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Protective Antioxidant Effects of Kombucha on Seminal Vesicle in Silver Nanoparticles-Treated Mice: A Stereological study

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ABSTRACT

Silver nanoparticles (AgNPs) are able to cause oxidative stress on the reproductive system. The aim of this study was to investigate the protective effect of Kombucha extract as an antioxidant against the toxicity of AgNPs on the Seminal vesicle of adult mice. In this experimental study, adult NMRI mice were randomly divided into 4 groups (n=6), control, AgNPs, Kombucha extract and AgNPs + Kombucha extract, and treated for 35 days. At the end of the treatment period, seminal vesicles were removed and their histological examination was performed using stereological methods. In addition, Malondialdehyde (MDA), total antioxidant capacity level and testosterone level in blood serum of mice were measured. A significant diminution in the volume of vesicular fluid ($p<0.01$) and a significant raising in the volume of connective tissue, muscular tissue ($p<0.05$) and blood vessels ($p<0.001$) were observed in AgNPs group compared to control group. MDA levels in mice receiving AgNPs also enhanced significantly ($p <0.05$), and vice versa total antioxidant capacity and testosterone level declined significantly ($p <0.05$). The mentioned parameters were normalized in the AgNPs + Kombucha group to the control ones. The Kombucha extract was able to protect against the seminal vesicle damage caused by AgNPs.

Introduction

Nanomaterials are part of an industrial revolution that aim is to produce a lightweight but strong material for different purposes Mohammed, et al. [1]. Given these physical and chemical attributes of materials on a nanometer scale, these materials can be used to generate new consumer products that are also utilized in biology and biotechnology Mohammed, et al. [1]. Among nanomaterials, AgNPs have the highest commercial application due to their antimicrobial activity Magnusson [2]. The widespread usage of AgNPs in industrial and medical products causes AgNPs to be absorbed directly into the body through the gastrointestinal tract and respiratory system Martirosyan, et al. [3]. The nanoscale dimensions in these particles facilitate the easy passage of the biological membrane and affect cell physiology so that as the diameter decreases, the contact surface increases and permeability of these particles boost Martirosyan, et al. [3]. Previous research has shown that AgNPs cause toxication in many animal species as well as cultured cells Magnusson [2]. The toxicity of silver particles often dependent on the emission of silver ions which is due to the production of active oxygen species accompanied by the induction of oxidative stress Magnusson [2]. Given that AgNPs can directly affect the normal functioning of body cells and disorder in the organs function therefore, evaluating their toxicity in the vita media is very important Yarmohammad, et al. [4].

Some studies have shown the toxic effects of AgNPs on the liver, kidney, heart, brain, testicular tissue and appendages, and sperm parameters in laboratory animals Mohammed, et al. [1,5,6]. The toxicity of AgNPs in the reproductive system causes apoptosis in sperm, reduces in sperm functional parameters, changes in the expression of spermatogenesis involved genes, degenerates in testicular tissue and declines in germinal cells, and lipid peroxidation in testis Habibian, et al. [6,7]. Kombucha is a beverage derived from the fermentation of tea and sugar and the coexistence of acetic bacteria and fungi which has beneficial effects on human health Dufresne, et al. [8]. The fermentation and oxidation processes carried out by Kombucha microorganisms produce a wide span of acids, vitamins, and enzymes Gharib, et al. [9]. Kombucha is rich in antioxidants such as vitamins C and E, beta-carotene, carotenoids, polyphenols and other antioxidant compounds Dufresne, et al. [8,9]. Scientists have found that the antioxidant activity in Kombucha is 100 times higher than vitamin C and 25 times higher than vitamin E Dufresne, et al. [9]. Various studies have shown that Kombucha can improve the damage caused by environmental pollutants such as trichlorethylene, alloxan, cadmium and gamma particles, lead acetate, the effect of magnetic fields and the side effects of drugs such as thiostamide and paracetamol and it is useful for people who are exposed to these substances Gharib, et al. [9,10]. Despite the various therapeutic benefits of Kombucha extract and the fact that it is widely used in many populations around the world, its beneficial effects and adverse side effects have not yet been adequately studied, especially on the reproductive system Kabiri, et al. [10]. Therefore, in this study, the effect of Kombucha extract was investigated as a powerful antioxidant in downregulating the adverse effects of AgNPs on the tissue structure of the seminal vesicles of adult mice and oxidative stress indicators of serum.

Material and Methods

Animals, Treatments and Material

To carry out this experimental study, Adult male NMRI mice weighing 210–240g were purchased from Pasteur's Institute, Tehran, Iran. The animal was kept in Arak University Animal House in a plastic cage at 12 hours' light/dark cycle, 24 °C ±2 °C temperature with access to water and food freely. Animal divided to 4 groups (n=6 each group) including 1-control, 2-AgNPs (500mg/kg/day), 3-Kombucha extract (9mg/kg/day) and 4-AgNPs (500mg/kg/day) + Kombucha extract (9mg/kg/day). The doses used in this study were selected based on past studies (17, 18- 19). The mice were treated orally for AgNPs group and intraperitoneal injection for Kombucha extract at 24-hour intervals for 5 weeks. For treatment, AgNPs with a purity of 99.99% with a diameter of 20nm purchased from Pishgaman Iranian Nanomaterials Company (Product name: Silver (Ag) Powder Product Number: US1038 Silver (Ag) CAS #: 7440-22-4) were used. kombucha was also purchased from Agida Shiraz. The protocol of this research was designed in accordance with the principles approved by the International Committees on the Protection of the Rights of Laboratory Animals. Every effort has been made to minimize the pain and stress of the animal.

Histopathology Study

After treatment, weighing mice and then anesthetized by diethyl ether. Then, to remove the seminal vesicle located on both sides, except for the vas deferens section, the meniscus duct and the surrounding connective tissue was taken for quantitative evaluation. The wet weight and immersion weight of the seminal vesicle were measured. Seminal vesicles were placed in Bowen's solution fixative for fixation. After fixation, the orientation method was used to obtain isotropic uniform random sampling (IUR, Howard and Reed) Noorafshan, et al. [11]. Eventually, from each of the semen sacs, 9-10 sections were obtained and as well as round pieces of semen sacs were created by Trocar Noorafshan, et al. [11,12]. The final, these sections were used to measure the volume of wrinkles in the semen sac tissue after the tissue processing steps. In the first, these sections and fragments obtained by the Trocar were subjected to tissue processing (Leica), the next, placed in paraffin blocks, then these blocks were cut by a Microtome set in sections of 5 and 20µm thicknesses. The next these slices were stained with Heidenhain's Azan method Mahmoodi, et al. [12].

Stereological Study

Estimating the Volume of Parenchyma and Vesicular Fluid

Then, histopathologic assessments were performed to point estimate the volume of parenchyma and vesicular fluid Noorafshan, et al. [11,12]. In the end, the volumetric deduction of these segments was estimated using optical microscopy (Olympus, BX51) by the magnification of 10. For this purpose, a spot probe was randomly placed on microscopic images and then the points encountered with the parenchyma or vesicular fluid surface and all sections of the seminal vesicle were counted and the following formula estimated their volume fraction (volume density) (Vv):

$$V_{V(structuregland)} = \frac{\sum_{i=1}^{n} p_{(structure)}}{\sum_{i=1}^{n} p_{(gland)}}$$

$$V_{V(structure/gland)} = P_{(structure)} \Big/ P_{(gland)}$$

Which $\sum_{i=1}^{n} p_{(structure)}$ total number of points encountered with parenchyma profiles or vesicular fluid and $\sum_{i=1}^{n} p_{(gland)}$ is the number of points encountered with the total sections of the seminal vesicle.

Then, the volume of parenchyma and vesicular fluid was obtained by multiplying the volume density in the final volume of the seminal vesicle. To estimate the volumetric fraction of parenchymal segments, 14-10 microscopic fields in each gland were examined with a magnification of 40. To select microscopic fields, a systematic random selection method was used. To assay the volumetric density of parenchymal segments, the point-counting method was used according to the following formula Noorafshan, et al. [11,12]:

$$V_{V(structureparenchyma)} = \frac{\sum_{i=1}^{n} p_{(structure)}}{\sum_{i=1}^{n} p_{(parenchyma)}}$$

$$V_{V(structure/parenchyma)} = P_{(structure)} \Big/ P_{(parenchyma)}$$

Which $\sum_{i=1}^{n} p_{(structure)}$ the sum of the number of points is encountered with the profiles of epithelial cells, arteries, connective tissue, and smooth muscle tissue, and $\sum_{i=1}^{n} p_{(parenchyma)}$ is the sum of the number of points encountered with parenchymal profiles. To calculate the volume of the above segments, the volume fraction of each parameter was multiplied in the total volume of parenchyma.

Biochemical Evaluation

Assessment of Serum Malondialdehyde Level (MDA): The valuation of lipid peroxidation of blood serum samples of different groups of mice was measured by spectrophotometry of thiobarbituric acid. In the first, the TCA-TBA-HCL reaction solution, including trichloroacetic acid (TCA) 15% g/ml, thiobarbituric acid (TBA) 0.375% g/ml and chloride acid (HCL) 0.25 Nm (Merck KGaA, Darmstadt, Germany) were prepared. Then, 250µl of the sample was mixed with 500µl of TCA-TBA-HCL solution and the samples were placed in a bain-marie for 15 minutes. The next, after removing the samples from the bain-marie, they were cooled by using cold water and centrifuged for 10 minutes. In the end, the surface fluid was carefully separated and its absorption was measured by a spectrophotometer at 535 nm against a blank solution containing all but sample Buege, et al. [13].

Measurement of Total Antioxidant Power (FRAP method) of Serum: Total blood serum antioxidants were measured by the Ferric Reducing of Antioxidant Power (FRAP) method that first it was explained by Benzie in 1996. In the first, to perform the 10 ml acetate buffer, 1ml of the reactive solution 2, 4, 6- tri (2-pyridyl) -1, 3, 5- triazine (TPTZ) (Sigma Aldrich, St. Louis, MO, USA), 1ml of ferric chloride solution and 1.2ml of distilled water were mixed and FRAP reagent was prepared. In the next step, the standard solution of ferrous sulfate was prepared in serial dilutions. Then 100 microliters of each standard sample or solution were mixed with 3ml of FRAP reagent and added to the quartz cuvettes. The absorption of the samples at a wavelength of 593 nm against Blanc was read by a spectrophotometer and then the FRAP rate was calculated using the regression formula obtained from the standard curve and expressed in mmol/L Benzie, et al. [14]. One of the limitations of this study was the lack of measurement of the levels of gonadotropin hormones (LH and FSH) and the secretory factors of semen activity such as fructose.

Evaluation of Testosterone Level: Serum testosterone levels were evaluated using DRG ELISA kit (USA, EIA-1559) based on the manufacturer's instructions. The findings were expressed as ng/ml.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD) for six animals per group. One-way analysis of variance (ANOVA) followed by Tukey's test was used to assess.

Results

Histopathology Study

Histological observations showed that in the control group of epithelial cells were cylindrical, the volume of fluid was high and monotonous, as well as the structure and thickness of connective and muscular tissue were normal (Figure 1A). However, in the AgNPs group, hyperplasia and fibrosis of connective tissue, decreased vesicular fluid volume and increased muscle wall of the seminal vesicle was observed, indicating a reduction in the exocrine function of the seminal vesicle. In addition, an enhance in the volume of blood vessels was visible in this group (Figure 1B). In the group that received both AgNPs and Kombucha extract at the same time, the tissue structure was almost similar to the control group, and the volume of vesicular fluid increased significantly compared to the AgNPs group (Figure 1C). Histological observations in the group receiving Kombucha extract also indicated the normal tissue structure of the seminal vesicles in this group (Figure 1D).

Stereological Study

There was no significant difference in the comparison of mean body weight in different groups of mice ($p > 0.05$). Also the mean weight and total volume of the seminal vesicles in the AgNPs group showed a noticeable decrease compared to the control group, but this decrease was not statistically significant ($p > 0.05$). As well as The mean parenchymal volume in the AgNPs was not significantly different compared to the control group ($p > 0.05$). However, the vesicular fluid volume in the AgNPs decreased significantly compared to the control group ($p < 0.01$) (Table 1). There was no significant alteration in the comparison of the mean volume of epithelial cells in different groups ($P > 0.05$). However, vascular

volume increased significantly in the AgNPs group compared with the control group ($p < 0.01$). In addition, in the AgNPs group, muscle volume and connective tissue volume showed a significant increase compared to the control group ($p < 0.05$). Finally, in the group that received Kombucha extract at the same time as the AgNPs, the measure of these parameters decreased significantly compared to the AgNPs group ($p < 0.05$) and was close to the routine level of the control group (Table 2).

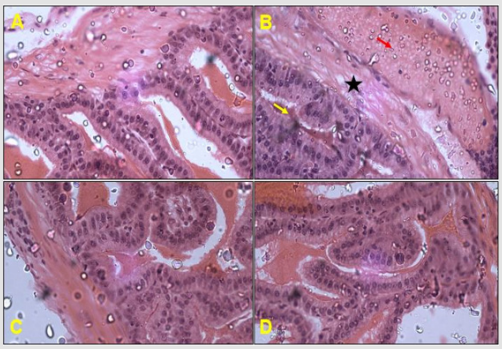

Figure 1: Microscopic images of the Seminal vesicle tissue of adult mice in different groups, treated with Silver Nano (500mg/kg/day) and Kombucha extract (9ml/kg/day) and Silver Nano+Kombucha. The thickness of the incisions is 5 microns, the hematoxylin-eosin is staining and the magnification is 400X.

A. Normal Seminal vesicle tissue in the control group,

B. Decreased vesicular fluid (yellow arrow), increased connective and muscular tissue wall (star) and increased vascular volume (red arrow) in the silver Nano group,

C. Tissue structure similar to the control group in Silver Nano+Kombucha group, in which an increase in vesicular fluid and the natural structure of connective and muscular tissue is seen,

D. Natural tissue structure in the Kombucha group.

Table 1: Comparison of the average Body weight at the end of treatment, Seminal vesicle weight, Total volume of the seminal vesicle, Parenchyma volume and Vesicular fluid volume (mm3) in various mice groups treated (for 35 days) whit Silver Nanoparticles (500mg/kg/day) and Kombucha extract (9ml/kg/day), Date one present as mean ± SD, ANOVA, Tukey's test, the mean with the same superscripts do not differ significantly.

Groups	Body Weight	Seminal Vesicles Weight	Total Seminal Vesicles Volume	Parenchyma Volume	Vesicular Fluid Volume
Control	35.0±8.9a	0.0±25.02a	170.10±26.21a	35.11±12.35ab	135.12±13.61a
Silver Nano	33.1±7.8a	0.0±21.04a	146.30±01.1a	50.12±5.78b	95.20±5.46b
Silver Nano+Kombucha	36.2±5.8a	0.0±24.04a	167.25±05.77a	39.6±14.03ab	127.24±9.56a
Kombucha	36.2±2.4a	0.0±23.03a	171.21±6.13a	33.10±46.6a	138.17±1.95a

Table 2: Comparison of the Epithelial cells volume, Blood vessel volume, Connective tissue volume and Muscular tissue volume in various mice groups treated (for 35 days) whit Silver Nanoparticles (500mg/kg/day) and Kombucha extract (9ml/kg/day), Date one present as mean ± SD, ANOVA, Tukey's test, the mean with the same superscripts do not differ significantly.

Groups	Epithelial Cells Volume	Blood Vessels Volume	Connective Tissue Volume	Muscular Tissue Volume
Control	23.10±68.53a	0.0±23.04a	1.0±37.39a	9.1±66.98a
Silver Nano	35.9±13.8a	0.0±47.11b	1.0±96.33b	13.2±07.98b
Silver Nano+Kombucha	29.4±44.78a	0.0±21.12a	1.0±39.22ab	7.2±94.0a
Kombucha	25.10±66.52a	0.0±26.05a	1.0±07.43a	6.0±45.77a

Biochemical Evaluation

In the AgNPs group, evaluation of serum malondialdehyde level in different mice groups showed a significant enhancement (Figure 2) and vice versa total antioxidant capacity and testosterone level showed a significant diminishing (Figures 2 & 3) compared to the control group ($p < 0.05$). In the AgNPs + Kombucha group, the total antioxidant capacity did not change significantly compared to the control group, indicating that Kombucha, with its heavy antioxidant effects, was able to compensate for the antioxidant capacity level and upregulate its value to the control group. Also in this group, an upregulation in testosterone level and also a downregulation in MDA were noticeably observed compared to the control group, but these altered were not statistically significant ($p > 0.05$) (Figure 4).

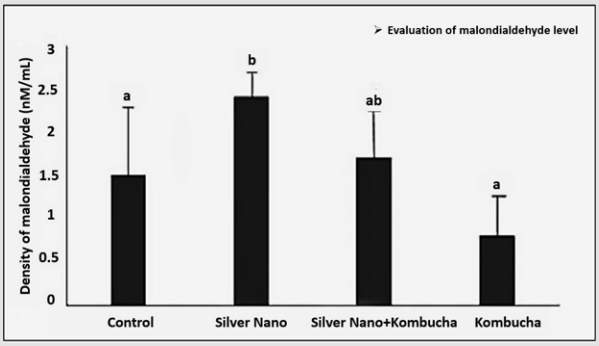

Figure 2: Comparison of Serum malondialdehyde (nM/mL) levels in various mice groups treated (for 35 days) whit Silver Nanoparticles (500mg/kg/day) and Kombucha extract (9ml/kg/day), Date one present as mean ± SD, ANOVA, Tukey's test, the mean with the same letters do not differ significantly.

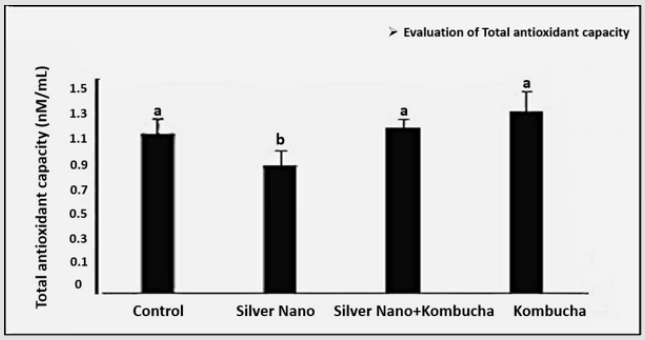

Figure 3: Comparison of Total antioxidant capacity of serum (nM/mL), using the FRAP method, ($p<0.04$) in various mice groups treated (for 35 days) whit Silver Nanoparticles (500mg/kg/day) and Kombucha extract (9ml/kg/day), Date one present as mean ± SD, ANOVA, Tukey's test, the mean with the same letters do not differ significantly.

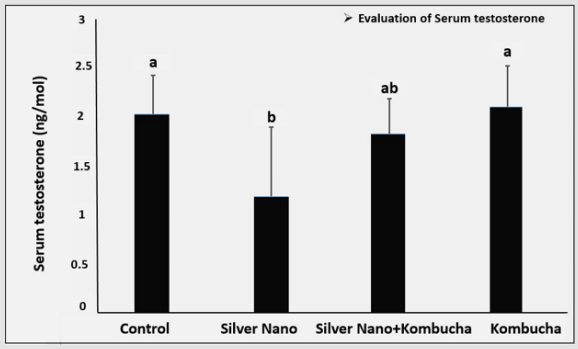

Figure 4: Comparison of serum Serum testosterone (ng/mol), ($p<0.04$) in various mice groups treated (for 35 days) whit Silver Nanoparticles (500mg/kg/day) and Kombucha extract (9ml/kg/day), Date one present as mean ± SD, ANOVA, Tukey's test, the mean with the same letters do not differ significantly.

Discussion

The evaluation of the semen sac is an important indicator of the quality of male fertility so that the failure of the seminal vesicles leads to a decrease in male fertility Mahmoodi, et al. [12]. Therefore, it is important to investigate the effect of environmental pollutants on the function of this tissue. In this study, changes were observed in the seminal vesicle tissue of mice treated with AgNPs (500mg/kg/day), including significant declines in fluid and vesicular fluid volume and a significant enhance in vascular volume, connective tissue volume, and muscle tissue volume relative to the control group Mahmoodi, et al. [12,15]. The seminal vesicle produces and secretes a large amount of fluid that the composition of which is specific to each organ, these secretions play an important role in various processes such as formation and maturation of sperm and involvement in suppressing immune responses Mohamad, et al.

[15]. A significant reduction in fluid and vesicular volume in the AgNPs group indicates that epithelial cell secretions are affected by treatment with these nanoparticles. Proteins, enzymes and amino acids are an important part of seminal vesicle secretions and it has been shown that both nanoparticles and silver ions (Ag+) can interact with proteins and amino acids. Nanoparticles cause the formation of the protein corona, opening up the structure of the protein and altering its function McShan, et al. [16]. The interaction of nanoparticles with proteins is an important toxicity mechanism for nanoparticles McShan, et al. [16].

Therefore, it may reduce the secretions of the seminal vesicles by effecting on proteins. Also, the secretory activity of the seminal vesicles is regulated by the nervous system and cholinergic and adrenergic neurons Gonzales [17]. Because AgNPs with oxidative stress created by the production of free radicals can neurotoxicity and apoptosis of neurons Rahman, et al. [18], the decrease in semen volume may be due to the same destructive mechanism and affects the nervous system, which reduces the secretion of this gland that of course, requires further investigation Rahman, et al. [18]. Another result of this study was a significant increase in the volume of blood vessels in the seminal vesicles of mice receiving AgNPs. In other reviews, AgNPs caused changes including dilation in the central vein and hypertension of the liver tissue Awasthi, et al. [19,20], as well as hypertension and dilation of blood vessels in the tissue of kidney mice Seyedalipour, et al. [5]. As well as, a significant elevation in the volume of connective and muscular tissue in mice in the AgNPs group compared to the control group was another result of the present study. Atrophy of the seminal vesicles is reported to be histologically associated with decreased seminal vesicle secretion, epithelial destruction, and increased fibro-muscular stroma Mahmoodi, et al. [12]. There was also a significant reduction in serum testosterone levels in the AgNPs recipient group, which is consistent with El-Azab and Elmahalaway study El-Azab, et al. [21].

The only research on the effect of AgNPs on the histology of the seminal vesicles was a study by Mohammed and colleagues in 2016 which AgNPs in doses of 0.6 and 0.4mg/kg body weight were given to albino rats and histological studies on prostate glands and seminal vesicles showed that AgNPs dilated prostate alveoli and flattened its cubic epithelium at low doses, destroying prostate alveoli and leaking prostate secretions into the interstitial connective tissue in the high doses of AgNPs Mohammed, et al. [1]. Histopathological changes were also observed in the tissue of the seminal vesicles, including shortening of the alveolar folds and hyperplasia of the connective tissue of the seminal vesicles, especially at high doses, which attributed these effects to toxic oxidative stress in AgNPs Mohammed, et al. [1]. The results of the present study also showed an increase in malondialdehyde and a decrease in total antioxidant capacity in the blood serum of mice treated with AgNPs, indicating oxidative stress by AgNPs, which is consistent with other studies Ahmadi, et al. [22-24]. Therefore, according to the results of this study and other studies, AgNPs may have caused semen atrophy through the production of free radicals and the formation of oxidative stress. AgNPs have been shown to induce ROS production, thereby inhibiting regenerated glutathione (GSH), DNA damage, protein carbonylation, and membrane oxidation McShan, et al. [16,25]

The nanoparticle also accumulates in the outer mitochondrial membrane, causing direct damage to the mitochondria and impairing respiratory chain function, eventually leading to ROS production and oxidative damage McShan, et al. [16]. In fact, AgNPs at higher doses and smaller sizes cause more tissue damage than larger nanoparticles due to their higher surface-to-volume ratio Ahmed, et al. [25]. The dissolution of AgNPs is related to its size, and the oxidative dissolution of smaller particles increases the production of hydrogen peroxide as well as protons, which ultimately leads to the production of intracellular ROS Ahmed, et al. [25]. Bodyweight data showed that at the end of the treatment period there was no significant difference between any of the different treatment groups. This result indicates that the treatment of adult mice with a dose of 500 mg/kg of AgNPs for 35 days did not significantly affect the metabolic activity of mice and thus their weight change. This result was consistent with the results of some previous research Almansou, et al. [6,26]. For example, in the study of Azza et al Even treatment of doses of 100,500 and 1000mg/kg of AgNPs for 28 days did not alter the mice's body weight Attia [26]. In this study, in mice that received both AgNPs and Kombucha extract simultaneously, Kombucha extract significantly upregulated the volume of vesicular fluid and significantly downregulated the volume of connective tissue and muscle (in the normal range). Also, the blood vessel volume in this group did not differ significantly from the control group. Kombucha is a powerful antioxidant that diminishes the damage induced by oxidative stress.

The results of the present study also showed a downregulate in malondialdehyde level and an upregulated in the total antioxidant capacity of blood serum in mice that simultaneously received Kombucha extract with AgNPs. The only study to investigate the protective effect of Kombucha extract on reproductive tissues by Alikarami [27] was performed this year Alikarami [27], which confirms some of our results. Also, studies have shown its protective effect on various tissues using inhibition of oxidative stress Gharib, et al. [9,28] Bhattacharya et al., 2011). A study by Gharib, et al. [9] also found that Kombucha extract was able to improve kidney damage induced by trichlorethylene due to the presence of acetic acid in the extract, which was able to bind to toxins, dissolve and remove toxins from the body Gharib, et al. [9]. Also, Bhattacharya et al (2011) showed a protective effect of Kombucha extract on the toxicity of tert-butyl hydroperoxide on the production of free radicals, apoptosis, changes in mitochondrial membrane potential,

cytochrome C release, activation of caspases 3, 9 and Apaf-33 (Bhattacharya et al., 2011). The antioxidant activity of Kombucha extract is due to the presence of tea polyphenols, ascorbic acid (vitamin C) and DSL (1,4-lactone D-Saccharic acid) Jayabalan, et al. [29,30]. Kombucha extract has been shown to have stronger antioxidant activity than unfermented tea Değirmencioğlu, et al. [30], which can be due to the production of low molecular weight compounds and structural changes in tea polyphenols by enzymes produced by bacteria and yeasts during the fermentation process Jayabalan, et al. [29].

The phenolic compounds present in Kombucha have scavenged activity against radical DPPH and radical superoxide Jayabalan, et al. [29,31]. Vitamin C and beta-glucan (β-Glucan), as a by-product of Kombucha tea fermentation, are strong radical free radicals and non-specific stimulants of the body's immune response Ibrahim [28]. On the other hand, vitamin C can increase testosterone levels Rizk, et al. [32]. Kombucha's protective mechanism can also be dependent on the presence of hyaluronic acid, which reduces the damage induced by free radicals and provides protection against oxidative stress Ibrahim, et al. [28,30,31]. Glucuronic acid, as a powerful antioxidant and another important acid in Kombucha, is able to facilitate the detoxification process in the body Gharib, et al. [9,28]. The catechins in Kombucha have antioxidant, anti-cancer, anti-diabetic and anti-atherosclerotic properties and perform their properties better in an environment that contains acetic acid and glucuronic acid Watanabe, et al. [33]. Tocopherol and ascorbic acid in Kombucha has a strong synergistic effect on the antioxidant activity of Catechins in an environment containing linolenic acid Bhattacharya, et al. [34-40].

Conclusion

As the results of the present study showed, AgNPs significantly reduced the mean volume of vesicular fluid and also significantly increased the mean volume of connective tissue, muscle tissue, blood vessel volume and also decreased serum testosterone level compared to the control group. In vice versa, Kombucha extract also improved the above parameters in the AgNPs + group of Kombucha extract to the control group. The potent antioxidant effect of Kombucha extract, which was observed in the results of this study, as well as its detoxifying properties due to the presence of organic acids (glucuronic acid and acetic acid), can both reduce the toxicity effects of AgNPs on the histological parameters of semen.

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