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Research article

Characterization and Cytotoxic of Synthesized Silver Nanoparticles by Using the *Ocimum basilicum*

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ABSTRACT

The preparation of nanomaterial using herbal plants is considered one of the most effective methods. The preparation of nanoscale silver nitrate based on plant herbs will open new horizons in the safe synthesis of nanomaterial. The current study highlighted the green synthesis of silver nanoparticles (AgNPs) using the *Ocimum basilicum* (aqueous extract). Here, AgNPs were green synthesized by the precipitation method based on the reduction of silver ions (AgNO3) by *O. basilicum* extract. The toxicity effects of green synthetic AgNPs were evaluated by using a micronucleus assay, the effect on mice's liver and kidney, and lethal dose 50 (LD 50) post administrated to experimental mice with AgNPs orally. The green synthesized AgNPs were relatively uniform in size in the range of 25-70 nm. The results demonstrated that there is no significant effect of green synthetic AgNPs on the number of polychromatic erythrocytes of micronuclei cells. The 1 mg of green synthetic AgNPs killed 50 % of experimental mice (LD 50= 1 mg/100 mg of mice weight). The results showed that *O. basilicum* extract has a good ability to produce safe silver nanoparticles.

Keywords: AgNPs, Kidney, Liver, Micronucleus assay, Mice.

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1. INTRODUCTION

Basil, also known as *Ocimum basilicum*, is an African and Asian native. Nowadays, there are several types, from tiny-leafed Greek basil to sturdy 2-foot-tall plants with large succulent leaves. The leaves of certain species are a rich purple color. The majority of blooms are tiny and pale, although some might be pink or vivid magenta. The ability to dry leaves for subsequent use. Frost may be particularly damaging to basil [1]. The incorporation of green chemistry approaches and techniques into nanotechnology, which has attracted a lot of attention over the past 10 years, is of significant interest [2]. The use of toxic reagents less frequently or never at all a practice known as "green chemistry" has significantly reduced the quantity of residues that are hazardous to both human health and the environment [3]. AgNPs have been synthesized using a variety of approaches, including physical, chemical, and green (biological). All procedures include reducing the silver ions to silver elements using reducing agents, followed by nucleation and growth processes to produce stabilized nanoparticles. It is possible to create particles with the appropriate properties using a variety of physical and chemical techniques that have been used in the production of nanoparticles [4]. Unfortunately, they are unpromising procedures since they utilize pricey and harmful compounds as reducing and stabilizing agents. A viable alternative synthesis method that has recently received a lot of interest and use is the green synthesis of AgNPs by microbes and plant extracts. Sugars, alkaloids, phenolic acids, terpenoids, polyphenols, and proteins are only a few of the many plant metabolites that are present [5].

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The current study aims to determine the cytotoxicity of AgNPs by using micronucleus assays and in vivo other methods.

2. MATERIALS and METHODS

2.1. Plant Materials and Extraction.

Aerial parts of *O. basilicum* were obtained from local markets in Baghdad city, Iraq in March 2016. The collected plants were classified by a botanist, at the College of Science, University of Baghdad, Iraq. Air-dried leaves of *O. basilicum* (100 g) were extracted by a percolation process with water consecutively for 3 days at 25°C. Then, the extracts were filtered by using filter paper (Sigma-Aldrich) and the filtrate was evaporated in a vacuum at 56°C by means of a rotary evaporator and preserved at −25°C.

2.2. Green Synthesis of AgNPs.

The AgNPs were green synthesized by the precipitation method based on the reduction of silver ions (AgNO3) through *O. basilicum* extract according to the standard method of Sulaiman et al. [6]. Ten milliliters of the extract were mixed with 90 ml of AgNO3 (1 Mm, *Sigma-Aldrich*) and kept at 21 ºC in the dark for 18 h to reduce silver ions. The formation of AgNO3 is indicated by the extract's color transition from pale yellow to dark brown to black. A spectrophotometer with a wavelength range of 300–700 nm was tested in order to observe the effects of color changes on the absorbance of the solution. The nanoparticle-containing solution was centrifuged for 20 minutes at 11000 gx, and the supernatant was then discarded.

2.3. UV-vis Spectroscopy Analysis.

The conversion of the Ag ions to AgNPs was approved by measuring the surface plasmon resonance (SPR) of the generated AgNPs using a UV-vis spectrophotometer. As a result, 0.3 ml of the NPS solution was diluted with 3 ml of normal saline before being subjected to UV-vis spectrum examination using a spectrophotometer (Shimadzu UV2550, Japan) in the 300–700 nm wavelength range.

2.4. X-Ray Diffraction (XRD) Analysis.

XRD analysis was used to investigate the presence of green synthesized AgNPs by *O. basilicum* extract. This method examines the stepwise formation of biodegradable nanoparticles. The crystal structure of the green synthesized AgNPs was investigated by considering the Ka ray source of a copper lamp with a wavelength of X beams in λ = 1:54 A0 by a XRD device model 2000 APD (Italy).

2.5. Half lethal dose 50 (LD50).

It is one of the common methods for measuring the toxicity of substances used for human or animal consumption. The principle of this method is based on the dose that causes the killing of 50% of the animals used in the experiment. The standard method of Abal et al. (2017) was followed. The linear method was used to calculate the LD50 of green synthesized AgNPs.

2.6. Micronucleus assay.

This method is considered one of the most important methods that determine the genotoxicity of the materials used in the genetic material. Mice were used to accomplish this method. The In the current study, in vivo micronucleus assay. The standard method of Sousa et al., (2016) with little medication was followed. Four groups of mice were used in this experiment. Briefly, mice (n:3) were injected intraperitoneally with 100 µl of green synthesized AgNPs (1 mg/ml). The control group of mice was injected intraperitoneally with 100 µl of phosphate buffer saline (PBS, 0.1 M, pH 7.2). The mice were sacrificed 48 hours post-administration and the bone marrow cells were collected immediately. In a serial endotoxin-free tube previously bone marrow material was suspended with PBS containing 5 % fetal bovine serum until homogeneous (this step was repeated three times). Smears were prepared by dripping off 2 drops of suspension on the tip of a slide and the slides were air-dried and stained by Leishman's stain (Sigma-Aldrich). Micronuclei were measured in 1,000 polychromatic erythrocytes (PCEs) / animal in the bone marrow of adult mice [8].

2.7. Effect of AgNPs on kidney and liver

This method was performed to determine the toxicity of the green synthesized AgNPs. The mice (n; 3) were injected interpersonally with 100 µl (1 mg/ml) of green synthesized AgNPs dissolved in PBS (0.1 M, 7.2 pH). The control group is mice injected interpersonally with 100 µl of PBS (0.1 M, 7.2 pH). The mice were sacrificed four days post-injection. The pieces of Kidney and liver were collected the standard method of Ayres-Silva (2011) was followed to check the histomorphological changes in the liver and kidney post-injection [11].

3. RESULTS

The presence of a peak at 419 nm by UV-vis spectroscopy confirmed the synthesis of AgNPs (Fig. 1). The peak formed at a wavelength of 400 to 450 nm indicates the formation of AgNPs and is related to the surface plasma on the resonance of AgNPs, that attributed to the remark of free electrons in nanoparticles.

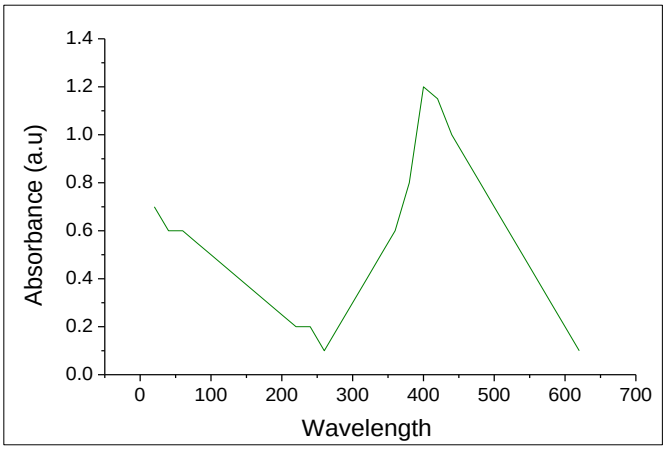

Fig 1. Ultraviolet absorption by silver nanoparticle green synthesized from the *O. basilicum* extract.

The XRD analysis of AgNP green synthesized from the *O. basilicum* extract. The XRD pattern showed that peaks 113, 206, 221, and 331 at 29.15°, 46.1°, 68.1°, and 79.2° corresponded to nanocrystals and silver cubic structures. The non-appearance of other peaks confirmed the purity of AgNPs used in the analysis. The size distribution of the green synthesized AgNPs was in the range of 10-65 nm.

Half lethal dose 50 (LD50) was used to check the toxicity of green synthesized AgNPs. The present study showed that the percentage of death in experimental mice increased when the level of dose increased. 50 % of mice died after injection intraperitoneally with 1 mg of green synthesized AgNPs per 100 mg of mice weight (Fig 2).

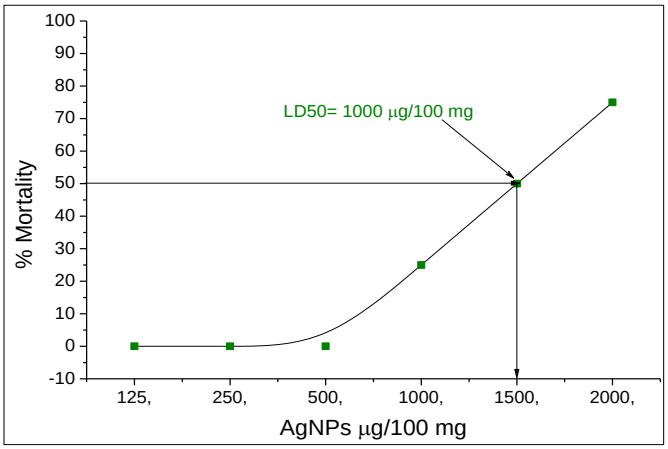

Fig 2. Nonlinear regression fitting procedure to determine LD50 that is showing dose response mortality curve of oral AgNPs nanoparticles in mice. Percentage lethality values were plotted against the dose of the AgNPs nanop-articles.

Micronucleus assay was used to check the genotoxicity effect of green synthesized AgNPs. Fig 3 approve that there is no genotoxicity effect of LD50 dose of green synthesized AgNPs as there is no significant difference in the percentage of micronucleated polychromatic erythrocytes (PCEMNs) in the bone marrow of mice injected with green synthesized AgNPs and the percentage of micronucleated polychromatic erythrocytes (PCEMNs) in the bone marrow of mice that injected with PBS intraperitoneally.

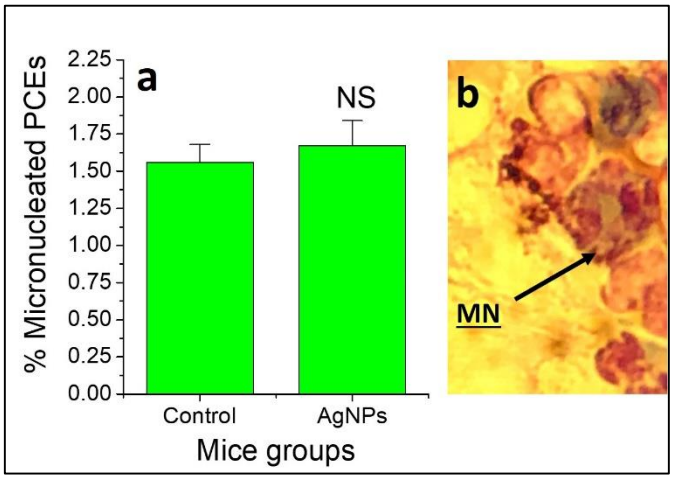

Fig 3. Percentages of micronucleated polychromatic erythrocytes (PCEMNs) in the bone marrow of mice after 48 hours post-injected intraperitoneally with PBS (control) and AgNPs (1mg/100mg). NS, the non-significant difference from control group. b, micronucleus (MN) in PCEMN.

4. DISCUSSION

The current study is aimed at green synthesis and characterization of the AgNPs using the *O. basilicum* methanolic extract. The obtained results demonstrated that the complete reduction of Ag^+ ions to AgNPs was performed by changing the color of the culture medium and spectroscopy. The change in color of the sample to dark brown is a clear sign of the synthesis of silver nanoparticles.

The use of any medication in the treatment must be investigated for its toxicity. No medicine can be approved, whatever its therapeutic effectiveness, without passing through a number of tests to determine its toxicity. This is done through a number of methods used in previous studies [12].

Due to their size, structure, and morphology, nanoparticles have a significant influence on every area of human existence. Metal nanoparticles, such as those made of silver, gold, platinum, and palladium, have several uses in a variety of scientific fields, including medicine and engineering [13]. Due to some of their biological properties, such as their anticancer, antibacterial, antioxidant, and other impacts, silver nanoparticle manufacturing has long been popular. Several researchers have recently examined using plants as sustainable and accessible sources for the creation of biocompatible nanoparticles, and the benefits of this approach include biocompatibility, affordability, nontoxicity, and synthesis of high-purity nanoparticles [13].
In the present study, the green synthesis AgNPs prepared by *O. basilicum* is safe because no effect we saw on the number of micronucleus cells, and also the LD50 was higher. The effectiveness and safety of green synthesis AgNPs encourage our staff to go ahead of check the possibility of using this material in treating the infected host with different pathogens.

5. Conclusion:

Generally, the outcome of the current study showed that *O. basilicum* extract has a good ability to produce silver nanoparticles. The AgNPs produced have no significant cytotoxicity against mice cells. Therefore, these particles could open a door in the treatment of patients suffering from infectious diseases as they are safe and effective.

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Conflict of interest
The authors declare that they have no conflict of interests.

Ethical statement
The study was conducted following approval from the animal ethics committee of the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq (Reference number 945, Date: 5/04/2017).

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