to 4-µm thickness, stained with hematoxylin-eosin, and examined under a light microscope. The diameter of seminiferous tubules and the number of Leydig cells were counted and compared between groups. Additionally, atrophy, spermatogenic cell status, duct atrophy, sperm content within tubules, and cellular changes were evaluated.

2.8 Statistical Analysis

Statistical analyses were performed using SPSS software version 26 with significance set at $p< 0.05$. Data were expressed as mean ± standard deviation. Normality was assessed using the Shapiro-Wilk test, and variance homogeneity was checked with Levene's test. Welch's test was used when variances were unequal. The Kruskal-Wallis test was applied for non-normally distributed variables. Pairwise comparisons were conducted using the Bonferroni or Dunnett test, depending on variance homogeneity.

3. Result

3.1 Testosterone and oxidative stress parameters

The current study demonstrated a significant reduction in testosterone levels in the AgNP-only group compared to the controls ($P<0.001$). Conversely, testosterone levels were significantly elevated in the group receiving resveratrol alone, relative to the AgNP-only group ($P<0.001$). Notably, the testosterone levels in the resveratrol-treated group did not differ significantly from those in the control group ($P=0.982$), with values being nearly comparable. In the group administered both AgNPs and resveratrol, testosterone levels were increased

compared to the AgNP-only group, although the increase was not statistically significant (P=0.06) (Table1I).
According to Table I, the AgNP-only group exhibited significantly elevated SOD levels compared to the control group in terms of oxidative stress markers (P=0.001).
The group receiving Resveratrol alone showed no significant difference in SOD levels relative to the control group (P=0.695). In the group administered both AgNPs and resveratrol, SOD levels were reduced compared to the AgNP-only group (P=1.000.); however, this reduction was not statistically significant. Also, no significant differences between the groups were observed in TAC (P=0.051) and MDA (P=0.065) levels. (Table 1)
8-OHdG levels were significantly elevated in the AgNP-only group compared to the control and resveratrol-only groups (P=0.045).
The combined treatment group also showed a significant increase in 8-OHdG levels compared to the control (P=0.012), although the elevation was less pronounced than in the AgNP-only group (P=1.000, Table1).

Table 1. Comparison of testosterone levels and oxidative stress biomarkers in the study groups (n=6/each).

Serum biochemical parameters	Control group	AgNP-only group	Resveratrol group	AgNPs + Resveratrol group	P-value
Testosterone	0.04±0.64	0.23±0.02[a]	0.68±0.05[b]	0.33±0.02[a,c]	<0.001
SOD	370.41±30.86	748.11±42.20[a]	520.43±54.07	627.62±61.44[a]	0.001
TAC	0.69±0.10	0.96±0.01	0.68±0.09	0.84±0.06	0.051
MDA	192.97±10.71	143.35±18.82	143.98±10.17	186.08±21.05	0.065
8OHdG	0.18±0.01	0.65±0.06[a]	1.26±0.13[a,b]	0.54±0.06[a]	<0.001

Note: Data are expressed as mean ± SEM.*: P values are related to the comparison of factors distribution among the four groups, which were calculated by Kruskal-Wallis and Welch tests, as appropriateAgNPs: silver nanoparticles
SOD: Superoxide dismutase
MDA: Malondialdehyde
TAC: Total antioxidant capacity
8OHdG: 8-hydroxy-2′-deoxyguanosine
a: significantly different from the control group
b: significantly different from the nanoparticle group
c: significantly different from the resveratrol group

3.2 Testis histopathologic changes

Compared to the control group, there was no significant difference in testicular weight among the groups treated with AgNPs, resveratrol, or their combination. The diameter of the seminiferous tubules was significantly reduced in the AgNP-only group compared to the control group (P<0.001).
No significant difference was observed between the resveratrol and control groups. Although the difference was not statistically significant, the seminiferous tubule diameter in the combined treatment group was greater than that in the AgNP-only group(P=0.73), yet remained lower than in the resveratrol and control groups, as Table 2 shows the epithelial thickness in the resveratrol group was significantly higher than in the AgNP-only group, but similar to the control.
In the combined treatment group, epithelial thickness was significantly higher than in the AgNP-only group (P<0.001).
Also, the number of spermatogonia did not differ significantly among groups.
The AgNP-only group had significantly fewer primary spermatocytes compared to the controls (P<0.001). In contrast the resveratrol group and the combined group showed significant increases compared to the AgNP-only group (P<0.001).
Spermatozoa counts were significantly reduced in the AgNP-only group versus the control group (P<0.001), but increased in the resveratrol and combined groups, although the latter remained lower than the control and resveratrol groups.
The number of Sertoli and Leydig cells was significantly decreased in the AgNP-only group (P<0.001), while resveratrol treatment significantly restored their numbers (P<0.001). The combined group showed partial recovery, but values remained below those of the control and resveratrol-only groups (Table 2 and Figure 1A–D).

Table 2. Comparison of testis weight, seminiferous diameter, epithelium thickness, and histopathologic analysis results between the study groups (n=6/each).

Group	Control group	AgNP-only group	Resveratrol group	AgNPs + Resveratrol group	P-value
Testis weight	0.15±0.47	0.43±0.18	0.53±0.22	0.46±0.17	0.068
Seminiferous diameter	4.43±251	172±4.77[a]	220±5.01[b,d]	185.2±4.42[a,c]	<0.001
Epithelium thickness	70.21±4.31	29.3±5.5[a]	72.33±4.88[b]	63.81±5.12[b]	<0.001
Spermatogonia	21.33±4.41	17.79±4.19	22.11±4.32	19.16±3.87	-
Primary spermatocyte	16.62±4.61	10.27±4.4[a]	14.96±4.3[b]	13.94±3.13[a,b]	<0.001
Spermatid	24.31±4.7	11.16±3.87[a]	22.88±4.91[b,d]	15.34±5.1[a,b,c]	<0.001
Sertoli cells	11.19±4.3	6.22±2.1[a]	12.09±4.14[b,d]	7.14±2.61[a,c]	<0.001
Leydig cells	25.91±3.88	10.12±4.11[a]	27.3±3.7[b]	14.9±3.3[a,c]	<0.001

Note: Data are expressed as mean ± SEM.*: P values are related to the comparison of factors distribution among the four groups, which were calculated by Kruskal-Wallis and Welch tests, as appropriate.
a: significantly different from the control group
b: significantly different from the nanoparticle group
c: significantly different from the resveratrol group

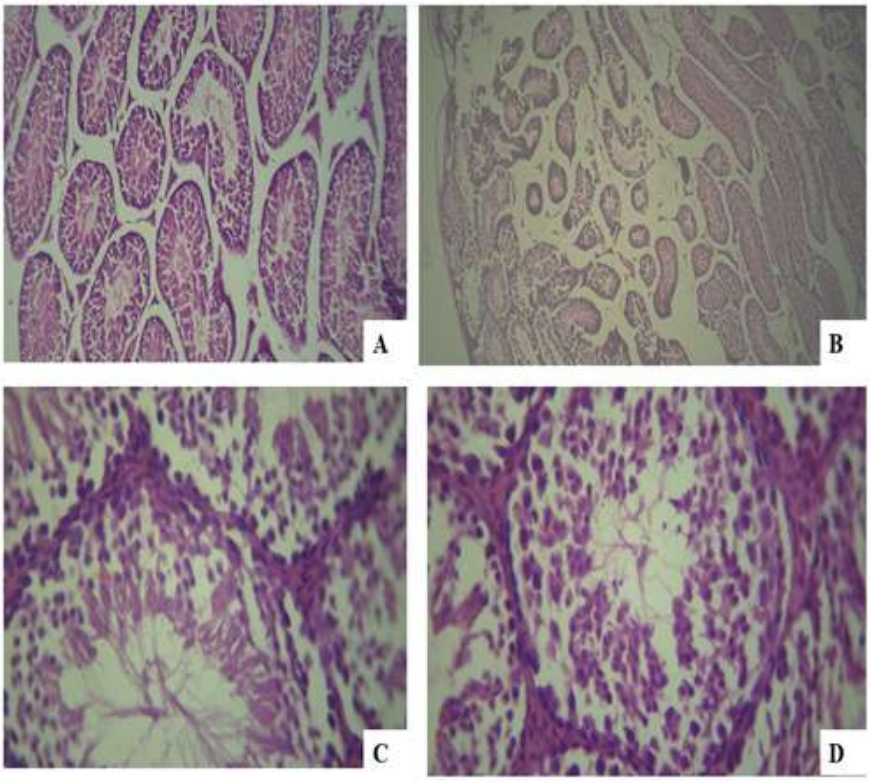

Figure 1. Effects of silver nanoparticles and resveratrol on testosterone levels and testicular histology. (**Prepared by Authors, 2026**).

Note: A: Control group: The testis and the epithelium of the rat seminiferous tubules appear natural and healthy (H&E 100x).
B: AgNPs treated group: Reduction in the thickness of the seminiferous tubule epithelium, a decrease in the number of cells, irregular arrangement, and an increase in interstitial space between the seminiferous tubules are observed (H&E 40x).
C: AgNPs +Res group: The testis and the thickness of the rat seminiferous tubule epithelium (spermatogonia, spermatocytes, spermatids, and spermatozoa, and Sertoli cells) appear natural and healthy (H&E 400x).
D: AgNPs +Res group: An increase in the thickness of the tubule epithelium and regular arrangement of cells and seminiferous tubules is observed (H&E 400x).

4. Discussion

The present study demonstrated that the administration of AgNPs caused significant testicular damage by reducing testosterone levels, the epithelial thickness of the seminiferous tubules, disrupting cellular organization, and increasing oxidative stress (with increased levels of 8-OHdG and SOD). Co-administration of resveratrol partially improved these parameters - especially testosterone levels, histopathological structure, and oxidative stress markers, although these improvements were not statistically significant in all markers such as TAC and MDA.

These findings align with prior research indicating that oxidative stress is a key mechanism by which AgNPs impair testicular function. Due to the presence of unpaired electrons these particles, have a strong tendency to react with essential body macromolecules, initiating oxidative stress and inducing cellular toxicity, DNA damage, and apoptosis. Some of this damage is due to the increase in MDA concentration and the decrease in antioxidant factors such as SOD and catalase **(3, 10)**. AgNPs can easily pass through the sperm membrane and have detrimental effects on sperm quality parameters such as count, morphology, and motility **(11)**.

Consistent with our findings, studies have shown that these particles can decrease concentrations of sex hormones such as testosterone and LH. A study in 2002 showed that testes of mice treated with AgNPs showed histopathological changes such as reduced thickness of the seminiferous tubule epithelium, reduced cell number and irregular arrangement, edema, and increased interstitial space between seminiferous tubules compared to controls **(12)**. Özatik and co-workers also showed that resveratrol partially ameliorated the histopathological changes induced by AgNPs in testicular cells **(13)**. Assar and colleagues showed a significant decrease in testicular weight and testosterone and estradiol levels in the groups receiving AgNPs compared to the controls in the adult male rat model **(14)**. Another study demonstrated a significant decrease in germinal epithelium thickness, seminiferous tubular shrinkage, and decreased sperm count in adult male rats. In addition, testosterone levels were significantly lower than usual **(15)**.

The present study did not observe significant changes in TAC and MDA levels among the groups. However, 8-OHdG levels, which indicate oxidative DNA damage, were significantly increased in the AgNPs group and decreased with resveratrol treatment, although not considerably. Ansar and colleagues also reported reduced antioxidant enzyme activity and increased MDA levels following AgNPs exposure. In their study, co-administration of selenium partially mitigated these adverse effects **(16)**. Similarly, Lopes and others (2019) observed decreased testosterone and antioxidant levels, along with testicular tissue damage, in rats treated with AgNPs **(17)**.

Other studies have also highlighted the antioxidant and protective properties of resveratrol in different models of oxidative injury **(18-19)**. However, discrepancies in findings may be attributed to differences in species, exposure duration, nanoparticle concentrations, or analytical techniques. Fukui and co-workers investigated the protective effect of resveratrol against glutamate-induced neurotoxicity in rat hippocampal cells. They reported that glutamate can cause neurotoxicity in neurons through oxidative stress processes. On the other hand, resveratrol, by increasing the concentration of SOD, was able to significantly improve this type of neuronal damage, indicating its antioxidant properties **(18)**. The difference between the results of our study and this study may be due to the variation in the type of laboratory animal used. In a study by Wang and others, the protective effects of resveratrol in reducing COPD symptoms in Wistar rats were investigated. They reported that resveratrol, as an antioxidant, could have a protective effect on rats against cigarette smoke by increasing the levels of SOD and interleukins 6 and 8, along with reducing the concentration of MDA **(19)**. This is inconsistent with the recent study's findings, which may be due to a change in the dose or duration of resveratrol treatment.

Mojica-Villegas and colleagues investigated the protective effect of resveratrol on oxidative stress biomarkers and sperm quality parameters in rats receiving iron ascorbate. They reported that the groups treated with resveratrol before receiving iron ascorbate had better sperm quality parameters than the others. This group showed a significantly higher number of viable and motile sperm. In addition, lipid peroxidation levels, glutathione peroxidase levels, and ROS production were lower than in other groups. However, increase in SOD production was observed in this group, which is consistent with the findings of a recent study **(20)**.

5. Conclusion

The current study provides evidence that AgNPs induce oxidative stress-mediated testicular damage and hormonal disruption in male mice. While resveratrol exerts a protective role by mitigating some of these effects, its impact was limited and did not fully reverse AgNP-induced toxicity.

6. Declarations

6.1 Acknowledgments

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6.2 Ethical Considerations

The study proposal was approved by the ethics committee of Dezful University of Medical Sciences, Dezful, Iran (Code: IR.DUMS.REC.1401.046). All animal experiments were conducted in accordance with institutional and national ethical guidelines.

6.3 Authors' Contributions

S.M.P and M.A.B designed the study and conducted the research. S.M.P, M.A.B and S.J monitored, evaluated, and analyzed the results of the study. Further, S.M.P, M.A.B, S.J and R.R, reviewed the article. All authors approved the final manuscript and take responsibility for the integrity of the data.

6.4 Conflict of Interest

The authors declare that there is no conflict of interest.

6.5 Fund or Financial Support

The authors would like to acknowledge Dezful University of Medical Sciences for financially supporting this study.

6.6 Using Artificial Intelligence Tools (AI Tools)

Grammarly was used as an artificial intelligence–based tool to assist with language and grammatical review of the manuscript. The authors take full responsibility for the final content of the manuscript.

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