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Winter 2024, Volume 9, Issue 32 (36-41)

Investigation of Antioxidant and Cytotoxic Effects of Cerium Oxide Nanoparticles Synthesized Using Aqueous Extract of Hyssopus Officinalis Plant on MDA-MB231 Breast Cancer Cell Line

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DOI: 10.22034/pmj.2024.2024143.1031

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Submitted: 2024-03-02
Accepted: 2024-03-25

Keywords:
Cerium Oxide Nanoparticle
Breast cancer
Antioxidant
Hyssopus officinalis extract

How to Cite this Article:
M. Tourani, K. Eghbalpour, N. Eghbalpour, A. Neamati. "Investigation of Antioxidant and Cytotoxic Effects of Cerium Oxide Nanoparticles Synthesized Using Aqueous Extract of Hyssopus Officinalis Plant on MDA-MB231 Breast Cancer Cell Line" Personalized Medicine Journal, Vol. 9, no. 32, pp. 36-41.

Abstract:
Free radicals are naturally produced in the body and are inhibited by the body's antioxidants. The excessive production of free radicals and the inability of the body to remove them lead to oxidative stress in the body, which can lead to many diseases, including cancer. Nanoparticles are compounds that have been given much attention to cancer prevention and treatment, due to their specific biological characteristics and their small size. This study aimed to evaluate the cytotoxic and antioxidant potential of serum oxide nanoparticles synthesized using aqueous extract of Hyssopus officinalis. To perform the MTT assay, first, the MDA-MB231 cancerous cells were cultured, seeded and then treated for 24, 48 and 72 hours. Subsequently, MTT was performed and finally, absorption at 517 nm was recorded. The antioxidant potential of the CeO-NPs was evaluated by estimating the amount of ABTS and DPPH free radicals inhibiting in different concentrations of nanoparticles.
The results showed that the CeO-NPs were able to inhibit the ABTS and DPPH free radicals with a mean concentration (IC50) of about 62 and 31.2 µg / ml. Also, the CeO-NPs inhibited cancer cells with IC50 of about 400 µg/ml, 48 hours after exposure. According to the antioxidant results obtained from this paper, it is suggested that by performing further experiments, this nanoparticle can be used as an antioxidant supplement.

INTRODUCTION

The uncontrollable proliferation and growth of cells in some cases leads to the formation of a mass of cells known as neoplasm or tumor. Some tumors have the ability to spread to other parts of the body, which is called metastasis. Cancer involves many challenges due to the involvement of different cells, tissues and organs of the body, and for this reason its treatments are often non-specific (1). Cancer occurs as a result of disturbing the balance between proliferation and cell death, after which angiogenesis and feeding to cancerous tissue can cause its spread (2). Free radicals are chemically active molecules that are able to react with other molecules and become stable by receiving electrons. These radicals are produced naturally and as a result of natural metabolism in the body, and the body inhibits these radicals through antioxidant defense. But their excessive production causes oxidative stress and damage to vital body molecules, including lipids, proteins, enzymes, DNA and RNA (3). Oxidative stress can lead to damage, followed by diseases such as cancer, heart disease, neurological diseases (such as multiple sclerosis, Parkinson's disease), autoimmune disease, stroke, diabetes, etc (4).

Antioxidant compounds play an important role in preventing oxidative stress in the body. Vitamin E is one of the most important antioxidants in the body. Enzymes such as superoxide dismutase (SOD), glutathione peroxide (GPx) and catalase (5) are part of the body's enzymatic antioxidant defense systems (3). The science of nanotechnology and the use of nanoparticles with different size, shape and surface chemical properties can have wide applications in the field of medicine and treatment (6). The unique properties of nanoparticles have led to their use in rare cancer diagnosis and treatment. Studies show that anticancer drugs on nanoparticles play an effective role in enhancing the drug's performance and destroying

cancer cells. The results of some studies show that nanoparticles contribute to the performance of anticancer drugs in killing cancer cells by producing reactive oxygen species or other unknown mechanisms (7).

Chemical regeneration (8) laser ablation (9) and microwave waves (10) are among the methods used in making nanoparticles, but all these methods are toxic and have potential risks. for the environment (5). Today, the green synthesis method has replaced the previous methods due to its lack of chemicals, cheap price and environmental friendliness. Since this method occurs without the production of toxic substances and by enzymatic and sometimes non-enzymatic processes, it is also called green technology (11).

Cerium oxide nanoparticles are actually the oxidized form of the rare element cerium, which, due to their special characteristics, are able to mimic the activity of superoxide dismutase and catalase and are able to act as scavengers of reactive oxygen species (10) in many biological fields. act Such feature has caused the potential applications of this nanoparticle in biomedicine (12) Medicinal plants have been considered for the treatment of many diseases since ancient times. Hyssop medicinal plant (Hyssopus officinalis L) is a perennial plant of the mint family. The essential oil of this plant is used in the treatment of colds and coughs. It is also widely used as a flavoring agent and in the cosmetic industry. Pinocamphene (50%), alpha-beta-pinene, camphene and sesqui terpene alcohols are among the most important components of the essential oil of this plant. Also, this plant contains flavonoids, tannins and other substances such as diosmin, hyssopin and mucilaginous compounds.

In this research, cerium oxide nanoparticle was synthesized using the aqueous extract of hyssop plant (green method) and then its biological effects (cytotoxicity and antioxidant effect) were evaluated.

MATERIALS AND METHODS

Green synthesis of cerium oxide nanoparticles from aqueous extract of hyssop plant

First, the aqueous extract of hyssop plant was prepared by dissolving 10 grams of hyssop plant powder in 100 ml of distilled water, the resulting mixture was placed on a hot plate stirrer at a temperature of 100 degrees Celsius for 30 minutes, and then it was filtered using filter paper.

To make nanoparticles, 10 ml of prepared extract was combined with 100 ml of cerium nitrate solution and placed on a stirrer at 35°C for one hour. Next, the resulting sediment was separated by a centrifuge, then it was dried, and after confirming the formation of cerium oxide nanoparticles, its biological characteristics were investigated.

Examining the average size of nanoparticles

For this purpose, the powder prepared from nanoparticles in solution was used to check the size of the particles.

Examining the cytotoxic effect of cerium oxide nanoparticles:

MTT test was used to evaluate the toxicity of nanoparticles on cells. For this purpose, the cells were cultured and after reaching the logarithmic phase, they were transferred to a 96-well plate with a density of 5000 cells per well, after 24 hours, the cells were treated with different concentrations of nanoparticles, and then after a period of time Treatment and draining of the treatment medium, MTT solution was added to the cells. The cells were incubated for 4 hours and then the MTT solution was replaced with 100 µl of DMSO. Finally, the absorbance of each well was measured by ELISA reader at a wavelength of 570 nm and the viability of the cells was calculated from the following formula.

Cell viability (%) = (Mean optical absorbance of each well concentration / (mean optical absorbance of control wells)

Investigating the antioxidant capacity of cerium oxide nanoparticles

DPPH test

This test was performed based on the method of Brand-Williams and his colleagues(13). In this method, DPPH free radicals were first produced, and for this purpose, DPPH powder was dissolved in 96% ethanol to obtain a DPPH solution with a concentration of 0.1 mM. Next, the resulting solution was mixed with different concentrations of nanoparticles in an equal ratio, and after incubation for 30 minutes at 37 degrees Celsius, the absorbance of each solution was measured at a wavelength of 517 nm. In this method, BHA was used as a standard antioxidant.

ABTS test

This test was performed based on Miller et al.'s method (Miller & Rice-Evans, 1997). At first, ABTS stock solution was prepared, for this ABTS powder and potassium persulfate were dissolved in deionized distilled water and incubated for 16 hours in the dark at room temperature. After that, to prepare working ABTS, the resulting solution was diluted with distilled water until reaching the absorbance of 0.756 at the wavelength of 734 nm. Finally, the ABTS solution was mixed in a volume equal to different concentrations of nanoparticles, and after one hour of incubation at room temperature, its absorbance was measured at a wavelength of 734 nm.

RESULTS

Evaluating the size of nanoparticles

The results showed that the size of the synthesized

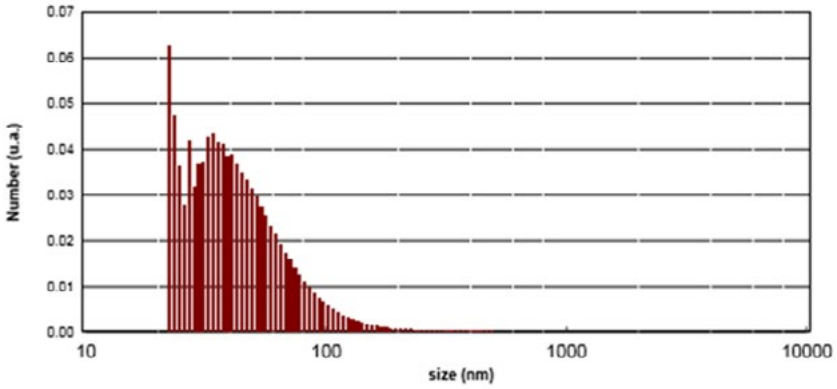

Fig1. Particle dispersion

particles is about 40 nm, which is the best size for biological and biomedical applications of nanoparticles.

Results from TEM and FESEM

Based on the results of TEM and FESEM electron microscopy, the nanoparticles synthesized using the aqueous extract of the hyssop plant have a spherical appearance.

Results from FTIR

FTIR spectrum was recorded in order to determine the effect of hyssop plant on cerium oxide ion. Figure 4-4 shows the FTIR spectrum of the nanoparticle obtained from the aqueous extract of the hyssop plant, in which the 2921/123 cm band is related to the alkyl chain. Also, the 3441/16 cm-1 band in the FTIR spectrum of the nanoparticle is related to the symmetric and asymmetric stretching vibrations of the C-H bond in the four and five positions of the imidazolium ring.

Investigating the toxicity effect of cerium oxide nanoparticles on breast cancer cells (MDA-MB231)

Figure 2 shows the effect of nanoparticle toxicity on breast cancer cells at different nanoparticle concentrations and different treatment times. As can be seen, with the increase in nanoparticle concentration, their toxicity on cells increases. The diagram shows that the nanoparticle at the initial concentration of 62.5 µg/ml is capable of significantly inhibiting cancer cells, and the inhibition rate increases with increasing concentration. In 24 and 48 hours, the IC50 is close to 500 µg/ml, but after 72 hours, the IC50 decreases to about 250 µg/ml, which indicates the effect of concentration- and time-dependent toxicity.

DPPH test

As seen in Figure 3, cerium oxide nanoparticles synthesized using hyssop plant are able to remove DPPH free radicals. As the nanoparticle concentration increases, the inhibition activity of the nanoparticle on free radicals also increases.

Examining the inhibition rate shows that the nanoparticle at a concentration of 62.5 µg/ml is able to inhibit about 50% of free radicals, which indicates the high antioxidant effects of the synthesized nanoparticle.

ABTS test

Figure 4 shows the inhibition of ABTS free radicals

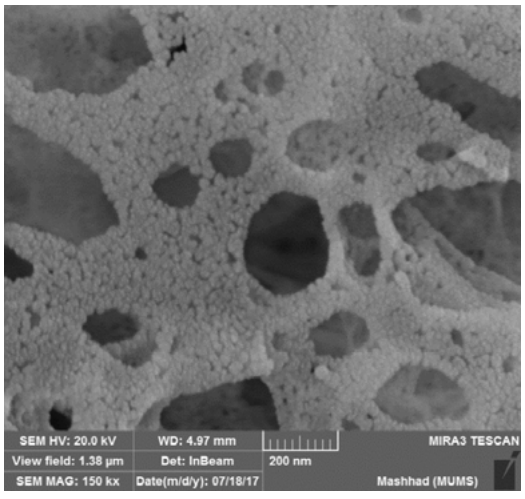

Fig2. Image of cerium oxide nanoparticle obtained from FESEM investigation

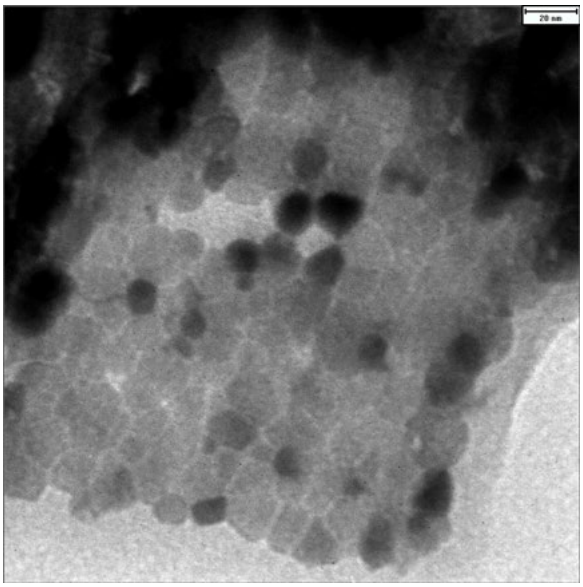

Fig 3. Image of cerium oxide nanoparticle obtained from TEM examination

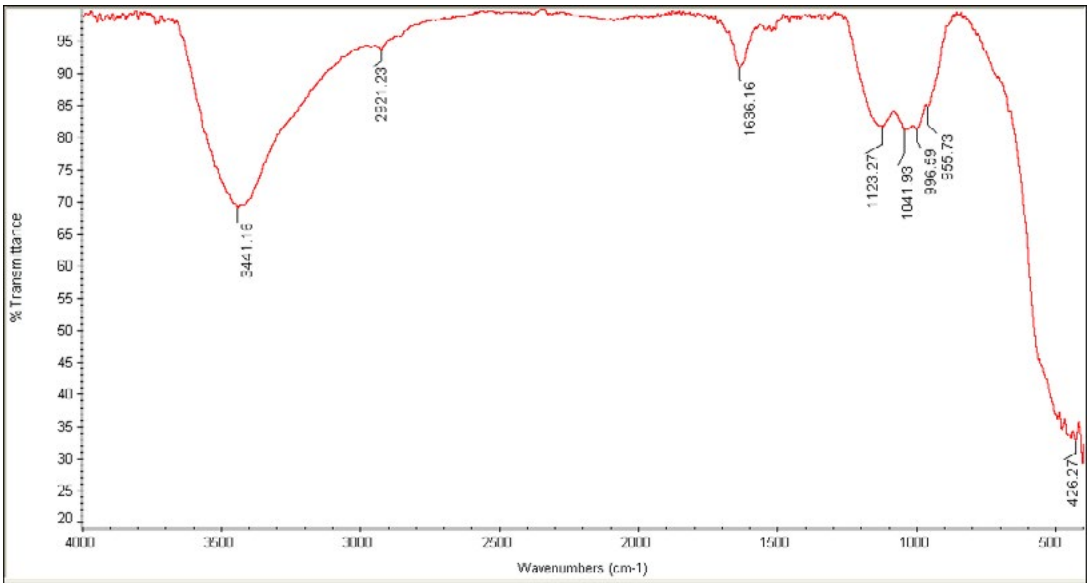

Fig4. FTIR spectrum of cerium oxide nanoparticles

by cerium oxide nanoparticles synthesized from hyssop plant. As can be seen in the figure, with the increase in the concentration of nanoparticles, the inhibition rate of free radicals also increases, so that at a concentration of 500 micrograms/ml of nanoparticles, the inhibition rate is around 95%. The IC50 calculated in this test is about 31.2 µg/ml, which indicates that about 50% of free radicals are inhibited in this concentration.

DISCUSSION

In this study, cerium oxide nanoparticles were first prepared from hyssop plant and then the size of the particles was investigated. After confirming the synthesis of nanoparticles, the effects of cytotoxicity and antioxidant power were evaluated. The use of nanotechnology in the synthesis and optimization of nanoparticles has attracted a lot of attention today. Since nanoparticles synthesized by different methods or from different sources have different biological effects, therefore, investigating the effects of new nanoparticles can open the way for pharmaceutical and therapeutic problems.

The use of nanoparticles with dimensions less than 100 nm in order to deliver and target diagnostic and medicinal agents in medical projects related to cancer has been greatly expanded. Recently, many nanoparticles have been used in targeted drug delivery to malignant tumor cells or reducing the systemic toxicity of anticancer drugs (14) . Examining the effect of toxicity of nanoparticles in inhibiting cancer cells has been evaluated in many studies, for example, Park et al. during a study synthesized cerium oxide

nanoparticles of different sizes (15, 25, 30 and 45 nm) and then the effect of this cytotoxicity Nanoparticles were tested on lung epithelial cells (BEAS-2B) with concentrations of 5, 10, 20 and 40 µg/ml. The results showed that nanoparticles inhibited the growth of cancer cells (15).

In a similar study, Lin et al synthesized cerium oxide nanoparticles with a size of 20 nm and found cytotoxicity on human lung cancer cells with concentrations of 3.5, 10.5 and 23.3 µg/ml at three times 24, 48 and checked for 72 hours. The results showed that the aforementioned nanoparticles induced cell death in a dose- and time-dependent manner similar to the result of cerium oxide nanoparticles synthesized from the hyssop plant (16). Recent studies show that silver nanoparticles also have cytotoxic effects on different cell lines, including Hela and MCF7 cancer cell lines (17). In 2014, Venkatesan et al investigated the anticancer effect of silver nanoparticles produced by green method using water extract of rose petals against human lung adenocarcinoma (A549) by MTT test. The calculated IC50 was 80 µg/ml (18).

In another study silver nanoparticles produced by the green method from cauliflower have antioxidant properties. Antioxidant activity of silver nanoparticles was investigated using DPPH radical scavenging ability and nanoparticles had potential ability to scavenge DPPH radical (19). Furthermore in a similar another study in 2014, the activity of ABTS radical scavenging by silver nanoparticles synthesized from Inonotus obliqqus mushroom was investigated. In this experiment, BHT was used as a standard. The results showed maximum inhibition at 1 mM concentration of about 76% and minimum inhibition at 0.125 mM concentration of about 60%. In general, in this study, similar to the current study, it was shown that increasing the concentration of nanoparticles increases the inhibition of free radicals (20).

In 2004, Giridharan et al used Dodonaea viscosa and Capparis decidua plants to prepare silver nanoparticles and then investigated the antioxidant effects of the synthesized nanoparticles. The results of DPPH and hydroxyl radical removal studies showed that silver nanoparticles obtained from both plants have significant antioxidant activity compared to standard antioxidants. Gold nanoparticles can be mentioned among other synthesized nanoparticles. During another investigation, Inonotus obliqqus plant was used for the synthesis of gold nanoparticles, and then the effect of the synthesized nanoparticles on the inhibition of ABTS free radicals was investigated. The results showed that with the increase in nanoparticle concentration, the amount of free radical inhibition also increases. Investigating the amount of inhibition of gold nanoparticles in different concentrations of 1, 0.5, 0.25, 0.125 mM showed the maximum inhibition of ABTS radical at 1 mM and the minimum inhibition at 0.125 mM). Similarly, Dipankar et al. studied the antioxidant effects of nanoparticles produced from the leaf extract of the blood leaf plant and the results showed that the nanoparticles potentially have concentration-dependent antioxidant activity (21). In another study in 2015, the effect of inhibition of DPPH free radicals by zinc oxide nanoparticles synthesized from Cassiafistula plant extract was calculated in different concentrations of 2, 4, 6, 8 mg in a size of 5-15 nm and the results showed that with increasing concentration from 2000 to 8000 micrograms, the inhibition percentage of DPPH free radicals increases. The IC50 in this study was calculated to be 2853 µg/ml, which is very weak in inhibiting DPPH free radicals compared to the IC50 of the present study (22).

In 2013, Ramamurthy et al. synthesized gold and silver nanoparticles using an aqueous extract of a type of eggplant and further investigated their antioxidant effects with various laboratory methods. The results showed that gold and silver nanoparticles have significant antioxidant power against hydroxyl, superoxide, nitric oxide and DPPH radicals (23).

In another study conducted by Lee et al. in 2013, zinc oxide nanoparticles were first synthesized from the medicinal plant Fagopyrum esculentum and its antioxidant effects were evaluated. Investigating the effects of biomass and the activity of oxidizing enzymes showed an increase in the activity of catalase enzyme and antioxidant glutathione in a concentration-dependent manner (24).

CONCLUSION

The results showed that in the synthesis method, particles with a size of about 40 nm were prepared (Figure 1), which are the appropriate size for further biological investigations. Further investigation of the toxicity effect on breast cancer cells showed that this nanoparticle is able to destroy cancer cells in a time and concentration-dependent manner (Figure 2). The results of the antioxidant power investigation showed that the nanoparticle is capable of inhibiting ABTS free radicals (IC50:31.2) more powerfully than DPPH (IC50:62.5) and overall, this nanoparticle showed high antioxidant effects.

Examining the results of the above studies shows the strong antioxidant properties of different metal nanoparticles. The results obtained from the present study also showed a very high antioxidant property of cerium oxide nanoparticles synthesized using the hyssop plant method, which is consistent with the results of previous studies.

Acknowledgments

This article is a part of the results of the master's thesis, titled as the investigation of the antioxidant properties

and toxicity of cerium oxide nanoparticle synthesized by green method by Hyssopus officinalis plant. The researchers express their thanks and appreciation to all the experts of the research laboratories of the Mashhad Branch, Islamic Azad University who have cooperated in this research.

Funding

This research has performed by personal fund.

Statements and Declarations

There is no statement and declaration.

Conflict of interest statement

There is no conflict of interest.

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