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

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ABSTRACT

Background: The evidence has shown that Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes due to their distinctive physical and chemical properties. Despite the advantages of NPs, a number of problems have raised the possibility of toxicity in both people and animals.
Objective: The primary aim of the current study is to assess the impact of AgNPs synthesized by green method from plant extract *Borago officinalis* on reproductive system.
Material and methods: AgNPs synthesized by using *Borago officinalis* flower extracts as reducing agents/stabilizers which be a friendly technique to environment. A total of 40 adult male rats are taken and divided to 4 equal groups: *Group 1* treated with distilled water, *Group 2* received orally only plant extract (5mg/kg), and *Groups 3 and 4* injected through intraperitoneal cavity with AgNPs 30 and 50 mg/kg respectively. We measured oxidative parameters of malondialdehyde (MDA) levels and blood testosterone hormone levels after 35 days.
Results: Our results demonstrated that AgNPs rise significantly the MDA value in the animal groups that treated with different concentrations of AgNPs in comparison to the control groups which treated only with Borage extract. Also, a non-significant decrease of testosterone levels in animals treated with Borage extract in contrast to control group which have a significant decrease in testosterone which treated with different concentrations of AgNPs.
Conclusion: The Borage flower crude extract are nontoxic, where AgNPs (even if it was produced from plant extract) have a negative impact on male reproductive system by alteration the testicular functions especially the testosterone.
Keywords: Silver nanoparticles (AgNPs), Green synthesis, Malondialdehyde (MDA), Testosterone, Experimental studies, Baghdad University.

INTRODUCTION

The male reproductive system can be divided to internal structures the external structures. The generation, storage, and ejaculation of sperm for fertilization as well as the production of critical androgens for male growth are promoted by the well-vascularized structures with numerous glands and ducts found in this system [(1)].

There are two main cell kinds in the testis. The first is called Leydig cells, and they are found in the interstitium of the testis, close to the seminiferous tubules, where they secrete testosterone, a key male androgen [(2)]. Sertoli cells, a second type of cell, are located on the outside edge of seminiferous tubules [(3)] which increase spermatogenesis that begins at the periphery of the tubules. Due to these important functions of the testis, the males have an important role in determining fertility. The larger potential to improve life, the discovery of novel molecules and the manipulation of those naturally present in man-size could be of interest [(4)].

Buraihi *et al.* [(5)] showed that nanoparticles have an extremely small size and high surface area hence their surface has been available for further modification with hydrophobic, hydraulic or any metal to the surrounding environment so they have many applications in biological science [(6)].

These applications such as biomedicine, diagnosing illnesses, gene therapy, as well as drug delivery, catalyst, cosmetics, food production, agriculture, pharmaceuticals, orthopedics, and antimicrobial therapy, etc. These nanoparticles NPs can be synthesis by many ways by using chemical, physical, mechanical and Biosynthesis methods that is an ecofriendly and environmentally beneficial process for making nontoxic and biodegradable nanoparticles [(7)].

This process (Biosynthesis) can be used bacteria, plant, fungi to produce nanoparticles. The silver nanoparticles AgNPs is one of the many nanoparticles that produced by green biosynthesis. AgNPs' impact on human health and the mechanisms underlying their function are poorly known.

To better evaluate the risk to humans, it is crucial to investigate their potential toxicity in live organisms, particularly in mammals. According to numerous studies, AgNPs can increase the generation of reactive oxygen species (ROS), which in turn induces oxidative stress and toxicity in a variety of cell types [(8)].

The primary aim of the current study is to assess the impact of *Borago officinalis L.* flower extracts and different doses of AgNPs synthesized by green method (from the same plant) on testis of male rats by studying the histopathological changes.

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MATERIALS AND METHODS

Experimental Design

A total of 40 male rats were divided into 4 groups randomly (n= 10 per group) of each group and treated as follows:

*Group G1:** Control animals that treated with normal slain three times per week during the entire experiment period.

*Group G2:** Animals that orally administrated with plant extract of Borago officinalis L. (5mg/kg) 3 times per week during the entire experiment period.

*Group G3:** Animal that treated with of AgNPs (30 mg /kg) 3 times per week during the entire experiment period.

*Group G4:** animal that treated with of AgNPs (50 mg /kg)/ 3 times per week during the entire experiment period.

Plant extracts preparation

The dried flower of *Borago officinal L.* was rinsed in running tap water to remove dust. We add 50 grams of dried flowers to 250 mL of deionized water and allowed to sit for 24 hours. An aqueous cold extraction was carried out by using deionized water which is considered as a very effective way for extracting of active ingredients [9]. The yielded solution was filtered firstly through sterile double layer of gauze to remove all large particles, and the second step of filtration was done through a Whatman filter paper. The next step is centrifugation of extract at 5000 rpm for 10 minutes to remove all large particles from the extract solution. The supernatant dispensed in to Petri dishes and incubated at 37-38 °C to facilitate evaporation of extract solution until formation of thick viscous material. The final output of *Borago officinal* flowers was collected from pettier dishes by silicon spatula and the yielded viscous material is kept in dark sealed lid containers and stored in refrigerator [10]. Using plants extract is one of the latest techniques used to synthesize nanoparticles. Many metal nanoparticles were produced by this way. Plant extracts have the ability to function as stabilizing and reducing factors in the creation of nanoparticles [11].

Synthesis of (AgNps) by green method

According to the standard procedure, AgNPs were prepared at two different molar concentrations (1.17 and 2.37) by dissolving 19.53 and 39.57 mg of precursor AgNO3 respectively in 100 ml of deionized water under magnetic stirring for two hours.

Mixed one part plant extracts stock (*B. officinalis*), act as a reducing and capping agents, with one part of AgNO3 solution (for both molar concertation) under magnetic stirrer for 15 min, after that a slight change in color is observed from light brown to dark brown, and this is evidence of the formation of silver nanoparticles. The resulting solution is stored in dark vials and sent for characterization at a later time [12].

Characterization of silver nanoparticles

Transmission Electron Microscopy (TEM) is a kind of microscopy where a beam of electrons is sent through a very thin sample, interacting with the sample as it does so. When electrons are sent through a sample and interact, a picture is created; the image is enlarged and focused onto a layer of photographic film and then exposed to an imaging medium, like a fluorescent screen. A Philips Model CM10 Transmission Electron Microscope was used to measure the AgNPs. A 314-square copper grid with a 3 mm diameter and 200 mesh was utilized with nanoparticles dispersions on carbon-coated TEM copper grid. The mixes were left to air-dry for a period of time.

UV-VIS spectroscopy

As part of optical characteristics, the absorption and transmission spectra of various AgNps samples by using evolving spectrophotometer for UV/Visible radiation (Amersham biosciences firm/Ultrospec 4300 pro). The test ranged from 190 nm to 1100 nm in wavelength.

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

All statistical analyses were performed using **MINITAB 19.1**. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as means and SD, and Analysis of variance (ANOVA) was used for comparison between groups. P value ≤0.05 was considered to be statistically significant.

RESULTS

Transmission electron microscopy (TEM) measurements

The results of this investigation show that the range of AgNPs width was between less than 10 nm and more than 50 nm. By using TEM images this study revealed a uniform size and distribution (**Figure 1 a** and **b**). The structure is also shown as a spherical configuration of AgNPs with numerous twinning planes where is the particles with sizes of 30 nm was the most abundant. The uniformity of the manufactured Ag NPs was tested using an energy-absorbing colloidal solution as illustrated in **Figure 2 a** and **b**.

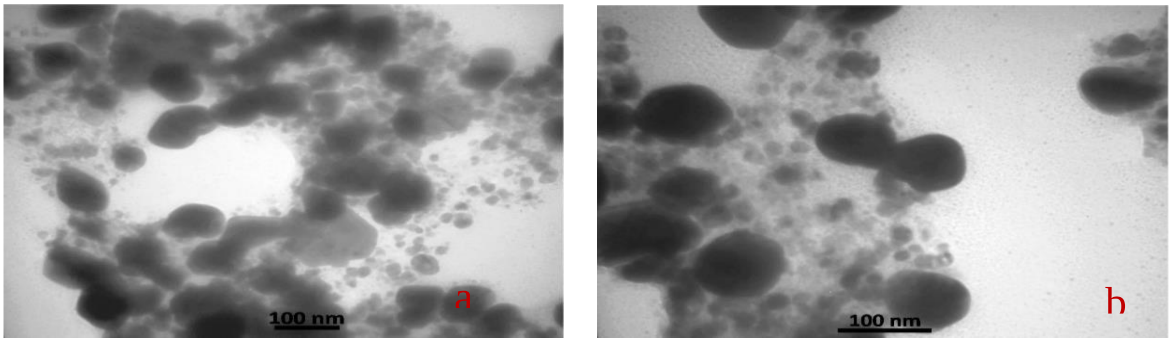

Figure 1. TEM images for Ag NPs with different molar ratio: (a) 1.17mg/ ml, (b) 2.37mg/ml.

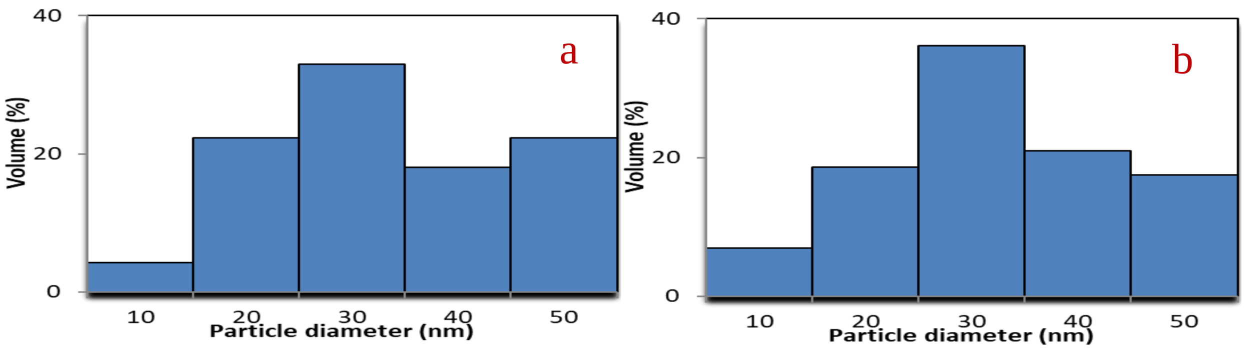

Figure 2. Particle size distributions resulting from the measurements of (200–300) particles of AgNPs for different molar ratio: (a) 1.17 M, (b) 2.37 M.

Optical properties of Ag NPs prepared by green synthesis.

UV-VIS.

The AgNPs absorbance spectra at wavelengths between 200 and 1000 nm are shown in **Figure 3**; the extract's performance increased with increasing extract concentration. When the concentration was high, a peak at 487,406 nm was seen; when the concentration was low, a peak at 474,402 nm was observed.

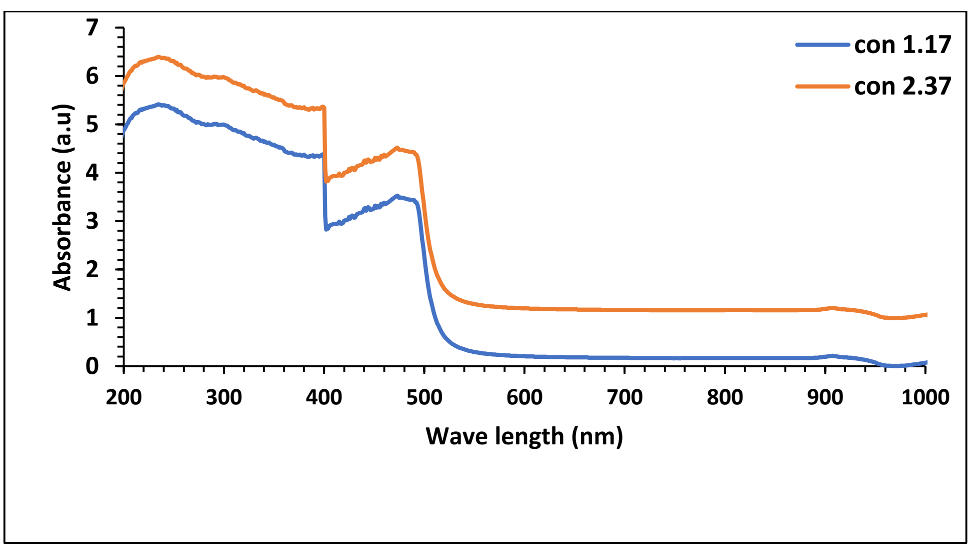

Figure 3. Absorption spectra of Borage extract AgNPs.

Biological Parameters

Testosterone

Figure 4 shows the results of testosterone hormone in experimental animals compared to control group. The results show a non- considerable reduction of testosterone levels in ***Group B*** which was 1.619 (SD 0.296) compared to control group (1.857, SD 0.285). The current study also revealed a significant decrease in testosterone levels (P ≤0.05) in Ag NPs groups animals (30mg\kg and50mg\kg) which were 0.767 (SD 0.166) and 0.141 (SD 0.059), respectively compared to control group animals.

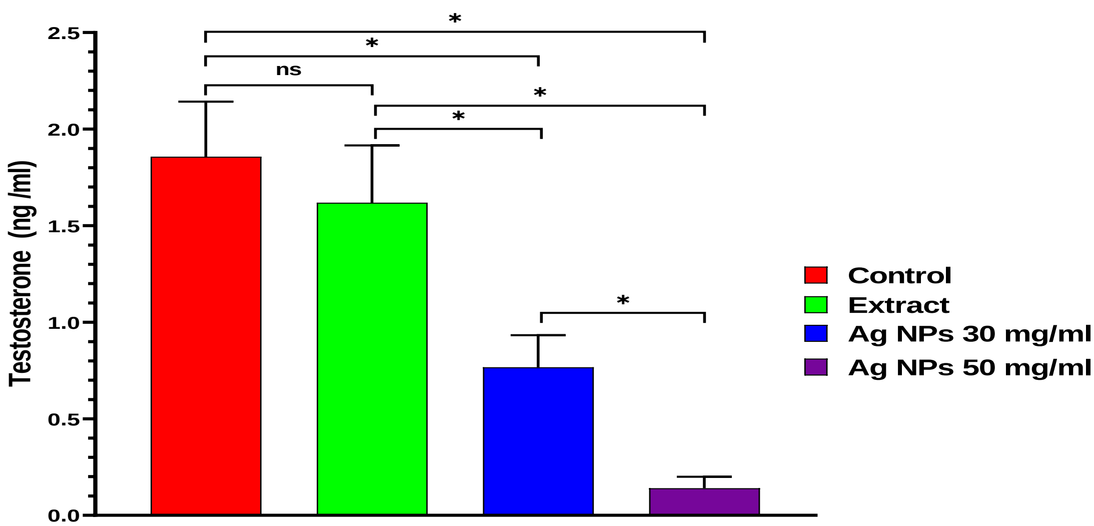

Figure 4. Testosterone hormone levels in different groups [The values were determined to be statistically important at P ≤ 0.05].

Malondialdehyde (MDA)

The results in **Figure 5** demonstrated a significant increase in the mean serum MDA levels (P ≤0.05) in AgNPs (30mg\kg) and (50mg\kg) which were 1.5512 (SD 0.2762) and 91.899 (SD 0.446), respectively compared with the control group (0.944, SD 0.220). While the outcomes demonstrated showed a significant difference in serum MDA levels between the Borage extract treated group and the control group which was 0.676 (SD 0.096).

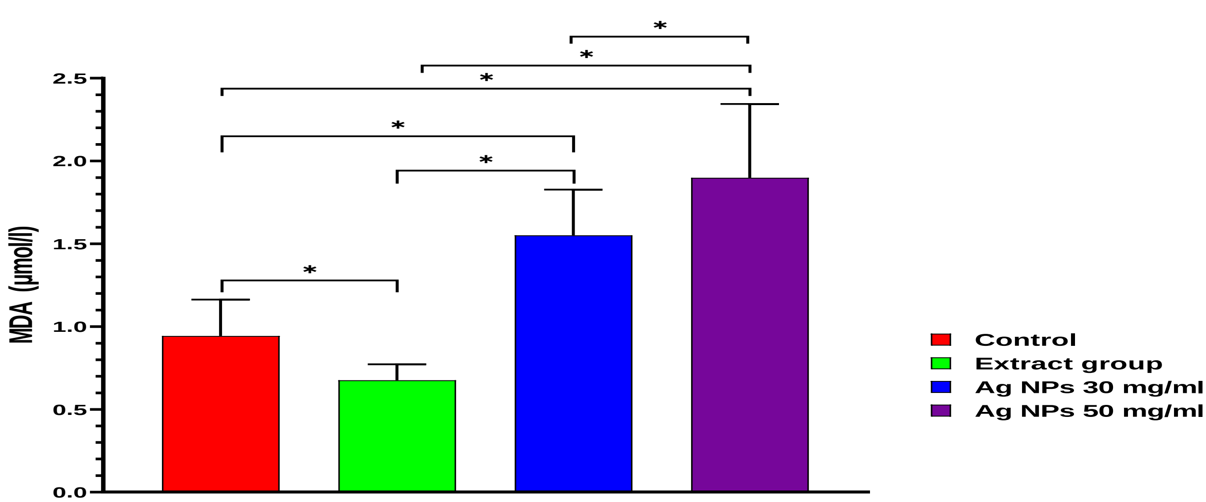

Figure 5. Malondialdehyde hormone levels in different groups [The values were determined to be statistically significant at P ≤0.05].

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 interacts with NPs's surface resonant light and that lead to peak formation (13). 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.

Gamma-linolenic acid (GLA), Oleic acid and Erucic acids are only a few of the many distinct types of fatty acids that *Borago officinalis* is famous (14). One of the crucial fatty acids in this plant, gamma-linolenic acid (GLA) has the ability to reduce cholesterol and have anti-inflammatory, antithrombotic, antiproliferative and lipid-lowering potentials effects. Additionally, GLA regarded as one of the biogenic fatty acids that make up mitochondrial membrane, cell membrane phospholipids and that the enhance fluidity and integrity of the membrane. Linoleic acid is converted to GLA in the human body acid and is thought to be a precursor to prostaglandins PGE. The generation of prostaglandins which limit testosterone production by having a direct impact on testicular interstitial cells may be the cause of the decrease in for testosterone levels.

According **Meikle *et al*** (15) refers that oleic acid acts to reduce the proportion of testosterone synthesis in Leydig cells. The presence of these fatty acids, which are in Borage constituents, may be the reason for the cause of decrease in the testosterone levels in the group of animals that treated with Borage extract.

Our study demonstrated that testosterone levels were a significant reduced in animals treated with Ag NPs (30mg\kg and50mg\kg) decrease in testosterone. The same outcomes were discovered by **Baki *et al*.** (16) and **Dziendzikowska *et al*** (17) who found that administering AgNPs intravenous reduced the number of Leydig cells, changed the concentration of sex hormones in the plasma and testes, and drastically decreased the testosterone levels and dihydrotestosterone inside the testes. Furthermore, **Ferdous and Nemmar** (18) state that AgNPs can accumulate in several organs, quickly overcome biological barriers, and cause toxic stress in a variety of tissues. Furthermore, numerous studies have shown that Ag-NPs have negative effects on reproductive indicators, such as altering the structure of the testes, decreasing the expression of genes involved in spermatogenesis, lowering serum testosterone, increasing apoptosis in germ cells, and interfering with the hormonal regulation of reproductive processes in male rats.

In addition, the majority of nanoparticles raise ROS levels, particularly superoxide, which accelerates the oxidation of molecules like proteins or even DNA (19) and that causes a decline in the number of Leydig cells, which in turn affects the production of testosterone. According to certain research, nanoparticles can affect the expression of the Steroidogenic Acute Regulatory protein (StAR) (20). This protein is a transmit protein that controls the transfer of cholesterol into the inner mitochondrial membrane and improves the steroid hormones' synthesis. **Baki *et al*.** (16) explained that testosterone levels will decline as a result of silver nanoparticles preventing cholesterol transport into the inner mitochondrial membrane by reducing StAR protein expression and, ultimately, halting lipid conversion to steroid hormones.

One of the main causes of aging and etiopathogenesis of many chronic illnesses is oxidative stress (21).

According to **Zamboni (2008)**, functional damage to cells under oxidative stress is caused by improved lipid peroxidation in addition to oxygen free radicals and an imbalanced redox potential (22,23).

Among the many biological targets of oxidative stress, lipids are the class of macromolecules that are most engaged. Numerous secondary chemicals are created during the of lipid oxidation of lipid. Most of these chemicals are aldehydes, which might cause oxidative damage predominate among these substances, and they can increase oxidative damage (24). Because of their longevity and high reactivity, these compounds may interact with biomolecules including nucleic acids and proteins, acting both within and outside of cells and usually causing irreparably damage to the delicate systems required for cell operation. MDA is the most prevalent and researched by-product of polyunsaturated fatty acid peroxidation MDA.

Furthermore, the protective action of Borage which is connected to its role in scavenging free radicals and high amount of linolenic acid may be responsible for decreased MDA levels show in rats treated with Borage extract [25]. This finding is consistent with previous studies [26] which found that Borage one of the most abundant plants that included γ -linolenic acid, where linolenic acid is required for the creation of hormone-like prostaglandins, which control the levels of antioxidants, guard against prooxidants, and regulate certain elements of metabolism. **Komaki *et al*** [27] explained that the presence of polyphenols, flavonoids, and compounds like linolenic acid in Borage flowers extract increases the plant's calming properties.

MDA levels on the other hand a significantly increased in both groups that were treated with silver nanoparticles, these finding are consistent with those of **Adeyemi and Faniyan** [28] who found that administration of silver nanoparticles significantly increases the levels of MDA in serum and tissues. Their finding was depending on the concentration and duration of treatment and they hypothesized that this could be a sign of oxidative stress.

The increase in MDA levels in groups that received silver nanoparticle treatment could be explained by the ability of silver nanoparticles to harm essential cell biomolecules like proteins that are oxidatively modified in three ways; oxidative alteration of a particular amino acid, peptide breakage driven by free radicals, and production of protein cross-linkage as a result of contact with lipid peroxidation products. Lipid peroxidation occurs with the polysaturated fatty acids contained in cell membranes, which is subsequently followed by a radical chain reaction. According to theory, hydroxyl radicals initiate ROS and take hydrogen atoms from lipid radicals to make diene conjugates. In addition, the presence of oxygen generates the peroxyl radical which attacks an additional fatty acid to form lipid hydroperoxide and a fresh radical. By triggering the synthesis of MDA molecule, the generation of intracellular Reactive Oxygen Species (ROS) destroys the lipid membrane of the target cells [29].

Additionally, our findings are consistent with several studies on the impact of silver nanoparticles on various organs, showing a considerable increase in MDA levels in comparison to control groups and a marked decline in total antioxidant levels [30]. They interpret the toxicity that occurs after the delivery of AgNPs as being caused by an excessive production of oxidative-free radicals and related ROS, which can trigger immunological activation, inflammation, and cell death processes like apoptosis.

In conclusion, the effects of borage flower crude extract are nontoxic. AgNPS 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 changes In the Testosterone hormone levels and the serum MDA levels.

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