ISSN NO: 2771-8840
Date of Publication: 13-04-2022

Evaluation the Breast Cancer Cell Line DNA Damage by Biosynthetic Silver-Green Nanoparticles

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Abstract: The prompt and remarkable development of nanotechnology has accompanied a great deal of interest in its use as a drug delivery procedure, anti-cancer, and bio-reducing agent. Therefore, this study was conducted at the Iraqi Center for Cancer and Medical Genetics Research for the period from 1-7-2021 to 15-1-2022 on cancerous and normal cell lines to assess the DNA damage. Comet Assay single-cell gel electrophoresis is a sensitive and versatile technique for measuring DNA damage and repair at the single-cell level, the genetic material of cancer cells. The biosynthetic nanoparticles were pretreated and measured by TEM. The nm20 nanomaterial created via the green technique was of a variety of sizes, allowing it to be stable and overcome challenges about nanoparticle toxicity. After extracting the active material from the basil plant using a GC-Mas instrument, the active substance was put onto silver. This study concluded that the biosynthetic material is effective for Michigan Cancer Foundation-7 (MCF-7) cells and safe for normal Human Foreskin Fibroblast cells (HFF) by evaluating the DNA damage by Comet Assay, making it an effective anti-cancer material.

Keywords: Cancer cell, DNA damage, GC-MS, Biosynthetic, green nanoparticles, Comet Assay.

Introduction

The meager pharmacological properties of conventional anticancer drugs due to poor solubility, stability and metabolism pose various challenges in their cytotoxic efficacy, inefficiency and limited bio distribution. It is necessary to develop effective formulations that can address the challenges and provide selective targeting of tumor sites without significant damage to healthy tissues [1]. Nanoparticles are scientifically significant because they exist between the huge volumetric, atomic, and molecular structures of the material. It should also be noted that when the material size approaches the nanoscale, the percentage of surface atoms becomes increasingly relevant. [5,2,3,4]. Silver nanoparticles have positive effects in eliminating breast cancer, which has become a source of high incidence among women in the world and an increase in the death rate as a result of this disease [6]. Plant materials have been employed by several researchers as safe and efficient treatments for a variety of microbial illnesses and malignancies [7]. Plants have also been employed in the synthesis of silver nanoparticles, which is referred to as green chemistry. [8,9]. The aqueous leaf extract method can be used for the rapid production of silver nanoparticles, and the size of the nanoparticles can be modified by using different amounts of the extract. As Zhang and his colleagues,2016 showed that silver nanoparticles are one of the many commercially used nanomaterials [10]. In order to Create a new medical agent for treatment present study has in designed

Procedures:

Collecting the plant sample and preparing the extract

Ocimum basilicum was obtained from Baghdad nurseries. The species was confirmed by classifying it by the Herbarium (College of Science - University of Baghdad) and based on the book Flora of Iraq. The leaves were collected, washed with distilled water, and then dried at room temperature for one week. The dried leaves were ground into a fine powder. The extract was prepared by boiling 25 g of the powder in 250 ml of distilled water for 15 minutes. Then the extract was filtered using NO: 0.1 filter paper. The filtered extract was stored in a refrigerator at 4°C for subsequent use in the biosynthesis of AgNPs.

Detection of the active compounds of the plant

The active chemicals in *Ocimum basilicum* leaves were discovered utilizing a gas chromatography-integrated mass spectrometer (GC-MS) and an alcoholic extract of the leaves. The leaves were extracted using saxolites, hexane and methanol as solvents, respectively, according to the method extraction

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mentioned Uddin and his team with some modifications [11]. 100 grams of leaf powder were placed in a filter paper made in the form of a funnel and closed tightly to avoid the exit of the vegetable powder. After that, it is placed in the Saxolith apparatus and 500 ml of solvent is placed on it for 24 hours. Then, the solution was concentrated by a rotary evaporator and the solvent was separated, where a dark brown oil was produced.

Biosynthesis of silver nanoparticles (AgNPs)

The biosynthesis process was carried out according to the method used by Explained by Pillai and his team with some modifications [12]. 100 ml of the previously prepared plant extract was heated to 60-70°C on a magnetic stirrer. When the temperature reaches 60 degrees Celsius, 10 grams of silver nitrate AgNO3 are added to it. It is boiled until it turns into a white gel. Then, the dough was washed with distilled water and placed in a hot oven at 400°C for two hours to obtain a white powder of AgNPs loaded with active compounds from the plant extract. The material was also examined by force transmission microscope (TEM) and showed the shape and size of the biosynthetic material. The size of silver nanoparticles is about 20 nm in Figure (1).

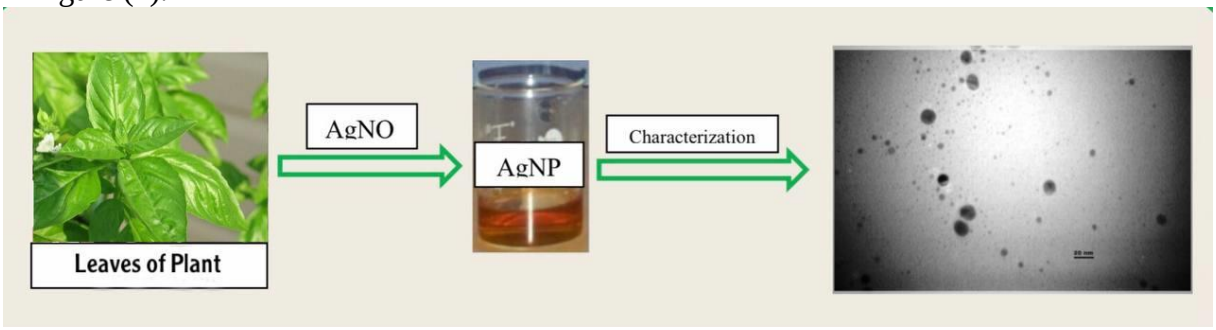

Figure (1) shows the steps of AgNPs biosynthesis and measurement by TEM

Genotoxicity Assay

The effect of Genotoxicity Assay of silver nanoparticles on DNA was studied by using Alkaline Comet Assay according to Grzesiakowska and his team, with some modifications [13]. The cells of the cancerous and normal cell lines treated with different concentrations of silver nanoparticles were harvested in Aquarose in the molten state at a concentration of 1×105mL and a ratio of 1:10 (V/V) at 37°C. The prepared samples were then transferred to a comet slide and these slides were immersed in 4°C lysis solvent for 1 hour, followed by addition of alkaline lysate solution pH >13.0 at room temperature for 20 minutes. After completion of the preparation steps, the slides were placed in an electrophoresis container containing NaOH-EDTA solution. Electrophoresis was performed under appropriate conditions 21 V for 30 minutes, cells in dried Aquarose stained with Sybr Green solution at room temperature for 30 min. After completing the pigment steps, a confocal laser scanning microscope was used to visualize the samples. The results data were analyzed by using Comet Assay software.

Results and discussion

GC-Mass analysis of essential oils.

The individual components of the leaf extract were identified by card matching with the commercial Wiley GC/MS, 3 Mass Finder and Baser commercial mass spectral libraries of internal essential oil components which include more than 3,200 authentic compounds with mass spectra and retention data of pure. The results appeared the presence of active compounds in the extract as shown presence of Phytol, 2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane and Phthalic acid, di(6-methylhept-2-yl) ester. The results were identical to results [14]. It acts as an anticancer through the active compounds and is an anti-proliferative agent of cancer cells, and the presence of the active compounds in *Ocimum Basilicum* can stimulate the signals that induce cell death, which are produced because cancer cells may mutate in the p53 protein, *Ocimum Basilicum* extract also contains compounds that act as a chemopreventive agent by protecting lymphocytes from induction of cancer. As well as the presence of essential oil compounds that can suppress tumors by improving the performance of the Human Natural Killer (immunstimulant), in order to destroy cancer cells. [15,16]

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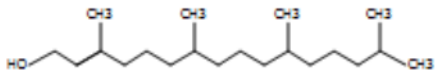
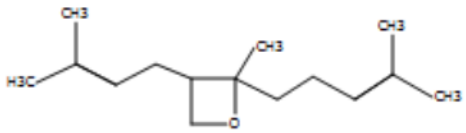
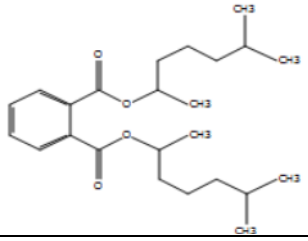
Table (1) shows the active compounds of *Ocimum basilicumL* leaf extract in the GC-Mass apparatus.

No	*Component*	*Chemical formula*	*Structural formula*
1	Phytol	C20H40O	
2	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	C15H26O	
3	Phthalic acid, di(6-methylhept-2-yl) ester	C24H38O4	

Ocimum basilicum belongs to the Lamiaceae family and is an aromatic herb that can be used fresh or dried as a spice. Essential oils extracted from fresh leaves and flowers can be used as aromatic additives in food, medicine and cosmetics [17]. Some of these phytochemicals are believed to be responsible for their high therapeutic efficacy as they contain potent antioxidant, anti-lipid peroxidation, immunomodulation, hypotensive, wound healing, antibacterial, and antidiabetic activities [18]. The strong antioxidant activities of *Ocimum basilicum* are due to the presence of phenolic compounds and polyphenols [8].

TEM analysis

The results obtained from the TEM study indicated the size and shape of the formed AgNps are derived from *Ocimum basilicum*. Representative TEM images indicated that most of the particles were spherical in shape and were mostly monodisperse [8]. The particles displayed a distribution of mean sizes with a size of 20 nm shape. (1) These results show that it is possible to prepare stable AgNps with a size of less than 20 nm by changing the ratio of AgNO3.

Genotoxicity Assay

The genotoxic effect of AgNPs on DNA was investigated by using Comet Assay to detect DNA single-strand breaks, base damage and nuclear repair loci. This technique is fast and sensitive to determine the damage caused by certain factors in the genetic material at the level of the single cell. The DNA of the damaged cells shows an increase in migration from the nucleus, forming a comet shape [19]. When applying this technique to assess the level of damage in the DNA of cells exposed and treated with AgNPs, it was found that there was a significant difference from the control group, $p < 0.01$.

The results of DNA breast cancer cells treated with a concentration of 31.43 µg/ml are shown as shown in Figure (2) (a) the normal cell line HFF, (b) the picture of the MCF-7 cancer cell line. The effect on cell lines is evident after treatment with AgNPs. As the cells appear in the form of a tail. These results indicate nuclear breakage and cell death due to the toxic effect of AgNPs on cells. This is due to the induction of base oxidation in DNA, which leads to cell death. These results are in agreement with the findings reached by [20]

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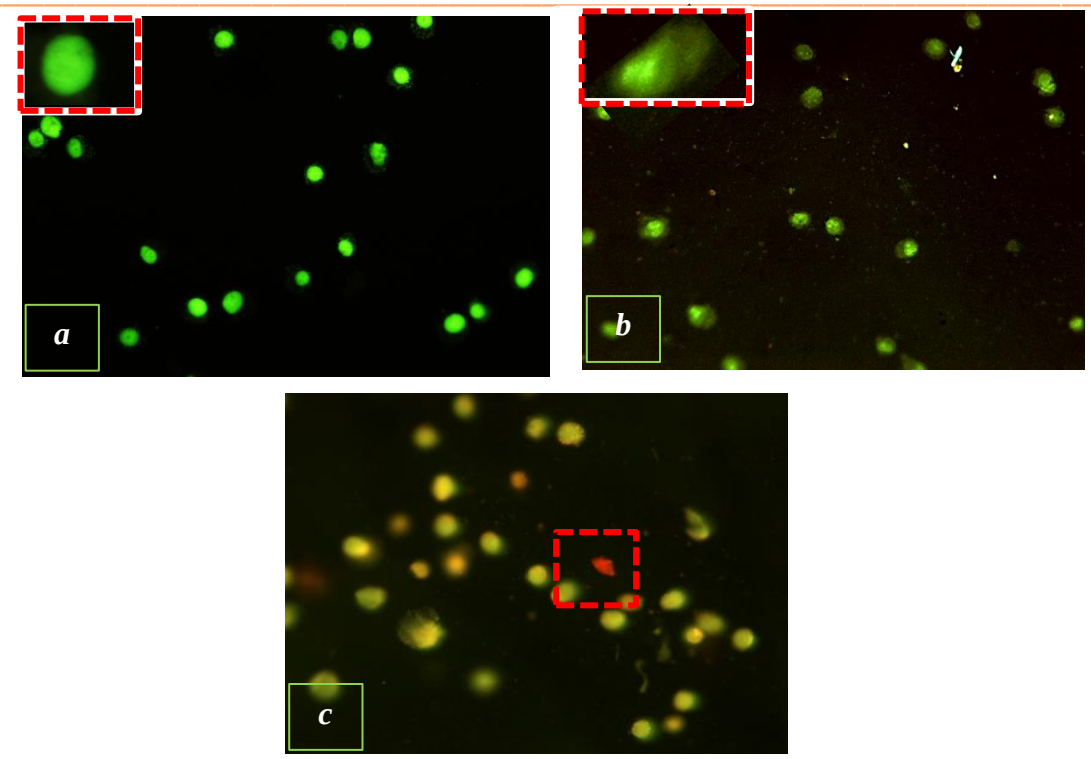

Figure (2): (a) HFF normal cell line without treatment with AgNPs 400X HD, (b) MCF-7 cancer cell line cell after treatment with AgNPs, at a concentration of 31.43 µg/ml, (c) Apoptosis phenomenon 400X HD.

Figure (2) shows (c) cell death, as the damage and swelling in cancer cells treated with AgNPs is evident by the corona estimation process, which determines the amount of damage in the DNA. It causes cancer cells to die when compared to normal cells. The image shows an orange pigment in the early stage of apoptosis where it differs in shape from normal cells due to damage to the walls and structure of the cancer cells. Cells show condensation and distortion in their structure, which confirms the increased effect on cancer cell damage when compared to other images due to stimulating the formation of reactive oxygen species (ROS) radicals, reducing chromosomal condensation, and inducing double-stranded DNA break in cancer cells.

The reason for this is that silver nanoparticles, which are easily and safely prepared in small sizes, stimulate the blockage of the tumor cell cycle and programmed cell death by reducing the regulation of the gene expression of oncogenes [21]. The main mechanism by which nanoparticles cause cell damage, including DNA damage, is that it adheres to the membrane of the cancer cell due to the nature of its acidic environment, whereby a change in permeability occurs and penetrates the cell. Thus, the substance ions are released and once they enter the cell cause structural and biomolecular damage, changes in respiration, and oxidative stress by increasing the radicals of reactive oxygen species. These results were confirmed by the study carried out by Manke and his colleagues [22]. Furthermore, the interaction between nanoparticles and DNA can cause DNA shearing or denaturation and disrupt uncontrolled cell division resulting in inhibition of the deterioration of the physiological status of cancer cells [23,24].

This study concluded that the biosynthetic AgNPs is effective against cancer cells (MCF-7) and safe on normal cells (HFF) through evaluating DNA damage by the Comet Assay, which makes there hope for eradicating breast cancer.

Acknowledgment:

I Thank The staff of the Iraqi Center for Cancer and Medical Genetics Research for their assistance during period of the experiment.

ISSN NO: 2771-8840
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Date of Publication: 13-04-2022

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