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DNA damage assessment of Hela cancer cell line by biosynthetic zinc oxide nanoparticles

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***Abstract*---**The current study examined the genotoxic effect of the biosynthetic zinc oxide nanoparticles ZnONPs using the Vitex agnus-castus plant, which contains effective compounds that inhibit the growth of pure cancer cells. The phenotypic and structural characteristics of the biosynthesized nanoparticles were detected using scanning electron microscopy (SEM). Most of the ZnONPs were dense and spherical with sizes ranging from (20-61) nanometers, on the cervical cancer cell line Hela and compared with the normal line Human Foreskin Fibroblast cells (HFF) using green dye. Comet assay technique and genotoxicity experiments were carried out at the Iraqi Center for Cancer Research / Al-Mustansiriya University. The results of this study showed the presence of damage to the DNA of cervical cancer cells Hela, which stopped cell division and entry. Cells are in the stage of programmed death a result of the release of reactive oxygen radicals (ROS) and changing cell functions by linking the nanomaterial to the genetic material. This gives hope for discovering a successful treatment to eliminate cervical cancer Hela or any type of cancer using methods Biosynthesis of nanoparticles.

***Keywords*---**biosynthetic, green nanoparticles, cancer cell, hela, GC-MS.

Introduction

Cancer is a most common devastating diseases that millions of people suffer from every year. It is expected to be the second main reason of death in humans (Thandra et al.,2021). cervical cancer is the fourth popular cancer type causing death among women in the world. In low-income countries, cervical cancer is often the leading cause of cancer-related illness and death (Castle et al.,2021). Biomedical nanomaterials have recently attracted bigger attention due to their

outstanding biological properties and biomedical applications. When nanomaterials developed, metal oxide nanoparticles become promising far-reaching possibilities in biomedicine especially for antibacterial, drug delivery, anticancer gene, cell imaging and biosensing(Novio,2020; Ziad et al.,2019; Mohanad et al.,2019). Zinc oxide nanoparticles (ZnO NPs) are utilized increasingly in industrial products such as paint, rubber, and cosmetics in the past two decades (Bloh et al.,2021). ZnO NPs are commonly forms of metal oxide nanoparticles in biological applications because of its excellent biocompatibility, economy, and low toxicity. (Hamrayev et al.,2020). ZnO NPs have been a promising potential in biomedicine, particularly in the cancer and antibacterial fields in their powerful capability of stimulating the production and release of reactive oxygen radicals (ROS) and induce apoptosis (Hamrayev et al.,2021). The use of nanoparticles for targeted drug delivery is a fertile research area for effective treatment of cancer. This delivery to cancer cells could decrease treatment drug doses and its side effects. The authors summarized that the biosynthesized ZnO-NP effectiveness is an anticancer agent which is dose-dependent, increasing the concentration of ZnO-NPs efficiency against cancer cells (Wu and Zhang, 2018; Latif et al.,2021).

Methods of preparation

Extract preparation

The plant *Vitex agnus-castus* was prepared from Al-Kurayat nurseries in Baghdad governorate and planted in the home garden. The species was confirmed by classifying it by the botanical herbarium at the College of Science / University of Baghdad based on the Flora of Iraq book. The leaves were collected and washed with distilled water to remove any particles suspended in it, then it was dried at room temperature for a week. The dried leaves were ground into a fine powder. Also, 25 g of the powder was boiled in 250 ml of distilled water for 15 minutes to prepare the extract. Next, the extract was filtered using NO:0.1 filter paper, and the filtered extract was kept in a refrigerator at 4-C for later use in the biosynthesis of ZnO NPs.

Biosynthesis of zinc oxid nanoparticles (ZnO NPs)

According to the method of Pillai and his group [11] with some modifications, 100 ml of the previously prepared plant extract was heated to 60-70 ° C on a magnetic stirrer. As temperature became 60 ° C, we added 10 g of zinc nitrate Zn(NO3)2 to it. It was boiled until it turned into a creamy white paste. Then the paste is washed with distilled water and placed in a hot oven at a temperature of 400°C for two hours after which a white powder will form from ZnONPs loaded with active compounds from the plant extract as in Figure (1) which shows the biosynthesis of ZnONPs.

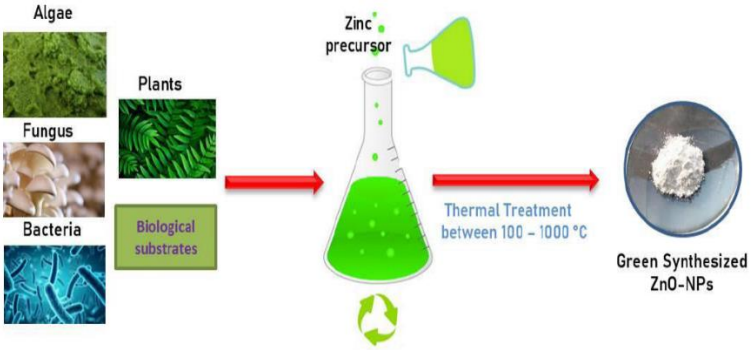


Figure 1. Biosynthesis of zinc oxide nanoparticles [7]

Scanning Electron Microscope (SEM)

The tests were carried out for the biosynthetic ZnONPs nanoparticles to show the phenotypic and structural properties of the biosynthetic particles using a scanning electron microscope (SEM) at Kashan University - Iran. The samples are made by electrons that collide with the sample atoms and transmit signals that analyze the surface structure. The detectors are able of converting the intensity of the electrons into a stored digital voltage forming three-dimensional images of 1 to 5 nanometers (Řiháček et al.,2021).

Genotoxicity Assay

The genotoxic effect of biosynthetic zinc oxide nanoparticles on DNA was studied by the alkaline comet assays. Cancer cell lines and normal cells treated with different concentrations of biosynthetic zinc oxide nanoparticles were collected in Aquarose Aquarose gel in the molten state at concentrations of 1 x 105. ml at a ratio of 1:10 (v/v) at a temperature of 37 ° C. Then samples were sent to the comet slide and the immersing slides in a lysis solution at a temperature of 4 ° C for one hour, followed by an alkaline disintegrating solution addition with a pH of > 13.0 At room temperature for 20 minutes. Then, we put the slides in electrophoresis containers with NaOH-EDTA solution. Electrophoresis was carried out under the appropriate conditions 21 volts for 30 minutes. Cells in the dried Aquarose were stained with green dye Sybr Green by room temperature for 30 minutes. After completing the staining stages, we used a confocal laser scanning microscope to image the samples. Comet Assay program was used to analyze the data.

Statistical Analysis

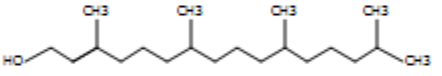
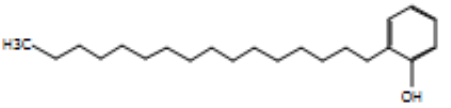
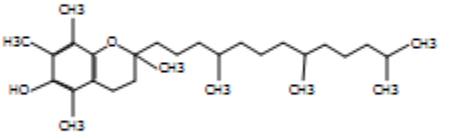
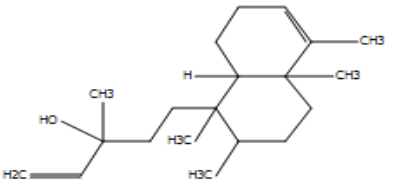
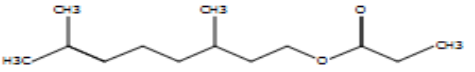
The data obtained using the unpaired t-test were analyzed using the statistical program (Graphpad Prism 6). The values were estimated as (arithmetic mean ± standard error) for the three replicates with a significant difference at the significance level $P < 0.05$
(Mitteer, and Greer,2022)

Results and Discussion

GC-Mass analysis results for essential oils

The individual components of the leaf extract were determined by computer matching and Wiley GC/MS library commercial mass spectrometer, MassFinder 3 library and Baser internal essential oil constituent library with more than 3,200 authentic compounds with mass spectra and retention data from purity. Table (1) shows the effectiveness in the extract as, so the results were identical to those of Ababutain and Alghamd (Ababutain, and Alghamdi,2021)

Table 1
The table shows the active compounds of the extract of the leaves of the plant *Vitex agnus-castus* L in the GC-Mass device

No	Compund name	Chemical formula	Structural formula
1	Phytol	C20H40O	
2	Phenol, 2-hexadecy	C22H38O	
3	Vitamin E	C29H50O2	
4	Kolavelool	C20H34O	
5	6-Octen-1-ol, 3,7-dimethyl-, propanoate	C13H24O2	

(SEM) Scanning Electron Microscope

The surface morphology of the biosynthesized ZnONPs nanoparticles was characterized by using scanning electron microscopy (SEM) as shown in Figure (2), as the image had a magnification power of (150 KX). It can be seen that most of the ZnONPs nanoparticles are dense and spherical in shape. Their sizes range

between (20-61) nanometers, and these results are identical to what was found by Gur *et al* (Gur et al.,2022).

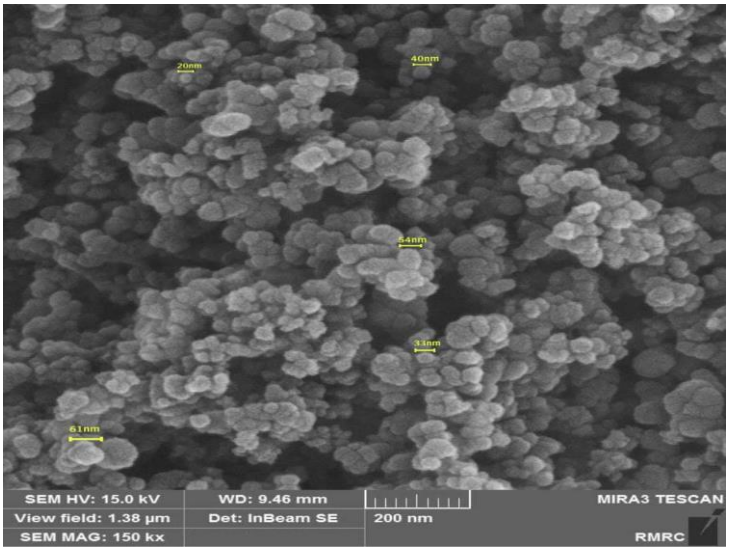


Figure 2. (SEM) image showing biosynthesized ZnONPs oxy-zinc nanoparticles

Genotoxic effects of biosynthetic ZnONPs on cell lines

Figure (3) shows the genotoxic effects of the biosynthetic ZnONPs on the cervical cancer cell line Hela and its comparison with the normal HFF line. Here, it shows the apoptosis of cancer cells treated with biosynthetic ZnONPs using the Comet assay technique that showed programmed death for cancer cells, where the color of the cells turns until they become orange. This proves that they entered the stage of programmed death. The reason for stimulating programmed death is due to the destruction of the chain of electron carriers in the mitochondria. This chain is related to the radicals of reactive oxygen species (ROS) inside the cells and zinc oxide particles work. The nanoparticles of ZnONPs enter cancer cells destroying the electron carrier chains releasing massive quantities of radical oxygen species (ROS), as the excessive amount of reactive oxygen species (ROS) radicals leads to damage to mitochondria. This leads to a loss of balance of protein activity, which eventually leads to death programmed cells (Jiang, and Cai, 2018).

The interaction of biosynthetic zinc oxide nanoparticles (ZnONPs) with DNA genetic material can cause DNA shearing or denaturation and disruption of the irregular division of cancer cells, which leads to inhibition of the proliferation and increases cancer cells as a result of increased expression of the tumor suppressor gene p53. Also, the increase of the roots of types Reactive oxygen (ROS) leads to the programmed death of cancer cells, as we mentioned previously, where the cells in the stage of programmed death appear in orange in comparison with normal cells and this is identical to what was stated in the results (Li et al.,2020; Mohanad,2022).

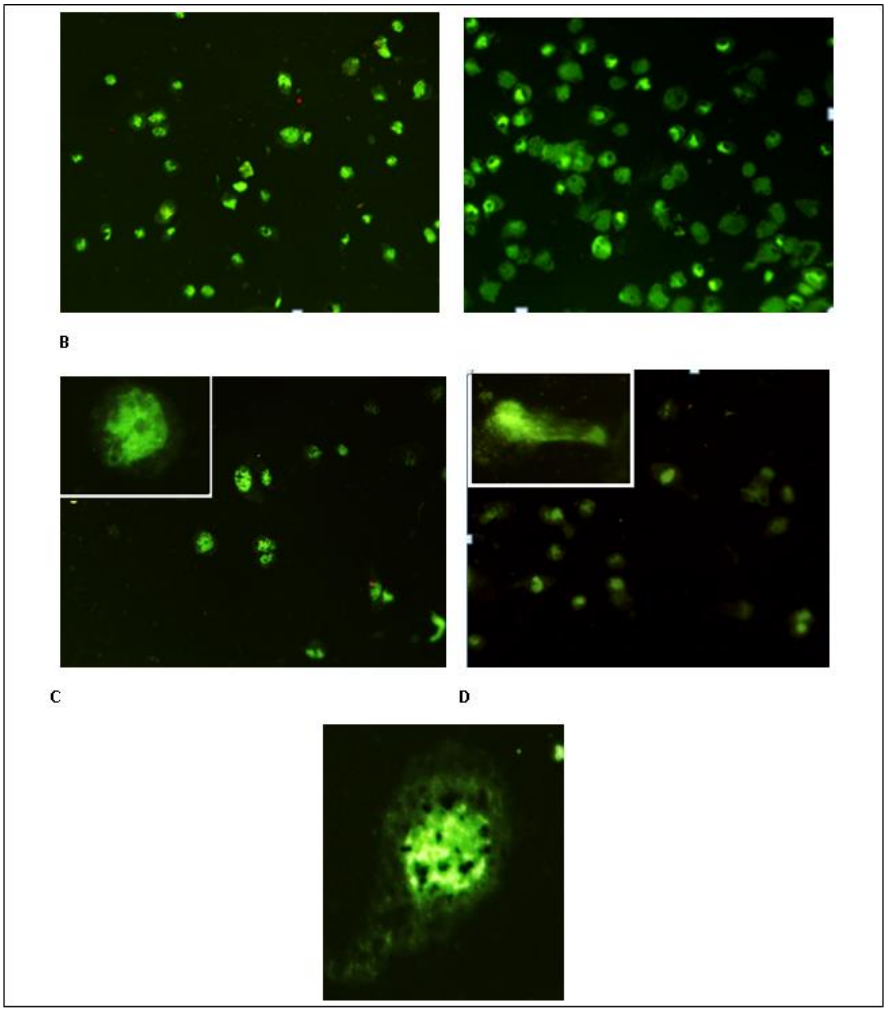


Figure (3) shows the genotoxicity of biosynthetic zinc oxide nanoparticles on the cervical Hela cell line and its comparison with the normal line as (A) HFF Hela cervical cancer cells before they were treated with biosynthetic zinc oxide nanoparticles; (B) HFF normal cells before being treated with zinc oxide nanoparticles; (C) Hela uterine cancer cells after being treated with biosynthetic zinc oxide nanoparticles at concentrations (50 µg/ml); (D) cells of the normal line after being treated with biosynthetic zinc oxide nanoparticles at a 50 µg/ml concentration and (E) cells of Cervical cancer cells in the stage of programmed death.

Conclusion

The biosynthesis of zinc oxide nanoparticles (ZnONPs) by biosynthesis method is better than the traditional physical and chemical methods because it is safer, less expensive and environmentally friendly. The biosynthesized ZnONPs nanoparticles are faster and more effective than the plant extract and the

physically prepared ZnONPs nanoparticles on cervical cancer cell line (Hela) by cytotoxicity assay.

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