Formulation of a novel anti-human bone tumor supplement by silver nanoparticles green-synthesized using Nigella sativa leaf aqueous extract

**Type**
Research paper

**Keywords**
Silver nanoparticles, chemotherapeutic drug, bone tumor

**Abstract**

*Introduction*

In the present research, we formulated a modern chemotherapeutic drug by silver nanoparticles (AgNPs) containing Nigella sativa aqueous extract for the treatment of bone tumor.

*Material and methods*

Characterization of AgNPs was done by UV–Visible Spectroscopy (UV-Vis), Fourier Transformed Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM), and Field Emission Scanning Electron Microscopy (FE-SEM). For investigating the antioxidant properties of AgNO3, N. sativa, and AgNPs, the DPPH test was used in the presence of butylated hydroxytoluene as the positive control. To survey the cytotoxicity and anti-bone tumor effects of AgNO3, N. sativa, and AgNPs, MTT assay was used on the human bone Ewing’s sarcoma (CADO-ES1 and MHH-ES1), human bone osteosarcoma (HOS and MG-63), and human bone chondrosarcoma (SW-1353 and CH-3573) cell lines.

*Results*

DPPH test revealed similar antioxidant potentials for N. sativa, AgNPs, and butylated hydroxytoluene. Silver nanoparticles had very low cell viability and anti-bone tumor properties dose-dependently against CADO-ES1, MHH-ES1, HOS, MG-63, SW-1353, and CH-3573 cell lines without any cytotoxicity on the normal cell line. The best result of anti-bone tumor properties of AgNPs against the above cell lines was seen in the case of the MG-63 cell line.

*Conclusions*

According to the above findings, the silver nanoparticles containing N. sativa aqueous extract can be administrated in humans for the treatment of several types of bone tumors.
Formulation of a novel anti-human bone tumor supplement by silver nanoparticles green-synthesized using *Nigella sativa* leaf aqueous extract

Yijiang Huang¹#, Ruimin Xu²#, Pei Fan¹, Liang Chen¹, Daosen Chen¹, Zhenxing Li¹, Huachen Yu¹*, Peng Luo¹*

¹ Department of Orthopaedic, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325000, China; Key Laboratory of Orthopaedics of Zhejiang Province, Wenzhou 325000, China.

² Emergency Department, The Second Affiliated Hospital of Hainan Medical University, Kaikou 570100, China.

# Yijiang Huang and Ruimin Xu contributed equally to this study and are considered as joint first authors

*Corresponding authors

1st corresponding author: Huachen Yu: Huachen.yu.dr@gmail.com

2nd corresponding author: Peng Luo: Peng.luo.dr@gmail.com
ABSTRACT

In the present research, we formulated a modern chemotherapeutic drug by silver nanoparticles (AgNPs) containing *Nigella sativa* aqueous extract for the treatment of bone tumor. Characterization of AgNPs was done by UV–Visible Spectroscopy (UV-Vis), Fourier Transformed Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM), and Field Emission Scanning Electron Microscopy (FE-SEM). For investigating the antioxidant properties of AgNO₃, *N. sativa*, and AgNPs, the DPPH test was used in the presence of butylated hydroxytoluene as the positive control. To survey the cytotoxicity and anti-bone tumor effects of AgNO₃, *N. sativa*, and AgNPs, MTT assay was used on the human bone Ewing’s sarcoma (CADO-ES1 and MHH-ES1), human bone osteosarcoma (HOS and MG-63), and human bone chondrosarcoma (SW-1353 and CH-3573) cell lines. DPPH test revealed similar antioxidant potentials for *N. sativa*, AgNPs, and butylated hydroxytoluene. Silver nanoparticles had very low cell viability and anti-bone tumor properties dose-dependently against CADO-ES1, MHH-ES1, HOS, MG-63, SW-1353, and CH-3573 cell lines without any cytotoxicity on the normal cell line. The best result of anti-bone tumor properties of AgNPs against the above cell lines was seen in the case of the MG-63 cell line. According to the above findings, the silver nanoparticles containing *N. sativa* aqueous extract can be administrated in humans for the treatment of several types of bone tumors.

KEYWORDS Silver nanoparticles; chemotherapeutic drug; bone tumor.
1 INTRODUCTION

Cancer is a genetic disease that includes 277 types of diseases. There are also more than 100,000 types of chemicals in our environment, of which only 35,000 have been analyzed and about 300 of them produce cancer. The remaining 65,000 chemicals in nature have not yet been tested. Cancer occurs due to uncontrolled cell division, which is the result of environmental factors and genetic disorders.\textsuperscript{[1-3]} The four key genes involved in cancer cell conduction include DNA repair genes, tumor suppressor genes, oncogenes, and programmed death genes.\textsuperscript{[2,3]} If a genetic mutation is produced in a cell, normal cells go out of their way and are affected by new commands that progress to cancer cells. In addition to chemicals, sunlight, shortwave, viruses and bacteria also have a special role in causing cancer.\textsuperscript{[4,5]} Cancers have existed since the beginning of mankind. In recent decades, advances in computer molecular medicine have been able to not only study the causes and mechanisms of this deadly disease but also to perform better in its early diagnosis and treatment.\textsuperscript{[5]} More than 50% of cancers are currently being treated, especially if diagnosed early. Cancer can be treated in several ways: surgery, chemotherapy, radiation therapy, immunotherapy, gene therapy, or a combination of these. Due to the relative inefficiency and very severe side effects of chemotherapy drugs, researchers and scientists have sought a new formulation of various compounds, especially metallic nanoparticles.\textsuperscript{[2-5]}

Nanoscience has shown that if we reduce the size to nanometers, unique properties such as optical properties, electrical conductivity, hardness, and chemical reaction will be obtained. Nanoparticles are widely used because of their high surface-to-volume ratio, small size, and excellent reactivity. One of the most important advances in nanotechnology is the production and application of nanoparticles in the biological sciences.\textsuperscript{[5-7]} Nanoparticles are generally effective in a wide variety of sectors that if their production is based on green chemistry, they have great applications in the fields of food, medicine, cosmetics and health. Nanoparticles centered on inorganic materials such as magnetic metals, their oxides and alloys, and semiconductors have the most studies and potential in biomedicine from diagnosis to treatment of diseases.\textsuperscript{[7-9]} The effects of nanoparticles should be predictable, controllable and get the desired results with minimal toxicity. Metallic nanoparticles used in treatment and diagnosis, in addition to being non-toxic, must be biocompatible and stable \textit{in vivo}. Also, by making appropriate changes in the surface of metallic nanoparticles, they will have a wide range of applications by binding to biomolecules and various carriers to cross the cell membrane and target the desired part in the body. One of the important points in the production of nanoparticles is the use of cost-effective and efficient precursors.\textsuperscript{[5-9]} There are three biological, chemical, and physical methods to synthesize the nanoparticles. Chemical and physical methods are time-consuming and costly. In addition, these methods use some toxic additive chemicals that cause adverse effects on medical applications by adsorption on the surface. Applying the principles of green chemistry has decreased the use of toxic compounds or hazardous solvents, provided optimal regeneration conditions and ameliorated materials for the chemical processes, and raised new sources for green synthesis.\textsuperscript{[6-10]} Therefore, one of the primary goals of green nanotechnology is to produce nanomaterials without harm to human health or
environment, and to develop and design nanomaterials and products that are suitable solutions to environmental problems. The synthesis of nanoparticles by similar biological methods results in greater catalytic activity and limits the use of toxic and expensive chemicals. In biological methods, plant extracts, enzymes or proteins carrying natural resources are used to produce or stabilize nanoparticles. The nature of the materials used to make nanoparticles influences the shape, structure and morphology of these nanoparticles.[5-9] Biological systems involved in the green synthesis of nanoparticles, plants and their derivatives, as well as microorganisms such as algae, fungi, and bacteria. Plant parts such as roots, leaves, stems, fruits, and tiny parts such as the kernel and skin of the fruit are suitable to synthesize the nanoparticles because their extracts are rich in phytochemicals that act as stabilizing and reducing substances.[6,8] The use of natural plant extracts is a cheap and environmentally friendly process and does not require intermediate groups. Short time, no need for expensive equipment, precursors, high purity product and excellent quality without impurities are the features of this method. This is possible very quickly, at room temperature and pressure as well as easily on a large scale.[5-10]

So far, any study hasn’t been done about the remedial capacities of silver nanoparticles containing natural compounds in the treatment of bone tumor. However, there are many studies about the anti-cancer properties of medicinal plants including Zingiber officinale, Viola tricolor, Vinca rosea, Urtica dioica L, Trigonella foenum-graecum L, Thymus vulgaris, Taverniera spartea D, Taxus baccata L, Silybum marianum, Thymbra spicata, Polygonum aviculare, Rosa damascenes Mill, Pegaum harmala L, Physalis alkekengi, Myrtus communis, Olea europae, Medicago sativa L, Mentha pulegium, Lagenaria siceraria Standl, Lepidium sativum, Ferula assa-foetida, Glycyrrhiza glabra, Crocus sativus L, Curcuma longa, Camellia sinensis, Citrullus colocynthis, Avicennia marina, Boswellia serrata, Artemisia absinthium L, Astragalus cytosus, Astrodaucus orientalis, Ammi majus, Allium sativum L, Achillea wilhelmsii, and Ammi visnaga.[11,12] In the present study, we decided to investigate the anti-bone tumor potentials of silver nanoparticles formulated by N. sativa against the bone tumor cell lines.

2 MATERIALS AND METHODS

2.1 Material

All materials used in the recent manuscript were achieved from Sigma-Aldrich Company of USA.

2.2 Synthesis of silver nanoparticles

First, the leaves of the N. sativa plant, after drying in the air, are pulverized using an electric grinder (model, Moulinex AR1066Q). 25 g of the leaves were soaked by maceration with distilled water and kept for at least three days with repeated stimulation to dissolve the solvent at room temperature. After three days, the mixture of extract and water was filtered using filter paper to separate the solids from the liquid. Finally, the excess solvent was
evaporated and concentrated using a rotary evaporate. With the help of a freezer dryer (Scientific LTE UK, Ltd), it was completely dried and turned into a powder. Finally, the dry powder was stored in a sealed glass container and refrigerated and used to prepare different concentrations.[9,10]

The green synthesis of the silver nanoparticles was initiated with a reaction mixture of 100 mL of silver salt (AgNO$_3$) in the concentration of $1 \times 10^{-3}$ M and 200 mL of aqueous extract solution of *N. sativa* leaf (20 µg/mL) in the proportion 1:10 in a conical flask.

The reaction mixture was kept under magnetic stirring for 12 h at room temperature. At the end of the reaction time, the black colored colloidal solution of Ag was formed. The mixture was centrifuged at 10000 rpm for 15 min. The precipitate was triplet washed with water and centrifuged subsequently.[9,10]

To analyze the silver nanoparticles, the common techniques of organic chemistry, i.e. FT-IR and UV-Vis. spectroscopy, FE-SEM, and TEM were used.

The biomolecules involved in the reduction of silver nanoparticles were detected by the FT-IR spectrophotometer (Shimadzu IR affinity.1). Silver nanoparticles were primarily confirmed using UV-Vis spectroscopy at a scan range from 200-800 nm wavelength (Jasco V670 Spectrophotometer).

The morphological features of silver nanoparticles in terms of surface, shape and sizes were exactly analyzed by common morphological testes i.e., FE-SEM (Fe-SEM ZEISS EVO18) and TEM (TEM FEI-TECNAI G2-20 TWIN) microscopic techniques.

### 2.3 Determination of the antioxidant property of silver nanoparticles

Free radicals are unstable atoms that have one or more unpaired electrons. These active species are very harmful due to their high reactivity. They are most often formed when oxygen molecules in the body split into separate unstable atoms. This process can turn into a chain reaction. Free radicals excessive production in the body causes cell damage and oxidative stress. Genetics and the environment affect the extent of free radical damage in individuals. These active molecules are produced as part of the body's natural biological processes. One of the most important free radicals is DPPH. DPPH is widely used to study the antioxidant activities of natural compounds and nanoparticles.[13]

In this method, the antioxidant activity of nanoparticles is measured for DPPH radical scavenging. The basis of the action is the reduction of the alcoholic solution of DPPH in the presence of hydrogen-giving antioxidants, especially phenolic compounds. To achieve the IC50 of the samples, 11 different concentrations of nanoparticles were prepared and the percentage of inhibitory versus concentration was used to plot. In practice, 300 µl of 1 M DPPH was combined with 100 µl of diluted sample to a final volume of 2 ml using methanol. After half an hour in the dark, the absorbance was read at 517 nm and the inhibitory percentage was obtained using the following formula:
In this formula, “Control A” shows the negative control of light absorption that lacks nanoparticles, and “Sample A” expresses the amount of light absorption of different concentrations of nanoparticles. \[^{[13]}\]

### 2.4 Determination of anti-bone tumor effects of silver nanoparticles

Investigation of cell proliferation and survival is one of the most important and basic techniques in cell laboratories. This study requires accurate quantification of the number of living cells in the cell culture medium. Therefore, cell survival calculation methods are necessary to optimize cell culture conditions, evaluate cell growth factors, detect antibiotics and anticancer drugs, evaluate the toxic effects of environmental pollutants, and study apoptosis. Many methods can be used for such purposes, but indirect methods using fluorescent or dye (chromogenic) markers provide very fast large-scale methods. Among these methods, measurement of cell survival by MTT method is the most widely used method. This method is a colorimetric method to study cell proliferation and survival, introduced in 1983 by Mossman. The method basis is based on mitochondrial activity. Mitochondrial activity in living cells is stable and therefore a raise or reduce in the living cells number is linearly related to mitochondrial activity. \[^{[14]}\] Mitochondrial dehydrogenases in living cells break the tetrazolium ring and yield NADPH and NADH, leading to forming a purple insoluble deposit called formazan. This precipitate can be dissolved by dimethyl sulfoxide or isopropanol. Dye formation is used as a marker of living cells. The color intensity produced is measured at a 540 to 630 nm wavelength and is directly proportional to the living cells number. High safety and providing a colorimetric and non-radioactive system are important advantages of this method. This kit is very easy to use, has high sensitivity and accuracy and can detect less than 950 cells. On the other hand, it has high efficiency for measuring cell proliferation, survival and mortality, and its implementation method does not require time-consuming washing steps and transfer from one plate to another. Examples studied in this method are adhesive or suspended cells and proliferating or non-proliferating cells. \[^{[14]}\]

In this research, the following cell lines have been used for investigating the cytotoxicity and anti-bone tumor effects of the AgNO\(_3\), *N. sativa* leaf aqueous extract, and silver nanoparticles using the common cytotoxicity test i.e., MTT assay:

I) Human bone Ewing’s sarcoma: CADO-ES1 and MHH-ES1.

II) Human bone osteosarcoma: HOS and MG-63.

III) Human bone chondrosarcoma: SW-1353 and CH-3573.

IV) Normal cell line: HUVEC.

Because nanoparticles is not soluble directly in 1640-RPMI medium and also the solvent of dimethyl sulfoxide nanoparticles (DMSO) itself has cytotoxic effects, to eliminate the effect of this substance on treated cells, its amount in the final solution is considered less than 1%. Dimethyl sulfoxide is not toxic to concentrations less than...
1% and the concentration of this solvent is important in this regard. For this purpose, 1000 µg of nanoparticle was dissolved in 100 µl of dimethyl sulfoxide solvent after weighing. Then 1 ml of culture medium was added for better dissolution and finally the volume of solution was increased to 24 ml using culture medium. Then, successive dilutions of this stock were used in the proportions of 1-1000 µg/ml. Eleven concentrations were used for the cell lines.\(^{[14]}\)

In this study, 100 microliters of culture medium containing \(10^4\) cells per plate 96 were placed. After 24 hours, incubation of concentrations of 1-1000 micrograms per milliliter of nanoparticles was added to the cells, and incubated for 24, 48, and 72 hours, respectively. After these times, 20 µl of MTT plate with a concentration of 5 mg/ml was added to each cell and incubated in the dark for another 4 hours. After some time, the MTT medium was carefully removed, and 200 microliters of acidified isopropanol were added to each plate to remove the purple formanes. After 15 minutes of incubation at room temperature, the light absorption of each well was read using an ELISA at 570 nm against a reference wavelength of 690 nm. The findings were reported as cell survival and IC50 (concentration that inhibits cell growth up to 50%) based on the concentration curve (µg/ml).\(^{[14]}\)

It should be noted that the effect of each concentration of the nanoparticles on cell lines was investigated in five independent experiments. According to the values of light absorption obtained by the ELISA reader, the percentage of growth inhibition related to each concentration was calculated using the following formula:

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

Finally, linear regression was done to gain IC50, which indicates the nanoparticles concentration, which causes 50% cancer cell growth inhibition. Using the curve, the line equation for cancer cells was obtained, respectively, then by replacing 50% inhibition in the equation, the IC50 value for cancer cells was obtained.\(^{[14]}\)

2.5 Statistical analysis

SPSS statistical software version 22 was used for data analysis and the findings were determined as the mean standard deviation of 5 replications. Data were analyzed using one-way analysis of variance and Duncan post hoc test and the significance level in the test was considered 0.05.

3 RESULTS AND DISCUSSION

3.1 UV-visible spectroscopy of silver nanoparticles synthesized using \(N. sativa\) leaf aqueous extract

UV-Vis is based on the irradiation of ultraviolet and visible photons on the sample and measures the rate of passage or absorption of matter at different wavelengths in the range of 200 to 1100 nm. It is possible to measure
UV-Vis spectroscopic analysis showed the presence of an absorption peak at 422 nm which confirmed the formation of the silver nanoparticles (Figure 1).

3.2 FT-IR analysis of silver nanoparticles synthesized using N. sativa leaf aqueous extract

FT-IR (Fourier Transform Infrared) has been a suitable technique for analyzing materials in the laboratory. An infrared spectrum represents the fingerprint of the sample under test with absorption peaks, which depends on our vibrational frequencies between the atomic bonds of that material. Since each substance has its own atomic bonds, no two compounds with the same infrared spectrum are alike. Hence, infrared spectroscopy can be effective in better identification (qualitative analysis) of different types of materials. Also, the peak sizes are in the range indicating the amount of material present. Advanced software algorithms make this spectroscopy a great tool for quantitative analysis.\(^{15-17}\)

The analysis of the IR spectra of the silver nanoparticles revealed the peaks at 615, 1149, 1388, 1611, and 3413 cm\(^{-1}\) related to the Ag-O, C-OH, C=O, C-O, and OH, respectively (Figure 2).

The IR spectra investigated for the silver nanoparticles revealed the absorption peaks at (I) 3287 cm\(^{-1}\) (OH group of alcohols and phenols); (II) 1623 cm\(^{-1}\) (C-O group of carboxylic acid group); (III) 1383 cm\(^{-1}\) (C=O stretching of carboxylic acid group); (IV) 1038 cm\(^{-1}\) (C-OH vibrations of the protein/polysaccharide).\(^{9,10}\)

3.3 Morphology analysis of silver nanoparticles

TEM (transmission electron microscope) is used for determining the structure and morphology of materials. TEM transmission electron microscope products enable microstructural studies with high resolution and high magnification such as studies of crystal structures, symmetry, orientation and crystal defects. TEM and SEM microscopes differ in how the beam passes and the information obtained from the sample. Scanning microscopes take pictures of the sample surface, while passing microscopes take pictures of the inside of the sample. The resolution and magnification of electron microscopes are higher than those of scanning electron microscopes. The electron beams in the scanning electron microscope scan the surface of the sample point-by-point, but the TEM
microscope beams hit and pass through the entire sample. In addition, sample preparation for the SEM microscope is easier than for the TEM microscope.[15-17]

The size of the nanoparticles (17-22 nm) calculated through TEM images (Figure 3). Furthermore, the histogram plot from the TEM image showed the particle size distribution of biosynthesized silver nanoparticles ranges of 14 to 28 nm.

In the previous studies, the size of silver nanoparticles formulated by aqueous extract of medicinal plants had been calculated in the ranges of 5-50 nm with the shape of spherical.[15-18] These reports support the results of the current work.

Figure 3

The FE-SEM device is one of the most powerful tools used in various fields, including nanotechnology, which uses electron bombardment to produce images of objects as small as 10 nanometers. The bombardment of the sample causes positively charged electrons to be released from the sample to the plate, where these electrons become signals.[15-17]

In the present study, the FE-SEM image of silver nanoparticles is shown in Figure 4. The silver nanoparticles appeared as an agglomerated structure. The hydroxyl groups present in N. sativa leaf aqueous extract could be responsible for agglomeration.[13] Also, FE-SEM images indicated a diameter of 17-22 nm and the shape of spherical for silver nanoparticles.

Figure 4

3.4 Antioxidant properties of silver nanoparticles

Oxidation is the electrons transfer from an atom and is the aerobic life and metabolism part of living organisms. Oxygen is the receptor for electrons in the electron transport system, which yields energy from ATP (Adenosine triphosphate) in the body. Under certain conditions, oxygen may become a single electron and release free radicals. When oxygen becomes a single electron, it is called reactive oxygen species (ROS). Oxidative loss to proteins, DNA, and other macromolecules is one of the internal causes of degenerative diseases such as aging, cardiovascular disease, cancer, immune system deficiency, cataracts, and abnormal brain function. Single oxygen, high-energy, mutagenic oxygen, can be produced by lipid peroxidation by the transmission of energy from light or the respiratory tract of neutrophils.[15-18] Some free radicals have positive roles such as regulating cell growth, phagocytosis, energy production, intracellular signals, or the important biological compounds synthesis. Antioxidants produced in the body fight free radicals with two systems: enzymatic defense and non-enzymatic defense. Superoxide dismutase, catalase, and glutathione peroxidase metabolize lipid peroxide, hydrogen peroxide, and superoxide and prevent the production of toxic hydroxyl radicals.[19-21] In non-enzymatic defense, there are two classes of fat-soluble antioxidants (such as carotenoids and vitamin E) and water-soluble
(glutathione and vitamin C) that trap free radicals. These two systems help neutralize oxidants. However, oxidants can escape from antioxidants and damage tissues. In this case, the activated antioxidant repair system (which is the enzymes lipase, protease, transferase and DNA repair enzymes), counteract the oxidant effects. However, due to deficiencies in the production of antioxidants in the body or due to physiopathological factors and situations (such as smoking, air pollution, UV radiation, diets containing high unsaturated fatty acids, inflammation, ischemia, bleeding, etc) that ROS are yielded in large quantities at the wrong place and time, oral antioxidants are needed to counteract the oxidative damage cumulative effects.[20-23]

In our study, the antioxidant effects of the silver nanoparticles synthesized using *N. sativa* leaf aqueous extract were evaluated by DPPH assay revealed concentration-dependent effects i.e., an increase in the concentration of the silver nanoparticles leads to an increase in antioxidant activities. In the concentrations of studied, the best result was seen in the high concentration or 1000 µg/mL (Figure 5).

Comparative analysis of the individual antioxidant assays showed significant variations in the exertion of radical scavenging effects. Among all materials tested (AgNO₃, *N. sativa* leaf aqueous extract, and silver nanoparticles), the silver nanoparticles indicated more excellent inhibition effects against DPPH. Standard (butylated hydroxytoluene) demonstrated similar antioxidant effects compared to the silver nanoparticles. The exact IC50 of *N. sativa* leaf aqueous extract, butylated hydroxytoluene, and silver nanoparticles were 317, 221, and 166 µg/mL, respectively (Table 1).

Figure 5

Table 1

### 3.5 Cytotoxicity and anti-bone tumor potentials of silver nanoparticles synthesized using *N. sativa* leaf aqueous extract

Nanotechnology is a new branch of science with a wide range of applications and nanoparticles with different compositions and sizes, shapes and surface chemical properties can have different biological and biomedical applications. Reducing the size of materials at the nanoscale can often cause electrical, magnetic, structural, morphological, and chemical changes. Nanoparticles typically have a higher percentage of atoms on their surface, which increases surface reactions.[21-24] Proper design of nanomaterials can be used to target specific cancer cells. Nanoparticles have antibacterial and magnetic properties by penetrating microorganisms due to their high surface-to-volume ratio and small size. Also, due to their photocatalytic, catalytic and ionic properties, they are widely used in the fight against human pathogenic microbes, bacteria, fungi and viruses.[24-26] Researchers have shown that silver nanoparticles kill Schwann cells by releasing Ag⁺. A study has shown that concomitant use of silver and doxorubicin reduces the reproductive toxicity of doxorubicin.[8-10] By producing active bases such as oxygen...
ions and hydroxides, silver nanoparticles disrupt the metabolism, proliferation and respiration of microorganisms by destroying organic structures and strongly interacting with enzymes and proteins in the electron transfer system, and it can kill more than 650 types of gram-negative and gram-positive bacteria resistant to common antibiotics in vitro up to 99.9%. Metallic nanoparticles in cell cultures and human tissues yield toxins that raise inflammatory products such as cytokines and oxidative stress, ultimately leading to cell death. Larger nanoparticles are seen by the nuclei and mitochondria, causing mutations in DNA, destruction of the mitochondrial structure, and even cell death. Solubility and density, surface baroelectricity, surface structure, shape, chemical composition, and size and dimensions are the key factors in determining the toxicity of nanoparticles. The exact effect of silver and silver nanoparticles on cancer cells is not fully understood, but increasing ROS production is one of the possible mechanisms. When nanoparticles are in contact with cancer cells, the cellular defense mechanism is activated to minimize damage. But, if the ROS production stimulation inside the cell by nanoparticles exceeds the cell antioxidant defense capacity, the cells are destroyed during the process of apoptotic cell death.

In the recent research, the treated cells with several concentrations of the present AgNO₃, N. sativa leaf aqueous extract, and silver nanoparticles were examined by MTT test for 48h regarding the cytotoxicity properties on normal (HUVEC) and common bone tumor (CADO-ES1, MHH-ES1, HOS, MG-63, SW-1353, and CH-3573) cell lines (Figures 6-12).

The absorbance rate was determined at 570 nm, which indicated extraordinary viability on normal cell line (HUVEC) even up to 1000μg/mL for AgNO₃, N. sativa leaf aqueous extract, and silver nanoparticles.

Figure 6
Figure 7
Figure 8
Figure 9
Figure 10
Figure 11
Figure 12
In the case of bone tumor cell lines, the viability of them reduced dose-dependently in the presence of AgNO₃, *N. sativa* leaf aqueous extract, and silver nanoparticles. The IC₅₀ of *N. sativa* leaf aqueous extract and silver nanoparticles against CH-3573 cell line were 549 and 367 µg/mL, respectively; against SW-1353 cell line were 422 and 249 µg/mL, respectively; against MG-63 cell line were 405 and 231 µg/mL, respectively; against HOS cell line were 438 and 252 µg/mL, respectively; against MHH-ES1 cell line were 461 and 281 µg/mL, respectively; and against CADO-ES1 cell line were 489 and 319 µg/mL, respectively (Table 2).

**Table 2**

### 4 CONCLUSION

*Nigella sativa* leaf collected was used for synthesizing silver nanoparticles as a suitable and safe material. After silver nanoparticles synthesizing, they were characterized by UV-Vis., FT-IR, TEM, and FE-SEM. The above analyses showed that silver nanoparticles were synthesized as the best possible form. Silver nanoparticles indicated remarkable antioxidant and anti-bone tumor properties against human bone Ewing’s sarcoma (CADO-ES1 and MHH-ES1), human bone osteosarcoma (HOS and MG-63), and human bone chondrosarcoma (SW-1353 and CH-3573) cell lines. It appears these nanoparticles may be administrated as a chemotherapeutic drug.

**Funding (Name of fund, subsidy number)**

1: Basic scientific research project of Wenzhou Science and Technology Bureau, (Grant Number: Y202098)

2: Zhejiang provincial public welfare technology research program / Social Development Fund, (Grant Number: LGF21H060012).

**REFERENCES**

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, *CA Cancer J Clin.* 2018, 68, 394–424.
[2] N. F. Boyd, H. Guo, L. J. Martin, L. Sun, J. Stone, E. Fishell, R. A. Jong, G. Hislop, A. Chiarelli, S. Minkin, M. J. Yaffe, *N Engl J Med.* 2007, 356, 227–36.
[3] Siu AL. *Ann Intern Med.* 2016, 164, 279–296.
[4] Jahanzeb M. *Clin Breast Cancer.* 2008, 8, 324–33.
[5] M. Moschini, G. Simone, A. Stenzl, I.S. Gill, J. Catto, *Eur Urol Focus.* 2016, 2, 19–29.
[6] M. M. Zangeneh, S. Bovandi, S. Gharehyakheh, A. Zangeneh, P. Irani, *Appl Organometal Chem.* 2019, 33, e4961.
[7] A. Zangeneh, M.M. Zangeneh, *Appl Organometal Chem.* 2019, 33, e5290. DOI:10.1002/aoc.5290.
[8] M. M. Zangeneh, *Appl Organometal Chem.* 2019, 33, e4963.
Gao J, Wang Z, Liu H, Wang L, Huang G. Liposome encapsulated of temozolomide for the treatment of glioma tumor: preparation, characterization and evaluation. Drug Discov Ther. 2015; 9(3):205-212.

Zangeneh MM. Appl Organometal Chem. 2020, 34, e5295.

W. Kooti, K. Servatayari, M. Behzadifar, M. Asadi-Samani, F. Sadeghi, B. Nouri, H. Zare Marzouni, J Evid Based Complementary Altern Med. 2017, 22, 982–995.

M. Thomson, M. Ali, Curr Cancer Drug Targets. 2003, 3, 67-81.

S.J. Hosseinimehr, A. Mahmoudzadeh, A. Ahmadi, S. A. Ashrafi, N. Shafaghati, N. Hedayati, Cancer Biother Radiopharm. 2011, 26, 325–329.

V. Arulmozhi, K. Pandian, S. Mirunalini, Colloids Surf B Biointerfaces. 2013, 110, 313–320.

Xinli DHZS. Applications of nanocarriers with tumor molecular targeted in chemotherapy. Chemistry. 2012; 75(7):621–627.

Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv. Drug Del. Rev. 2008; 60(15):1615–1626.

G. Mohammadi, M.M. Zangeneh, A. Zangeneh, Z.M. Siavosh Haghighi, Appl Organometal Chem. 2020, 34, e5136. DOI:10.1002/aoc.5136.

Lu Y, Wan X, Li L, Sun P, Liu G. Synthesis of a reusable composite of graphene and silver nanoparticles for catalytic reduction of 4-nitrophenol and performance as anti-colorectal carcinoma. Journal of Materials Research and Technology. 12 (2021) 1832-1843.
TABLE 1 The IC50 of AgNO₃, *N. sativa*, AgNPs, and BHT in antioxidant test.

| IC50 against DPPH | AgNO₃ (µg/mL) | *N. sativa* (µg/mL) | AgNPs (µg/mL) | BHT (µg/mL) |
|------------------|--------------|---------------------|---------------|-------------|
|                  | -            | 317                 | 166           | 221         |
TABLE 2 The IC50 of AgNO₃, *N. sativa*, and AgNPs in cytotoxicity test.

| IC50 against cell lines | AgNO₃ (µg/mL) | *N. sativa* (µg/mL) | AgNPs (µg/mL) |
|------------------------|--------------|---------------------|---------------|
| HUVEC                  | -            | -                   | -             |
| CADO-ES1               | -            | 489                 | 319           |
| MHH-ES1                | -            | 461                 | 281           |
| HOS                    | -            | 438                 | 252           |
| MG-63                  | -            | 405                 | 231           |
| SW-1353                | -            | 422                 | 249           |
| CH-3573                | -            | 549                 | 367           |
FIGURE 1 The UV–Vis spectrum of biosynthesized silver nanoparticles.

FIGURE 2 FT-IR spectra of biosynthesized silver nanoparticles.

FIGURE 3 TEM image of silver nanoparticles.

FIGURE 4 FE-SEM image of silver nanoparticles.

FIGURE 5 The antioxidant activities of AgNO₃, N. sativa, AgNPs, and BHT against DPPH.

FIGURE 6 The cytotoxicity activities of AgNO₃, N. sativa, and AgNPs against HUVEC.

FIGURE 7 The anti-bone tumor activities of AgNO₃, N. sativa, and AgNPs against CADO-ES1.

FIGURE 8 The anti-bone tumor activities of AgNO₃, N. sativa, and AgNPs against MHH-ES1.

FIGURE 9 The anti-bone tumor activities of AgNO₃, N. sativa, and AgNPs against HOS.

FIGURE 10 The anti-bone tumor activities of AgNO₃, N. sativa, and AgNPs against MG-63.

FIGURE 11 The anti-bone tumor activities of AgNO₃, N. sativa, and AgNPs against SW-1353.

FIGURE 12 The anti-bone tumor activities of AgNO₃, N. sativa, and AgNPs against CH-3573.
FIGURE 2
FIGURE 3
FIGURE 5

![Graph showing inhibition percentage vs. concentration for different substances: AgNO3, N. sativa, AgNPs, and BHT. The x-axis represents concentration in µg/mL, and the y-axis represents inhibition percentage.](#)
FIGURE 6

![Graph showing cell viability (%) against concentration (µg/mL) for AgNO3, N. sativa, and AgNPs.](image-url)
FIGURE 7

A bar graph showing cell viability (%) at different concentrations (μg/mL) for AgNO3, N. sativa, and AgNPs. The graph includes error bars indicating the variability of the data.
FIGURE 8

Cell viability (%) vs Concentration (µg/mL)

- AgNO3
- N. sativa
- AgNPs
FIGURE 9

The bar chart shows the cell viability (%) at various concentrations (µg/mL) of AgNO3, N. sativa, and AgNPs. The y-axis represents cell viability (%) ranging from 0 to 110, and the x-axis represents concentrations ranging from 0 to 1000 µg/mL. The bars indicate a decrease in cell viability with increasing concentration, particularly noticeable for AgNPs. The error bars suggest variability in the data points.
FIGURE 10

Cell viability (%) vs. Concentration (μg/mL)

- AgNO3
- N. sativa
- AgNPs
FIGURE 11

![Graph showing cell viability (%) against concentration (µg/mL) for AgNO3, N. sativa, and AgNPs.](image-url)
Figure 12

Bar chart showing cell viability (%) against concentration (μg/mL) for different samples: AgNO₃, N. sativa, and AgNPs. The chart indicates a decrease in cell viability with increasing concentration for all samples, with AgNPs showing the most significant decrease.