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Histological Effect of Green Synthesized Silver Nanoparticles Agnps on Albino Rat Testis

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ABSTRACT

Background: According to the data, AgNPs are being utilized more frequently for industrial, consumer, medical, food, and health care reasons as well as for other purposes due to their unique physical and chemical characteristics. Despite NPs' benefits, a variety of issues have increased the risk of toxicity in both people and animals.

Objective: main objective of this study is to evaluate the effects of AgNPs produced using a green technique from plant extract (Borago officinalis) on the reproductive system (testis).

Methods: Borago officinalis flower extracts are used as reducing agents and stabilizing agents in the production of silver nanoparticles, which is an environmentally beneficial process. 40 adult male rats are separated into 4 equally sized groups. The groups were given distilled water treatment, oral plant extract (5 mg/kg), and intraperitoneal injections of AgNPs 30, 50 mg/kg After 35 days, we evaluated the changes in the tissue of the testis. How were the four groups divided?

Results: The results showed that AgNPs significantly increased MDA levels in animal groups treated with various AgNP concentrations as compared to control groups treated only with Borage extract. Additionally, Testosterone levels in animals treated with Borage extract decreased non-significantly as compared to the control group, which experienced a significant decline in testosterone when treated with various AgNP concentrations.

Conclusion: despite being made from plant extract, AgNPs have a detrimental effect on the male reproductive system by changing the testicular functions, particularly the production of testosterone, in contrast to the benign nature of the crude Borage flower extract.

Keywords silver nanoparticles (AgNPs), green synthesis, tissue testis, TEM.

INTRODUCTION

There are internal and exterior structures in the male reproductive system. The well-vascularized structures with multiple glands and ducts found in this system enable the formation, storage, and ejaculation of sperm for fertilization as well as the production of essential androgens for male growth [(1)].

In the testis, there are primarily two types of cells. Leydig cells, the first kind, are situated adjacent to the seminiferous tubules in the interstitium of the testis and secrete testosterone, a significant male androgen [(2)].

Sertoli cells, a second type of cell, are located on the outside edge of seminiferous tubules [(3)]. may speed up the process of spermatogenesis, which starts at the tubule's edge. Males play a significant influence in determining fertility because of the testicles' critical roles in male development. The possibility of extending life, finding new molecules, and manipulating those naturally found in man-size organisms could all be of interest. [(4)].

Buraihi *et al*. [(5)] demonstrated that nanoparticles have a very tiny size and a large surface area, making it possible to further modify their surface with hydrophobic, hydraulic, or any metal to the surrounding environment. As a result, they have various uses in biological research [(6)]. Biomedicine, disease diagnosis, gene therapy, medication delivery, catalysts, cosmetics, food production, agriculture, pharmaceuticals, orthopedics, and antimicrobial therapy are a few examples of these uses. These nanoparticles, or NPs, can be created in a variety of ways, including through the eco-friendly and environmentally advantageous process of biosynthesis, which produces harmless and biodegradable nanoparticles [(7)].

Nanoparticles can be produced by bacteria, plants, and fungus using this mechanism (biosynthesis). One of the many nanoparticles created by green biosynthesis is the silver nanoparticle AgNPs. The effects of AgNPs on human health as well as the processes underlying their action are not well understood. It is critical to look at their possible toxicity in live organisms, especially in mammals, in order to more accurately assess the risk to humans. Numerous studies have shown that AgNPs can increase the production of reactive oxygen species (ROS), which causes oxidative stress and damage in a range of cell types [(8)].

The main objective of the current study is to evaluate the effects of Borago officinalis L. flower extracts and various doses of AgNPs made from the same plant using a green synthesis method on the testis of male rats by examining the histopathological changes.

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METHODS

Experimental Design

40 male rats in total were randomized into 4 groups at random (n = 10 per group), and each group received the following care:

*Group G1: control animals that treated-with normal slain three times per week during the entire experiment period.
*Group G2: animals that orally administration with plant extract of (Borago officinalis L.) (5mg/kg) (11) three times per week during the entire experiment period.
*Group G3: animal that treated with of AgNPs (30 mg /kg) three times per week during the entire experiment period
*Group G4: animal that treated with of AgNPs (50 mg /kg)/ three times per week during the entire experiment period.

Plant extracts preparation

To get rid of dust, dried flowers of Borago officinal L. were rinsed under running water. 250 mL of deionized water is combined with 50 grams of dried flowers, and the mixture is let to soak for 24 hours. Utilizing deionized water, a very efficient method for extracting active compounds, an aqueous cold extraction was performed [(9)]. The produced solution was filtered twice, once through a Whatman filter paper and once through a sterile double layer of gauze to get rid of all the big particles. The next step is to centrifuge the extract solution for 10 minutes at 5000 rpm to get rid of all big particles. In order to enhance the evaporation of the extract solution and the creation of thick viscous material, the supernatant was dispensed into Petri plates and incubated at (37–38 °C). The (Borago officinal) flowers' ultimate product was removed from pettier dishes using a silicon spatula, and the viscous fluid it produced was stored in dark containers with tight-fitting lids and maintained in the refrigerator [(10)]. One of the newest methods for creating nanoparticles uses plant extract. In this manner, several metal nanoparticles were created. Plant extracts can act as reducing and stabilizing agents in the production of nanoparticles [(11)].

Synthesis of (AgNps) by green method

The conventional approach was followed to create AgNPs at two distinct molar concentrations (1.17 and 2.37) by combining 19.53 and 39.57 mg of precursor AgNO3 with 100 ml of deionized water and swirling magnetically for two hours. After mixing one part of plant extract stock (B. officinalis) with one part of AgNO3 solution (for both molar concertation) under magnetic stirrer for 15 minutes, a minor shift in color from light brown to dark brown is noticed, and this is proof that silver nanoparticles have formed. The outcome is kept in opaque containers and forwarded for characterisation at a later date [(12)].

Characterization of silver nanoparticles

Transmission Electron Microscopy (TEM)

Is a type of microscopy in which an electron beam is passed through a very thin material while interacting with it. A picture is made when electrons are fired through a sample and interact. The image is then magnified, focussed, and exposed to an imaging medium, such as a fluorescent screen. The AgNPs were measured using a Philips Model CM10 Transmission Electron Microscope. On a copper TEM grid covered with carbon, a 314-square grid of copper with a mesh size of 200 and a diameter of 3 mm was used.

UV-VIS spectroscopy

The absorption and transmission spectra of several AgNps samples were measured as part of their optical properties using an evolving spectrophotometer for UV/Visible radiation (Ultrospec 4300 pro from Amersham Biosciences). The test's wavelength spanned from 190 nm to 1100 nm.

Ethical approval

The experimental animal procedures and handling were authorized by the collage of science research ethics committee of Baghdad University, Iraq (CSEC/1121/0063).

Statistical analysis

Using Minitab 19.1, all statistical analyses were carried out. Analysis of variance (ANOVA) was used to determine the importance of the various study groups.

RESULTS

Transmission electron microscopy (TEM) measurements

According to the investigation's findings, the width of AgNPs ranged from less than 10 nm to more than 50 nm. This study used TEM images to show a uniform size and distribution of……? Figure (1 a and b). The structure is also depicted as a spherical arrangement of AgNPs with multiple twinning planes, with the most common particle size being 30 nm. An energy-absorbing colloidal solution was used to test the generated Ag NPs' homogeneity, as shown in Figure (2 a and b).

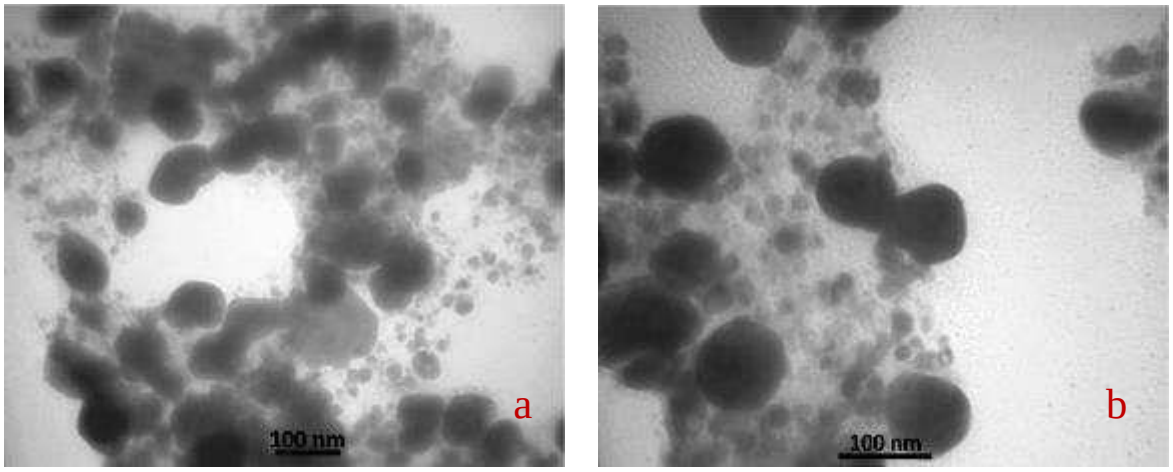


Figure (1) TEM images for Ag NPs with different molar ratio:(a) 1.17 mg/ ml, (b) 2.37 mg/ml.

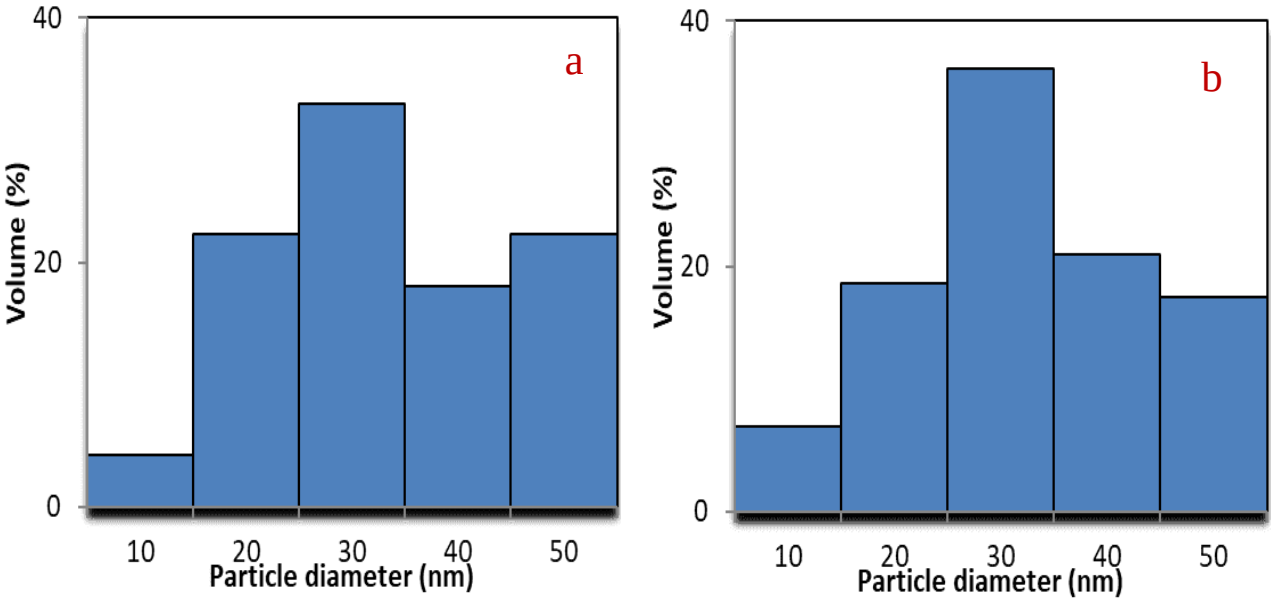


Figure (2) Particle size distributions resulting from the measurements of (200–300) particles of AgNPs for different molar ratio: (a (1.17 M, (b) 2.37 M

Optical properties of Ag NPs prepared by green synthesis.

UV-VIS.

Figure (3) displays the AgNPs absorbance spectra at wavelengths between 200 and 1000 nm; the performance of the extract improved with increasing extract content. A peak at 487,406 nm was noticed when the concentration was high, whereas a peak at 474,402 nm was seen when the concentration was low.

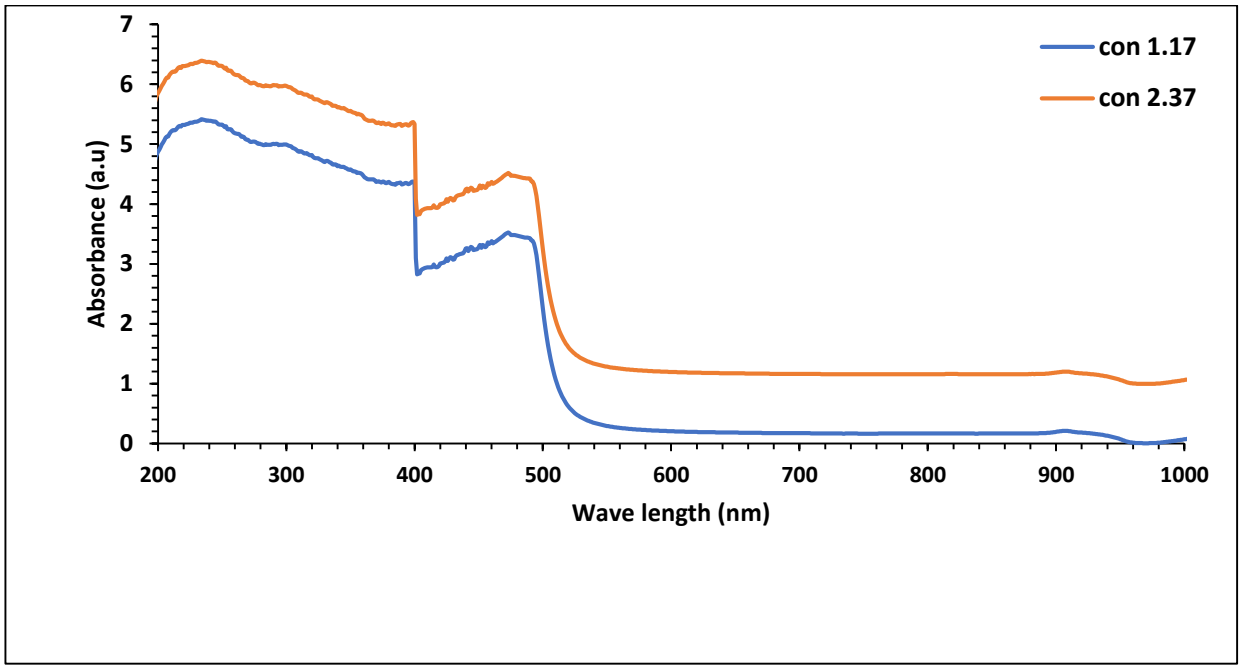


Figure (3) Absorption spectra of Borage extract AgNPs

Histological Examination

Histological sections of the testis tissues were prepared according to method of paraffin sections technique [13].

Control group

The microscopic examination for testes tissue for control groups (untreated animals) showed normal structural appearance, illustrating seminiferous tubules lined by stratified epithelium consists of two distinct population of spermatogenic cells (S) with spermatid (SZ) and Sertoli cell (St) and interstitial spaces between tubules with Leydig cell imbedded in plexus of blood and lymph capillaries(L).

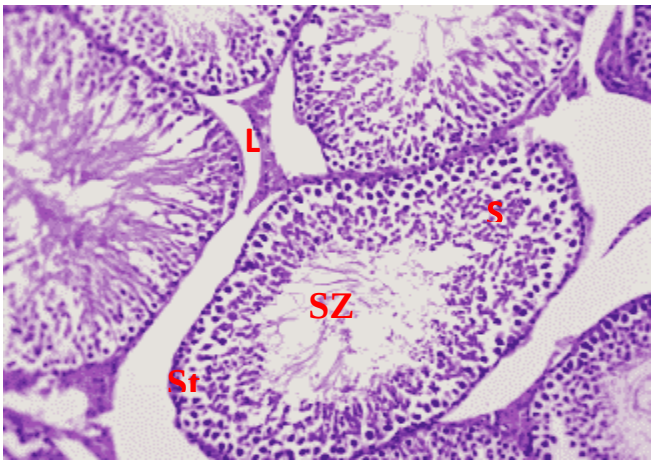
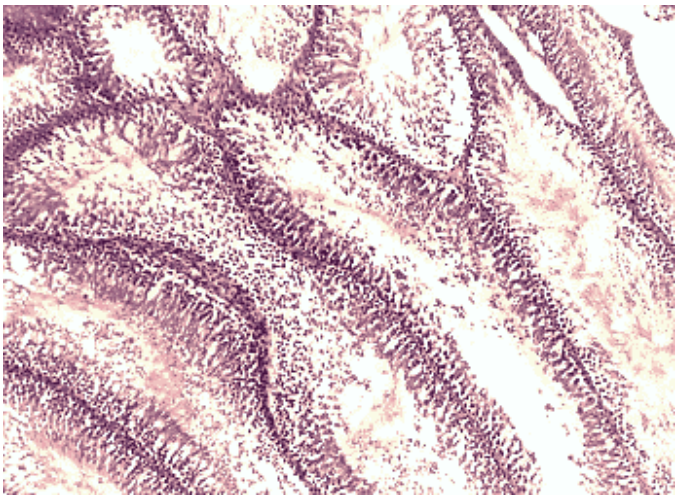


Figure (4) Microscopical section of testes control group show normal aspects of testicular structure without microscopic alteration (H&E, stain X: 10&40).(H&E, X:40).

***Borage officinalis* L. extract group**

Almost testis sections in the group that treated with Borago officinalis extract showed normal architecture compering with control group.



Figure(5) Cross section in the testes treated with Borago officinalis extract, showed section look like normal appearance which consist of seminephrous tubular with mature spermtogonia and presence of spearms inside the lumen (H&E, x:40).

While other section from this group revealed variable degree of tubular dilation and exhibited various

stage of spermatogenesis with prominence of sertoli cells tighter with leydig cell hyperplasia (Fig 6). Also with evidence of mature spermatozoa fill the lumen of seminiferous tubules associated with mild vacuolation of adjacent tubules (Fig 7).

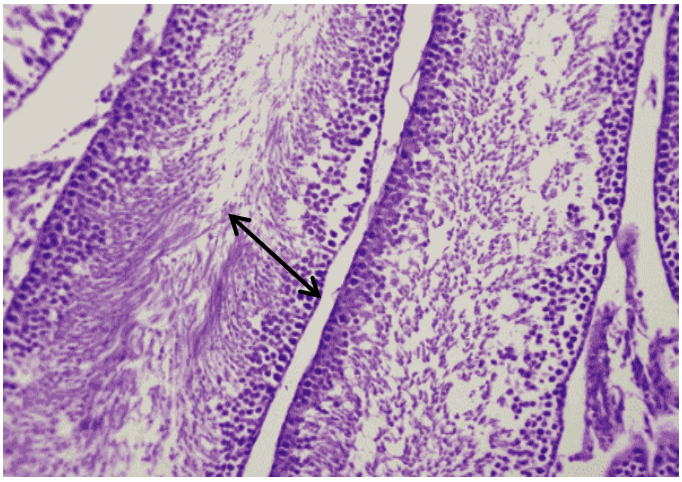


Figure (6) Cross section in the testes treated with Borago officinalis extract shows tubular dilation↔ with various stage of spermatogenesis→ and prominence of Sertoli cell with Leydig cell hyperplasia↔ (H&E, X:400).

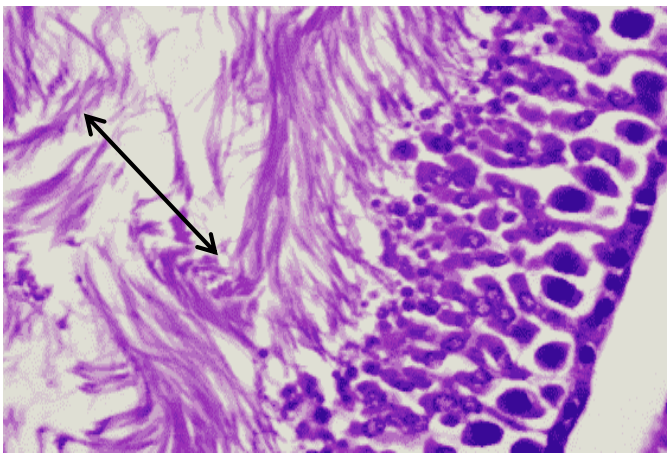


Figure (7) The testes' cross section after treatment with *Borago officinalis* extract shows mature spermatozoa fill the lumen of seminiferous tubule→ with mild vacuolation of adjacent tubule ↔ (H&E, X:400).

Animals treated with 30mg/kg silver nanoparticles.

Histological architecture of testes from this study revealed disarrangement of spermatocyte that express vacuolar degeneration and nuclear pyknosis with evidence of necrotic substance in lumen and subcapsular blood vessels congestion (Fig.9). other section showed apical sloughing and detachment of spermatogenic cells with complete loss of spermatid and Leydig cell atrophy (Fig.10).

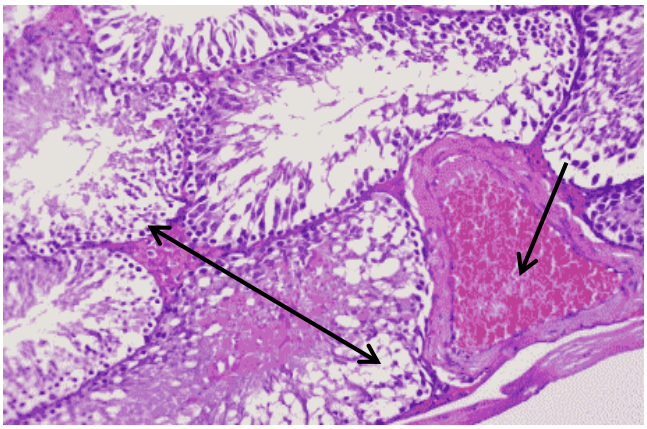


Figure (9) Cross section in the testes treated with AgNPs(30mg/ml) shows massive vacuolation and nuclear pyknosis with necrotic substance in lumen↔ and subcapsular blood vessels congestion→ (H&E, Stain X:10&40).

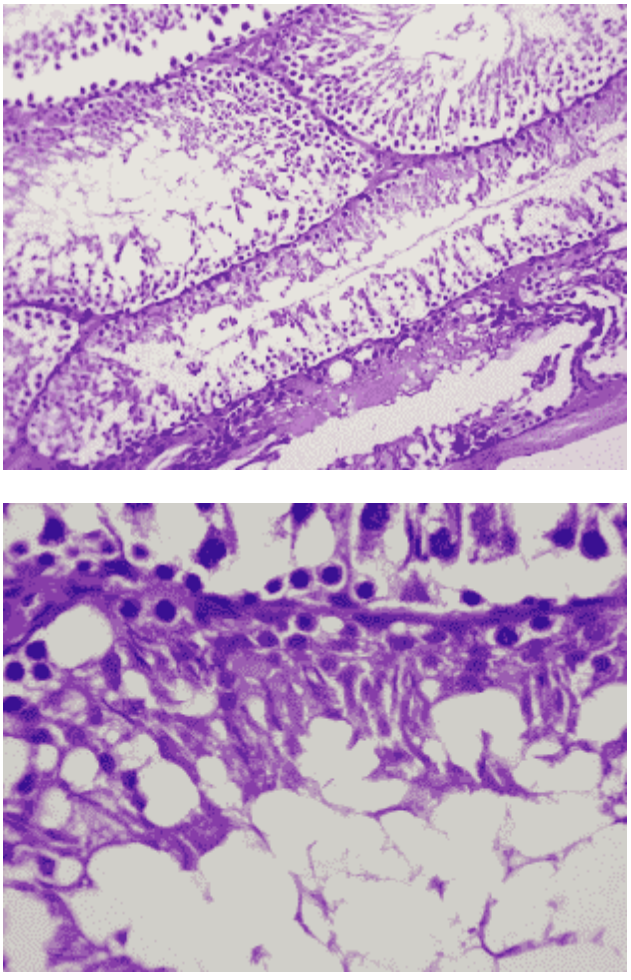


Figure (10) Cross section in the testes treated with AgNPs (30mg/kg) shows apical sloughing and detachment of spermatogenic cells with complete loss of spermatid and Leydig cell atrophy→ (H&E, Stain X:10&40)

Animals treated with 50mg/ kg silver nanoparticles

The individuals of animals that exposed to50 mg/kg of Ag NPs show similar histopathological changes that observed in individuals exposed to 30 mg/kg of Ag NPs. Furthermore, the percentage of vacuolized seminiferous tubules was increased and the testicular manifestation revealed massive vacuolar degeneration of spermatocyte lining with evidence of necrotic cells the lumen and giant cell formation (Fig.13).

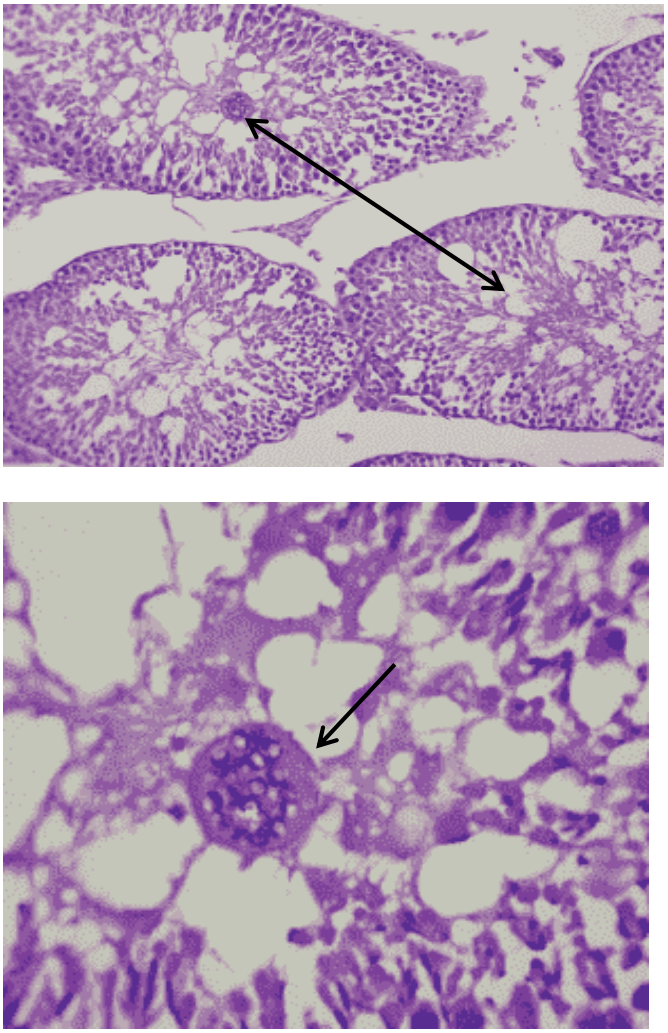


Figure (13) Cross section in the testes treated with AgNPs (50mg/ml) shows massive vacuolar degeneration of spermatocyte lining with necrotic cells in lumen↔ and giant cell formation→ (H&E, stain X :10&40).

DISCUSSION

The molar concentration ratio to generate AgNPs determines the difference in the effectiveness of the concentrations employed which have a significant effect on fluency, and this was linked to the surface plasmon resonance. For particles with diameters less than 100 nm, molecular concentration has a significant impact on size and distribution, which should be kept in mind. Mean diameter and dispersion of particles decrease with increasing concentration. The molar concentration of 1.17 M (0.195 mg/ml) is the best place to look for particles larger than 30 nm, whereas molar concentration of 2.37 M (0.395 mg/ml) is the best place to look for particles smaller than 10 nm

It's clearly from the Figure (3) that generated silver nanoparticles have a high surface plasmon resonance peak. When the conduction electrons in Ag metal interact with NPs's surface resonant light and that lead to peak formation [(14)]. AgNPs production is boosted by using this method. A chemical reduction produces a separate SPR peak at 487 nm. When SPR is used in conjunction with low concentrations, the peak shifts to 474 nm. Size and shape of the NP affect the SPD pinnacle's ability to change wavelength. The absorption peak can be used to estimate the NPs size It is possible that Ag+ binding to the Rosmarinic-diketone moiety is responsible for the appearance of the new band in the spectrum. During the procedure, increased absorbance at 400-490 nm indicated the formation of Ag NPs.

The results of current study that showed which represent normal appearance are constant with **Kubba** [(15)] which found the testis of mice that treated with Borago officinalis aqueous extract showed a recovery status after exposing animals to toxic compound (CCL4). This recovery status may due to ability of the plant extract to combat free radicals generated by many pathological conditions and toxic substances [(16)].

The findings also revealed seminiferous tubule dilatation, which is characterized by an increase in luminal tubule diameter. The seminiferous epithelium of the dilated tubules is delicate and compacted. The increasing volume of seminiferous tubule fluid within the lumen may have contributed to the dilation. Reduced fluid reabsorption in the efferent ducts, efferent duct blockage, decreased fluid emptying from the seminiferous tubules, or increased production of seminiferous tubule fluid by the Sertoli cells can all contribute to the increased fluid [(17)]. In addition to the previously mentioned factors, the high GLA content of Borago, which acts as an anticonvulsant, bronchodilator, and vasodilator, is the most logical explanation for seminiferous tubule dilatation in this group [(18, 19)]. Leydig's cell hyperplasia is characterized histologically by Leydig's cells with greater nucleoli, less smooth endoplasmic reticulum and less lipofuscin. Leydig's cell hyperplasia is hypothesized to be caused by a dysfunctional hypothalamic-pituitary-testicular axis, which leads in continual Leydig's cell stimulation [(20, 21)]. Leydig's cell hyperplasia can develop from elevated serum luteinizing hormone because it stimulates the production of androgens (such as testosterone) in Leydig's cells.

Our result for 30mg /kg is in one line with many studies. **Gromadzka-Ostrowska *et al***. [(22)] refer that, very

little amounts of Ag NPs may be damaging germ cells and that despite the various benefits of Ag NPs. According to studies by **Park *et al.*** [23], animals treated to different doses of Ag NPs for varying lengths of time either orally or by intraperitoneal injection substantially accumulated AgNPs in the testes tissue compared to controls, this accumulation of Ag NPs could be due to pass through blood-testis barrier [24].

Vacuolations (appearance of empty spaces in the seminiferous tubules), that appear in our testis section are smiler to vacuolation singe that found in the studies of **Ahmed *et al*.** [25] and where evaluate the effect of nanoparticles on testes of adult albino rats and they indicate of presence intracellular vacuolations in Sertoli and Leydig cells which are a spermatogenic cells., Vacuolation are considered as the first morphological sign of testicular injury. The presence of vacuoles in the cytoplasm of the Sertoli cell denoted direct damage to this cell and reflected its early response to injury as indicated by **Alam *et al.*** [26], **Nashwa *et al.*** [27]. This vacuolation was explained as a result of the autophagosomes created during the phagocytosis of necrotic germ cells by Sertoli cells. The enlargement and coalescence of intracellular membrane-bound organelles, such as the endoplasmic reticulum, may also be a cause of the vacuolization of Sertoli cells.

Additionally, pyknotic nuclei were showed in some section. pyknosis or karyopyknotic can be define as the nucleus becomes contracted, spheroidal, and filled with condensed chromatin. The irreversible condensation of chromatin, shrinking or disintegration of nuclear membrane and rarefaction of cytoplasm with massive loss of most organelles of a cell. It is followed by karyorrhexis, or the nucleus becomes fragmented and scattered throughout the cell undergoing necrosis or apoptosis Programmed cell death, usually by fragmentation [28, 29]. The damaged Sertoli cells would interfere with the spermatogenic cells' nourishment and maintenance, causing them to disintegrate, go through necrosis, and exfoliate into the lumen of the seminiferous tubule. Pyknosis either the damage to the Sertoli cells or the increased formation of reactive oxygen species (ROS) brought on by the delivery of silver nanoparticles could be the cause [30]. Silver nanoparticles are also known for their capacity to cause the pyknosis of germ cell nuclei due to DNA damage and disintegration [31, 32]. Our research suggests that sloughing of spermatogenic cells, which results in aberrant growth of the elongating spermatids, may be caused by Ag NPs effects on the Sertoli cell's microtubules and intermediate filaments. Also, disruption in the physical interaction of Sertoli - germ cell might have led to the sloughing of the germ cells from the seminiferous epithelium. Before finishing in the lumen of the seminiferous tubule, spermatogenesis goes through a number of stages. Ag-NPs' toxicity may act at these stages to cause the apoptosis of germ cells in the testis, which would result in lower testosterone levels [33]. The loss of spermatogenic cell that noticed in our sections may explained by the ability of Ag-NPs to pass the blood testicular barrier, and travel to the seminiferous tubules, where they might influence sperm production and quality [34-36]. The histopathologic alterations that observed in individual that treated with Ag NPs may related to Sertoli cell disfunctions and Leydig cells inhibiting testosterone synthesis efficiency.

CONCLUSIONS

We can conclude from this study that is evidence that the effects of borage flower crude extract are nontoxic. Silver nanoparticles have been shown to be harmful to testicles. Furthermore, AgNPS have a negative impact on male reproductive system by alteration of testicular functions and many histopathological changes in albino rat testis.

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