Anti-human ovarian cancer, cytotoxicity, and antioxidant effects of Nigella Sativa green-formulated Au nanoparticles: Describing a new chemotherapeutic supplement

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Abstract
Introduction
The recent research showed that the Gold nanoparticles (GNPs) formulated with Nigella Sativa aqueous extract having potent antioxidant and anti-human ovarian cancer activities in vitro condition.

Material and methods
For determinate the properties of the GNPs that were produced from the reaction between gold chloride solution with aqueous Nigella Sativa extract, we used UV–Visible Spectroscopy (UV-Vis), Field Emission Scanning Electron Microscopy (FE–SEM), Fourier Transformed Infrared Spectroscopy (FT–IR), and Transmission Electron Microscopy (TEM). For evaluating anti-ovarian cancer and cytotoxicity effects of GNPs, Au chloride, and Nigella Sativa aqueous extract, we used MTT assay.

Results
The result of this test showed that GNPs have no cytotoxicity on normal cell line (HUVEC) and have potent anti-ovarian cancer features dose-dependently against PA-1, SK-OV-3, and SW-626 cell lines. The IC50 of GNPs were 249, 361, and 433 µg/mL against PA-1, SW-626, and SK-OV-3 cell lines, respectively. For evaluating the antioxidant features of GNPs, Au chloride, and Nigella Sativa aqueous extract, we used the DPPH test, in this test butylated hydroxytoluene was a positive control, the results of this test showed that the GNPs have an effective antioxidant feature. In the antioxidant test, the IC50 of GNPs and BHT were 144 and 201 µg/mL, respectively.

Conclusions
 Probably, potent anti-human ovarian cancer activities of GNPs formulated with Nigella Sativa aqueous seed extract because of antioxidant properties. After evaluating the effectiveness of this formulation in clinical trial researches, it can be a good alternative to chemotherapy drugs.
Anti-human ovarian cancer, cytotoxicity, and antioxidant effects of
*Nigella Sativa* green-formulated Au nanoparticles: Describing a new
chemotherapeutic supplement

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This research showed that the Gold nanoparticles (GNPs) formulated with *Nigella Sativa* aqueous extract having potent antioxidant and anti-human ovarian cancer activities *in vitro* condition. For determinate the properties of the GNPs that were produced from the reaction between gold chloride solution with aqueous *Nigella Sativa* extract, we used UV–Visible Spectroscopy (UV-Vis), Field Emission Scanning Electron Microscopy (FE-SEM), Fourier Transformed Infrared Spectroscopy (FT-IR), and Transmission Electron Microscopy (TEM). For evaluating anti-ovarian cancer and cytotoxicity effects of GNPs, Au chloride, and *Nigella Sativa* aqueous extract, we used MTT assay. The result of this test showed that GNPs have no cytotoxicity on normal cell line (HUVEC) and have potent anti-ovarian cancer features dose-dependently against PA-1, SK-OV-3, and SW-626 cell lines. The IC50 of GNPs were 249, 361, and 433 µg/mL against PA-1, SW-626, and SK-OV-3 cell lines, respectively. For evaluating the antioxidant features of GNPs, Au chloride, and *Nigella Sativa* aqueous extract, we used the DPPH test, in this test butylated hydroxytoluene was a positive control, the results of this test showed that the GNPs have an effective antioxidant feature. In the antioxidant test, the IC50 of GNPs and BHT were 144 and 201 µg/mL, respectively. Probably, potent anti-human ovarian cancer activities of GNPs formulated with *Nigella Sativa* aqueous seed extract because of antioxidant properties. After evaluating the effectiveness of this formulation in clinical trial researches, it can be a good alternative to chemotherapy drugs.

**KEYWORDS** Gold nanoparticles; *Nigella Sativa* seeds; anti-human ovarian cancer; antioxidant; cytotoxicity.
1. Introduction

*Nigella Sativa* is an ethnomedicinal plant with several pharmacological features. It belongs to the *Plantae* kingdom, *Ranunculales* order, *Ranunculaceae* family, and *Nigella* genus. *Nigella Sativa* is an annual plant with delicate flowers that are usually yellow, white, pink, pale blue, or purple with 5 to 10 petals. The height of the plant reaches 20 to 90 cm. *Nigella Sativa* fruit has a large capsule with numerous seeds in it [1-3]. People have consumed *Nigella Sativa* to control and treat liver function, gastrointestinal, respiratory, cardiovascular, immune, and urinary systems diseases [4-12]. The seeds of *Nigella Sativa* and its products such as seeds tincture and roasted black seeds have been used to treat asthma, diarrhea, rheumatism, bronchitis, dropsy, loss of appetite, treatment of indigestion, dysmenorrhoea, amenorrhoea, treatment of skin eruptions and worms and, antiemetic. [4,13-15]. Chemical composition that isolated from *Nigella Sativa* are as fallow: thymoquinone, dithymoquinone, t-anethol, thymohydroquinone, dihydroxy-28-methy-lolean-12-enoate, p-cymene, 4-terpineol, α-pinene, thymol, carvacrol, thymol, sesquiterpene longifolene, nigellicine, nigellicimineN-oxide, nigellicimine, nigellidine, alpha-hederin, saponin, citronellol, carvone, limonene, protein, fat, carbohydrates, fiber, Cu, Zn, Fe, P, carotene, vitamin A, avenasterol-5-ene, oleic acid, dihomolinoleic acid, eicodadienoic acid, α-sitosterol, linoleic acid, campesterol, saponin, stigma-5, β-amyrin, obtusifoliol, β-amyrin, cycloartenol, aliphatic alcohol, terpenoids, melanthin, tannin, tirucallol, niggellone gramisterol, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16, citrostadienol, cycloart-23-methyl-7, Ns-D1, Ns-D2, etc [16-20]. Researches have shown that *Nigella Sativa* has many treatment properties such as antibacterial, antifungal, anticonvulsant and, neuro-pharmacological, testicular-protective, anti-asthmatic, pulmonary-protective, nephroprotective, hepato-protective,
gastro-protective, cardiovascular, immunomodulatory activity, analgesic and anti-inflammatory, anticancer, antidiabetic, antioxidant, and anti-schistosomiasis effects \[2, 27-38\].

Ovarian cancer is prevalent in all of the world. Predisposing factors for ovarian cancer are as fallow: obesity and overweight, gynecologic surgery, hormone therapy, breast cancer, age, family history, reproductive history, human, talcum powder, and papillomavirus \[39,40\]. Initially, ovarian cancer developed with the growth of an abnormal cell and then propagation to the whole uterus and all parts of the body \[40\]. Ovarian cancer has symptoms such as lethargy, weight loss, dyspnea, nausea, pain or pressure in the pelvis, backache, early satiety, abdominal pain, constipation, unexpected vaginal bleeding, urinary frequency, bloating and dyspnea \[41\]. Diagnosis of ovarian cancer base of these tests blood tests, biopsy, laparoscopy, and imaging examination \[42\]. A great number of doctors use chemotherapy, immunotherapy, and radiation therapy to treat ovarian cancer \[43\]. Chemotherapeutic drugs have a bad effect on the body, so today the formulation of an effective chemotherapy drug from GNP\(s\) is important \[44,45\].

Nanotechnology is the field to yield modern systems, tools, and materials by taking control at the atomic and molecular levels using the features that appear on those surfaces. Applications for nanotechnology in medical diagnostics, food, medicine, biotechnology, environment, energy, chemistry, physics, etc, introduce this technology as an interdisciplinary and cross-sectoral context. The interdisciplinary nature of nanoscience and nanotechnology as the field to yield modern systems, tools, and materials with precision atoms and molecules, will sooner or later affect the health and medical sector \[44,45\]. Drug use is currently volumetric, so most cells in the body need medication. In the new method, the drug is directed directly to specific cells with new injection devices and delivered to the required location. By this mechanism, small and large diseases can be diagnosed and treated at the beginning of their formation \[44-46\]. The
National Nanotechnology Project is being implemented in European countries, the United States and Japan with high priority in various fields. Nanotechnology and nanoscience emerging fields can move materials very accurately, to understand and control unprecedented fundamental components of physical objects. It seems that these developments will change the way we design and build everything from vaccines to computers. The plan would increase investment in nanotechnology about twice as much each year as last year. A branch of nanotechnology is the formulation of new drugs with metal nanoparticles [45,46]. Today, nanoparticles have become very popular due to their wide applications in biology, medicine and medicine. Structurally, their size is in the range of 100 nanometers. Several drugs such as small hydrophobic and hydrophilic drugs, molecules, and vaccines of biological nanoparticles can be administered by these nanoparticles. They are widely used in improving the treatment and diagnosis of diseases. Nanoparticles in nanoliposomes, carbon nanotubes, nanofibers, nanospheres have been widely used for drug carriers and in the manufacture of cell scaffolds [44-46]. Applications of nanoparticles in drug delivery include drug carriers in diseases such as cancer, cardiovascular disease, and Alzheimer's. The use of these nanocarriers is very effective for neurological diseases such as Alzheimer's. Due to their size, these nanoparticles can cross the blood-brain barrier, which has always been a barrier to the passage of drugs to the affected area in this type of destructive brain disease. Due to their small size, nanoparticles can also be used in brain cancers [46]. The goal in making nanoparticles is to control the surface properties, particle size, and release of a specific and efficient drug in a specific place and time for the drug to be as effective as possible. Nanoparticles are widely used in tissue engineering scaffolds, targeted drug delivery and disease diagnosis. At present, many drug delivery systems are made of nanoparticles and different materials have been used as drug stimulants or enhancers to
ameliorate the effectiveness of treatment and the durability and stability as well as the safety of anticancer drugs [45]. The substances used to release cancer drugs are divided into different polymers, magnetic, and biomolecules. These materials can also provide surface modifications such as binding to target antibodies and ligands to make the nanoparticles act purposefully to increase the effectiveness of the treatment [44-46].

GNPs as famous metallic nanoparticles with Pharmaceutic properties recently have been used for the treatment of some kind of cancers and tumors [44-46]. The results of a study showed that GNPs have anti-acute myeloid leukemia features in the cellular and molecular state, GNPs widely killed all malignant leukemia cells (32D-FLT3-ITD, Human HL-60/vcr, and Murine C1498) in the nano concentrations [46]. So far, there have been no reports of anti-human ovarian cancer properties of GNPs green-synthesized by herbs. However, several herbal medicines such as Turmeric [Curcuma longa], Quercetin [Quercus tinctoria], Genistein, Mayapple [Podophyllum peltatum], Shatavari [Asparagus racemosus], Camptotheca [Camptotheca acuminate], Ginkgo biloba, Lodhra [Symplocus racemosa], Pacific yew [Taxus brevifolia], Garlic [Allium sativum], Ashoka [Saraca indica], Green tea [Camellia sinensis], and Neem [Azadirachta indica] were used in ancient times to cure ovarian cancer [47]. In the current research, the properties of GNPs formulated by Nigella Sativa aqueous extract against common human ovarian cancer cell lines i.e., SK-OV-3, SW-626, and PA-1 were evaluated.

2. Material and Methods

2.1. Material
Bovine serum, antimycotic antibiotic solution, 2,2-diphenyl-1- pirkilhydrazil (DPPH), dimethyl sulfoxide (DMSO), decamplmaneh fetal, 4- (Dimethylamino) benzaldehyde, hydrolyzate, Ehrlich solution, and borax-sulfuric acid mixture, Dulbazolic mixture Modified Eagle Medium (DMED), all were afforded from the US Sigma-Aldrich company.

2.2. Synthesis of GNP containing Nigella sativa seed aqueous extract

To produce a green synthesis of GNP first stage is Extraction from Nigella sativa. The method that we used for achieving this purpose was extracted Nigella sativa seed with distilled water in the microwave. Generally, the method that we used for the synthesis gold nanoparticles containing Nigella sativa seeds extract are as follows in 50 mL of distilled water we dissolved 1.5 g of NaOH pellets and 100 mL of HAuCl₄×H₂O (1mM) and followed by this act, added 20 mL of Nigella sativa seeds extract and then stirred for 1 h at 25 °C. After this process, the solution color changed to the dark yellow color that indicated the formation of GNP. Finally, the solution was let to precipitate, then the precipitate was filtered and washed with ethanol, acetone, and distilled water. The final precipitate was dried for 14 hours at 90 °C to provide the best GNP powder [46].

2.3. Chemical characterization of GNP containing Nigella Sativa aqueous extract

Several techniques were used to indicate the characterizations of GNP are as follows: TEM, UV-Vis, and FT-IR.
The TEM is a method by which the size and shape of nanoparticles were determined.

UV-Vis spectroscopy analysis is a method by which the characteristic absorption bands of gold were evaluated.

FT-IR (Shimadzu IR affinity.1) was used to identify the potential biomolecules in the Nigella Sativa aqueous extract that participate in the reduction of gold nanoparticles.

2.4. Antioxidant activities of GNPs containing Nigella sativa seed aqueous extract

The free radical scavenging test was first performed by Blois in 1958, and after some modification by numerous studies in its current form. DPPH method is one of the most widely used methods for estimating antioxidant content. DPPH is a stable radical that reacts with hydrogen atom compounds. This test is based on the inhibition of DPPH, which causes the decolorization of DPPH solution by adding radical species or antioxidants. DPPH changes color from purple to yellow by taking an electron from the antioxidant compound. The free radicals in DPPH are adsorbed at 517 nm, which follows Beer Lambert's law, and decreased absorption is linearly related to the amount of antioxidants; the higher the amount of antioxidants, the more DPPH is consumed and the more purple turns yellow [46].

This experiment was performed with few changes in the method of Lu et al [46]. 0.5 ml of 0.1 mM DPPH solution prepared in 95% ethanol was mixed with 100 μl of Nigella sativa seed aqueous extract, GNPs, and gold salt at the concentrations of the 0-1000 µg/ml. The resulting solution was
kept in the dark at 38°C for 31 minutes. The absorbance of the samples was then read at 518 nm [46].

To compare the activity of *Nigella sativa* seed aqueous extract, GNPs, and gold salt, standard BHT compound was used as a standard antioxidant [46].

To determine the amount of IC50 (IC50 is defined as the concentration required to inhibit 50% of the antioxidant activity) for *Nigella sativa* seed aqueous extract, GNPs, and gold salt, experiments were performed at eleven different concentrations of the desired nanoparticle solution and BHT. Each experiment was performed in three shifts and the mean values were calculated [46].

Percentage of radicalization activity was calculated through the following equation [46]:

\[
\text{Inhibition} (\%) = \frac{\text{Sample A.}}{\text{Control A.}} \times 100
\]

In this regard, the blank adsorption indicates the adsorption of the control solution, which contains 0.5 ml of DMPH solution and 100 μl of 95% ethanol instead of *Nigella sativa* seed aqueous extract, GNPs, and gold salt solutions and adsorption of the reaction indicates the adsorption of the solution content of the *Nigella sativa* seed aqueous extract, GNPs, and gold salt samples [46].

2.5. *Anti-human ovarian cancer properties of GNPs containing Nigella sativa seed aqueous extract*
In this research, we used the following cell lines to evaluating anti-human ovarian cancer and cytotoxicity effects of *Nigella sativa* seed aqueous extract, GNPs, and gold salt using an MTT method.

a) Human ovarian cancer cell lines: PA-1 (ATCC CRL-1572), SW 626 (ATCC HTB-78), SK-OV-3 (ATCC HTB-77): A panel of 3 ovarian cancer cell lines with varying degrees of genetic complexity. They have genomic mutations in one or more of the following genes according to the Sanger COSMIC database: APC, CDKN2A, FAM123B, KRAS, MLH1, NRAS, PIK3CA, STK11, and TP53.

b) Normal cell line: HUVEC.

Each cell line was placed separately in T25 flasks with a complete culture medium (including DMEM (Dulbecco's Modified Eagle Medium, 10% complementary bovine fetal serum, and 1% penicillin-streptomycin solution) and at 37°C in the incubator, cell culture was incubated with 5% CO₂.

After obtaining 80% cell density, the sample was exposed to 1% trypsin-EDTA solution and after 3 minutes of incubation at 37°C in a cell culture incubator with 5% CO₂ and observation of cells removed from the bottom of the plate, the sample was centrifuged at 5000 rpm for 5 minutes and then the cell precipitate was decrypted by adding trypsin culture medium. Then, the cell suspensions after adding trypan blue dye were counted by neobar slide and cytotoxicity test was performed by MTT method. For this purpose, in each well of 98 cell culture plate, 10000 cells
were introduced with 200 µL from the complete cell culture medium and to achieve the cell monolayer density, the plate was re-exposed to 5% CO₂ at 37°C. After reaching 80% cell growth, the culture medium was removed and the cell surface was first washed with PBS buffer, again, in all wells, a complete two-concentration culture medium of 100 µl was introduced and 100 µl of solutions of *Nigella sativa* seed aqueous extract, GNPs, and gold salt at the concentrations of the 0-1000 µg/ml dissolved in PBS was introduced into well No. 1. After mixing the nanoparticles in the culture medium, 100 µl of it was removed and added to the second well. In the next step, 100 µl of the second well was removed after stirring the medium and added to well 3. This operation was performed up to well 11 and thus the amount of nanoparticles in each well was halved, respectively. Well No. 12 contained only one cell and complete culture medium of one concentration and remained as a control. The plate was again exposed to 5% CO₂ at 37°C for 24 hours and after 24 hours the cytotoxicity was determined using tetrazolium dye. 10 µl of tetrazolium dye (5 mg/ml) was added to all wells, including the control, and the plate was exposed to 5% CO₂ at 37°C for 2 hours. The dye was then removed from the wells and 100 µl of DMSO (Dimethyl sulfoxide) was added to the wells, the plate was wrapped in aluminum foil and shaken thoroughly in a shaker for 20 minutes. Finally, cell survival was recorded in ELISA reader at 540 nm [46]:

\[
\text{Cell viability (\%)} = \frac{\text{Sample A.}}{\text{Control A.}} \times 100
\]
Then, based on the absorption rate of each well and its comparison with the control, the inhibitory concentration of 50% (IC50) was obtained [46].

After collecting data, Minitab statistical software was used for statistical analysis. Evaluation of antioxidant results in a completely randomized design and comparison of means was Duncan post-hoc test with a maximum error of 5%. To measure the percentage of cell survival in factorial experiments with the original design of completely randomized blocks and compare the means, Duncan post-hoc test with a maximum error of 5% was used. The 50% cytotoxicity (IC50) and 50% free radical scavenging (IC50) was estimated with ED50 plus software (INER, V: 1.0). Measurements were reported as mean ± standard deviation.

2.6. Statistical analysis

The data were gathered and given into the “SPSS-24” a computer software program and was analyzed by “one-way ANOVA”, and then Duncan posthoc test (p≤0.01).

3. Results and Discussion

Cancer is now one of the leading causes of death worldwide. Existing treatments have not been able to meet the treatment needs for various types of cancer. Therefore, the use of new technologies
in the prevention and treatment of cancer can be helpful. Extensive research on nanoparticles has been conducted in recent years [44]. The advent of nanotechnology has had a profound effect on many areas of healthcare and scientific research. Common cancer treatments, including chemotherapy, radiation and surgery, may reduce the size of the tumor, but the effect of these methods is transient and has no positive effect on patient survival. Therefore, replacing more effective, more specific therapies with fewer side effects with higher anti-cancer activity is a dominant issue in clinical oncology [44,45].

The gradual maturation of nanotechnology has been considered not only for treating cancer but also for a wide variety of applications, especially for drug delivery and diagnostic and imaging cases. There are many types of nanoparticles available and choosing the right carriers according to demand is a key issue [46]. Nanoparticles are very close in size to biological molecules in terms of size and can easily penetrate into the cell, for this reason, one of the goals of nanotechnology is to mount molecules and drugs on nanoparticles and transfer them to the target cell [44-46]. It is also possible to create different surface properties for nanoparticles by attaching protective ligands to increase the nanoparticles' resistance to the immune system and increase their presence in the bloodstream, and even binding ligands to specifically bind the nanoparticles to the target tissue [44,46].
In this experiment, we formulated GNPs by using of *Nigella sativa* seed aqueous extract. Furthermore, *in vitro* condition, we evaluated the anti-human ovarian cancer activities of GNPs against common human ovarian cancer.

3.1. **FE-SEM analysis of GNPs containing Nigella sativa seed aqueous extract**

FE-SEM analysis is a method for assessment of the size and morphology of materials such as GNPs and is extensively utilized in chemistry, physics, and biology investigates. In this study, the FE-SEM image of GNPs containing *Nigella sativa* seed aqueous extract is shown in Figure 1. In this figure, the GNPs are spherical, 15–20 nm in size that closes together. Few GNPs seem to shape the agglomerated structure, which is caused by the hydroxyl group.

3.2. **TEM analysis of GNPs containing Nigella sativa seed aqueous extract**

TEM is a method for evaluating the size, shape, and distribution of materials. Figure 2 shows that the GNPs containing *Nigella sativa* seed extract are spherical and about 15–20 nm in sizes, furthermore a small number of them can be seen in other forms.

4.1. **UV-visible spectroscopy of GNPs containing Nigella sativa seed aqueous extract**
Figure 3 reveals the UV-Vis spectra analysis from GNPs containing Nigella sativa seed aqueous extract. In this study UV–Vis spectroscopic analysis indicated that an absorption peak at 519 nm, in this peak the formation of GNPs is approved, thanks to some researches that have been done the wavelength range for GNPs formation from pharmaceutic plants have been determined between 510 -550 nm [46].

4.2. FT-IR spectrum analysis of GNPs containing Nigella sativa seed aqueous extract

We used the FT-IR spectrum to determine potential biomolecules in the Nigella sativa seed aqueous extract which are responsible for reducing GNPs. Figure 4 shows the FT-IR spectrum of GNPs containing Nigella sativa seed aqueous extract in the wavenumber of 400-4000 cm\(^{-1}\). In figure 5, wavenumber 3396 is due to hydroxyl and phenolic groups. The bands at 2999, 1339-1602, 1057, and 531 cm\(^{-1}\) are referred to as asymmetric CH\(_2\) stretching, aromatic rings, C-O stretching, and Au-O respectively.

3.5. Cytotoxicity and anti-ovarian cancer activities of GNPs containing Nigella sativa seed aqueous extract

One of the cytotoxicity test methods to measure the rate of cell death is the MTT method, which is based on the formation of formazan dye by reducing the substance MTT (dimethyl thiazole 2

Preprint
and 5 diphenyltetrazolium bromide) or other tetrazolium salts [46]. By breaking the MTT tetrazolium ring by mitochondrial enzymes in living cells, insoluble purple formazan crystals are formed. The formation of these crystals indicates the activity of respiratory chain enzymes and is a measure of cell viability. By measuring the amount of absorption by spectrophotometer at specific wavelengths, the number of living cells can be determined. This test is performed according to ISO 10993-5 and its purpose is in vitro evaluation of cytotoxicity. Cytotoxicity test is performed according to ISO10993-5 standard and in three ways: NRU test, CFU test, MTT test and XTT test. The most common method for assessing cytotoxicity is to measure cell survival by MTT [46]. The basis of MTT method is based on the intensity of dye produced by the mitochondrial activity of cells, that measured at a wavelength of 540 to 630 nm and directly proportional to the number of living cells, the increase or decrease in the number of living cells is linearly related to the activity of cell mitochondria. MTT tetrazolium dye is revived in active (metabolically) cells. Mitochondrial dehydrogenases in living cells produce NADH and NADPH, leading to an insoluble purple precipitate called formazan. This precipitate can be dissolved by isopropanol or dimethyl sulfoxide [46]. Dead cells, on the other hand, are unable to perform this conversion due to the inactivity of their mitochondria and therefore do not show a signal. In this method, dye formation is used as a marker for the presence of living cells. In recent years, MTT testing has been the most important measurement method to evaluate the toxicity and anti-cancer effects of metal nanoparticles [46].
In this investigation, the treated cells with different concentrations of the present HAuCl₄, *Nigella sativa* seed aqueous extract, and GNPｓ were assessed by MTT assay for 48h about the cytotoxicity properties on normal (HUVEC) and ovarian malignancy cell lines i.e. SW-626, SK-OV-3, and PA-1. The absorbance rate was evaluated at 570 nm, which represented viability on normal cell line (HUVEC) even up to 1000μg/mL for HAuCl₄, *Nigella sativa* seed aqueous extract, and GNPｓ (Figure 5; Table 1). The viability of malignant ovarian cell line reduced dose-dependently in the presence of HAuCl₄, *Nigella sativa* seed aqueous extract, and GNPｓ. The IC50 of gold nanoparticles (GNPｓ) were 249, 361, and 433 μg/mL against PA-1, SW-626, and SK-OV-3 cell lines, respectively (Figure 5; Table 1). Metallic nanoparticles have various parameters such as shape, size, texture, etc. Sizes have very significant properties in the therapeutic effects of nanoparticles, some studies reported that small metallic nanoparticles have better entrance into cells and have significant anti-cancer activities. It has been assessed that particle size lower than 50 nm showed an excellent remedial feature in the corresponding cancer cell lines [49-53]. As you see in Figures 1 and 2 of our study, the average size of GNPｓ. synthesized by *Nigella sativa* seed aqueous extract is 17.5 nm. Gold nanoparticles have been utilized to treat various cancers including Lewis lung carcinoma, human glioma, human lung cancer, uterus cancer, lung epithelial cancer, colon cancer, and mammary carcinoma.

### 3.6. Antioxidant features of GNPｓ containing *Nigella sativa* seed aqueous extract
Oxidative stress is caused by an imbalance between the production of free radicals and metabolic reactions, which leads to damage to lipids, proteins and nucleic acids. These damages may be due to low levels of antioxidants or an excessive increase in the production of free radicals in the body [54,55]. In humans, oxidative stress is associated with chronic diseases such as diabetes and cancer. Therefore, the production of synthetic and natural antioxidants is necessary to prevent oxidative stress and its destructive effects. Antioxidants effectively and in various ways reduce the harmful effects of free radicals in the biological and food systems and cause detoxification [54-56]. In this regard, green nanoparticles can be used (using plant substrates to prepare nanomaterials that are environmentally friendly and do not contain any harmful chemicals) that show antioxidant properties. At present, the use of non-toxic substances in synthesizing nanoparticles to prevent biological hazards, especially in medical and pharmaceutical applications is considered [33-38]. Many researchers have focused on bioactive substances derived from plants or other sources such as bacteria, fungi and yeast for synthesizing nanoparticles. The green synthesis method is thought to increase the biocompatibility and performance of metal nanoparticles for biological applications due to removing harmful chemicals [56,57]. During the bioproduction stages of nanoparticles, their extracellular production using plants or their extracts is more beneficial and their production can be adjusted in a controlled way based on size, distribution and shape for different purposes [54-57].
The scavenging capacity of *Nigella sativa* seed aqueous extract green-synthesized gold nanoparticles and BHT at different concentrations expressed as percentage inhibition has been indicated in Fig. 6.

In the antioxidant test, the IC50 of GNPs and BHT were 144 and 201 µg/mL, respectively (Table 2).

Studies have shown that the antioxidant features of GNPs green-synthesized by pharmaceutical herbs are more significant than other metal nanoparticles. So far considerable antioxidant activities of GNPs green-synthesized by several pharmaceutic herbs like *Gundelia tournefortii* L., *Allium noeanum* Reut. ex Regel, *Falcaria vulgaris*, *Thymus vulgaris*, and *Camellia sinensis* have been confirmed [46]. GNPs green-synthesized by pharmaceutic herbs show noticeable antioxidant activities against free radicals formation in the living system [45,46]. The GNPs green-synthesized-formulated have important redox activities and have a noticeable role in free radicals dismantle [46].

Forgoing researches have revealed that phenolic and flavonoids compounds appended to the metallic nanoparticles have important antioxidant activities [46].

As was mention in the introduction *Nigella sativa* contains antioxidant compounds including thymoquinone, dithymoquinone, t-anethol, thymohydroquinone, dihydroxy-28-methy-lolean-12-enoate, p-cymene, 4-terpineol, α-pinene, thymol, carvacrol, thymol, sesquiterpene longifolene,
nigellicine, nigellicimineN-oxide, nigellicimine, nigellidine, alpha-hederin, saponin, citronellol, carvone, limonene, carotene, vitamin A, avenasterol-5-ene, oleic acid, dihomolinoleic acid, eicodadienoic acid, α-sitosterol, linoleic acid, campesterol, saponin, stigma-5, β-amyrin, obtusifoliol, β-amyrin, cycloartenol, aliphatic alcohol, terpenoids, melanthin, tannin, tirucallol, nigellone gramisterol, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16, citrostadienol, cycloart-23-methyl-7, Ns-D1, Ns-D2. It seems that the anti-human ovarian cancer effect of recent nanoparticles is due to their antioxidant compounds. Because tumor progression is so closely linked to inflammation and oxidative stress, a compound with anti-inflammatory or antioxidant properties can be an anticarcinogenic agent [54,55].

Many nanoparticles have pharmacological and biochemical properties, including antioxidant and anti-inflammatory properties, which appear to be involved in anticarcinogenic and antimutagenic activities [55,56]. Today, nanoparticles synthesized by biological methods play a vital role in treating many diseases, including cancer [55-57]. Nanoparticles synthesized by biological methods are no longer the only ones in traditional medicine, in addition, they have been able to adopt an industrial line of natural products for treating various cancers. Various cell lines from cancers of the prostate, ovary, lung, liver, and pancreas have been treated with metallic nanoparticles [56-58].
4. Conclusion

In this research, the GNPs were attained from the reaction between HAuCl₄ and Nigella sativa seed aqueous extract in vitro condition. TEM, FE-SEM, UV–Vis, and FT-IR methods were utilized to evaluate nanoparticle characteristics. The results of these techniques revealed that GNPs had been synthesized in the best way. Based on the FT-IR spectrum the presence of a great number of antioxidant compounds produced appropriate conditions for the reduction of gold. In the TEM technique, the mean size of gold nanoparticles (GNPs) was assessed to be 17.5 nm, which is favorable. The GNPs showed the best antioxidant activities against DPPH. In the antioxidant test, the IC₅₀ of GNPs and BHT were 144 and 201 µg/mL, respectively. GNPs had appropriate anti-ovarian cancer activities dose-dependently against SK-OV-3, SW-626, and PA-1 cell lines without any cytotoxicity on the normal cell line (HUVEC). The IC₅₀ of GNPs were 249, 361, and 433 µg/mL against PA-1, SW-626, and SK-OV-3 cell lines, respectively. After clinical study GNPs containing Nigella sativa seed aqueous extract can be utilized as an efficient drug in the treatment of ovarian cancer in humans.

Conflict of Interest

The authors declare that there is no conflict of interest.
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References


[36] Harzallah HJ, Grayaa R, Kharoubi W, Maaloul A, Hammami M, Mahjoub T. Thymoquinone, the Nigella sativa bioactive compound, prevents circulatory oxidative stress caused by 1,2-dimethylhydrazine in erythrocyte during colon postinitiation


Table 1. The IC50 of gold nanoparticles in the anti-human ovarian cancer test.

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<thead>
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<th>PA-1</th>
<th>SW-626</th>
<th>SK-OV-3</th>
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<tbody>
<tr>
<td>IC50 (µg/mL)</td>
<td>249</td>
<td>361</td>
<td>433</td>
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Table 2. The IC50 of gold nanoparticles and BHT in the antioxidant test.

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<th>Gold nanoparticles</th>
<th>BHT</th>
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<tbody>
<tr>
<td>IC50 (µg/mL)</td>
<td>144</td>
<td>277</td>
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</table>
Figure 1. FE-SEM image of GNPs green-synthesized by *Nigella sativa* seeds.
Figure 2. TEM image of Au nanoparticles green-synthesized by *Nigella sativa* seeds.
Figure 3. UV-Vis spectra of Au nanoparticles green-synthesized by *Nigella sativa* seeds.
Figure 4. FT-IR analysis of Au nanoparticles green-synthesized by Nigella sativa seeds.
Figure 5. The anti-human ovarian cancer properties (Cell viability (%)) of gold nanoparticles (Concentrations of 0-1000 μg/mL) against normal (HUVEC: A) and human ovarian cancer (PA-1 (A), SW-626 (B), and SK-OV-3 (C)) cell lines.

The numbers indicate the percents of cell viability in the concentrations of 0-1000 μg/mL of gold nanoparticles against several human ovarian cancer cell lines.
Figure 6. The antioxidant properties of gold nanoparticles (A) and BHT (B) against DPPH.

The numbers indicate the percents of free radical (DPPH) inhibition in the concentrations of 0-1000 μg/mL of gold nanoparticles (A) and BHT (B).