

RESEARCH PAPER

Preparation of Nano Magnesium Oxide using *Marjoram Herb* and Studying Its Effectiveness on Prostate Cancer Cells

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ABSTRACT

In this work, MgO NPs nanoparticles were prepared using marjoram herb extract in a novel, simple and inexpensive procedure involving techniques: co-precipitation and green chemistry. The following methods were used to characterize the produced nanoparticles: XRD, FT-IR, EDX and SEM. The average particle size measured in XRD was 3.69 nm, but the average particle size in SEM was 0.203 nm. The efficacy of the synthesized MgO NPs nanoparticles was evaluated against the drug Flutamide, which is administered for the treatment of prostate cancer in Iraq using PC3 cell line. The results showed the distinct efficacy of the nanoparticles and their superiority over the drug. In addition, they were characterized by their non-cytotoxicity when measured against the drug's toxicity on red blood cells in a cytotoxicity screening test. If the results indicated that flutamide had a cell killing value of 18.69%, 26.79%, 39.27%, 45.27%, 68.29% at 48 h, and for MgO NPs, respectively, the cell killing results were 30.18%, 39.97%, 47.73%, 60.88%, 84.9%, respectively, and the IC50 value = 70.74%.

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INTRODUCTION

Prostate cancer is the second most common cancer (after lung cancer) in men worldwide, with 1,276,106 new cases and 358,989 deaths (3.8% of all cancer deaths in men) in 2018 [1, 2]. The incidence and mortality rates of prostate cancer worldwide are age-related, with the median age at diagnosis being 66 years. Prostate cancer can be asymptomatic in its early stages and often has an indolent course, requiring minimal or no treatment. However, the most common complaints are difficulty urinating, increased urinary frequency, and nocturia, all of which may also arise from

prostate enlargement. More advanced disease may present with urinary retention and back pain, with the skeleton being the most common site of bone metastases. Many prostate cancers are detected on the basis of elevated plasma levels of prostate-specific antigen (PSA > 4 ng/mL), a glycoprotein normally expressed by prostate tissue. However, since men without cancer are also found to have elevated PSA, tissue biopsy is the standard of care to confirm the presence of cancer. Diet and physical activity play an important role in the development and progression of prostate cancer. Dietary factors are primarily

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associated with the observed global and ethnic differences in prostate cancer incidence rates [3,4]. Scientists have been working diligently to develop various therapeutic agents and raw materials for the treatment of cancer incidents [5]. Among the treatments, nanoparticle-based therapy is more successful due to its ability to target cancer cells and its low toxicity [6]. There are many physical or chemical methods for cancer treatment, but these techniques lead to side effects such as pain, skin redness, atrophy, and cause cytotoxicity to normal cells. Although nanoparticles/microparticles when inhaled cause deposition in human airways [7–9]. However, the correct use of a nanoparticle-based cancer drug or drug delivery system can target cancer cells broadly and induce eradication by increasing surface area and retention (EPR) without damaging normal cells [10–11]. Bio-nanoparticles are an effective source of antimicrobial, antioxidant, cytotoxic, biological repair, and wound healing [12–18]. Nano magnesium oxide is an inorganic material with extended band gap that is applied in many fields as catalysts, solid elements, toxic waste removal, recovery agents, and conductive antireflection sheets. Magnesium oxide nanoparticles (MgO NPs) are used as antimicrobial and antitumor agents. MgONPs are a bioactive agent with remarkable properties such as bactericidal, analgesic, anti-inflammatory, antioxidant, antidiabetic, and anticancer [19–21]. The US Food and Drug Administration (FDA) has declared that MgONPs (21CFR184.1431) are biocompatible, biodegradable, low toxic and can be used in pharmaceuticals [22–23]. The

presence of oxygen and superoxide on the surface of MgONPs induces antibacterial activity by damaging the cell wall membrane, inducing active oxygen, reactive oxygen species, lipid peroxidation, electrostatic interaction, alkalizing effect, and inhibiting or altering DNA replication, Krebs cycle, amino acid and nucleotide metabolism and cellular enzyme function [22–28]. In recent years, increasing environmental concerns have led to the development of environmentally friendly materials [29]. In recent biomedical research, there have been concerted attempts to improve the synthesis of nanoparticles using green chemistry that does not use toxic materials [30]. Biosynthesis of nanoparticles using microorganisms, plants and enzymes has been proposed as available alternatives to techniques using chemicals and physical materials that are risky and expensive [31]. Therefore, natural products play a leading role in the discovery of new medicinal molecules for the treatment of human diseases. Moreover, starting from 2004, the World Health Organization officially recognized alternative medicines as complementary therapies based on strong evidence of their benefits. This indicates a clear resurgence of interest and progress in herbal medicine. Phytochemical compounds with novel properties that can fight cancer have been widely recognized in cancer treatment [32]. In this study, we presented a method for the preparation of novel nanoparticles of MgO NPs using marjoram herb extract. These nanoparticles show great potential as a dedicated tool for the treatment of prostate cancer. Furthermore, the results were compared

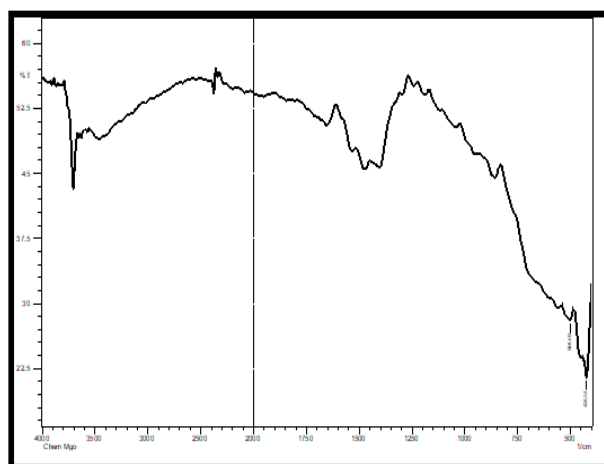


Fig. 1. FTIR spectrum of the compound MgO NPs.

with those obtained from the administration of flutamide, a drug commonly prescribed in Iraq for the treatment of malignant and benign tumors associated with prostate cancer.

MATERIALS AND METHODS

Magnesium chloride 98% India, sodium hydroxide 99% India, ethanol 99% Spain, hydrochloric acid 98% India, marjoram herb from Iraqi markets, phosphate buffer salt, deionized water, flutamide 250 mg tablets from Germany.

Marjoram extract

The extract is prepared in a ratio of (1:10) where (40 g) of marjoram plant is taken after washing and drying and dissolved in 400 mL of ionic water (deionized water). Place the mixture on a magnetic stirrer and leave it to stir for an hour at a temperature of 50 degrees Celsius and 400 rpm for an hour, then filter it and measure its pH, which was pH = 6.2. After that, filter the mixture and store the resulting liquid in a cool place.

Synthesis and Characterization of MgO Nanoparticles

A 0.3 M solution of $MgCl_2 \cdot 2H_2O$ was prepared by dissolving 3 gm in 50 ml of the extract. A 2 M solution was prepared by dissolving 2 gm of NaOH in 25 ml of the extract. The second solution was added by continuous distillation to the first solution with continuous stirring using a magnetic stirrer at a temperature of 50°C for an hour and the pH was adjusted to 7 using drops of HCl. It was then left for 48 hours to complete the digestion process. It

was filtered and washed with ionic water several times, then left to dry at a temperature of 180 and then burned at a temperature of 600 °C for 3 hours.

MTT assay for MgO-NPs

The MTT assay of MgO NPs nanoparticles used a concentration of 10 mg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide as the MTT dye. The MgO NPs nanoparticle samples were dissolved in a solution containing 0.2% dimethyl sulfoxide to determine the concentration levels. The concentrations were measured in parts per million (ppm) at 20, 40, 80, 160, and 320. A 200 µL sample of the suspended cells (1×10^4 cells/well) prepared in RPMI medium was distributed. The cells were cultured in an environment containing 5% CO₂ for 48 h at 37 °C. After adding 20 µL of MgO NPs nanoparticles to the cell cultures, they were incubated for 24 h under identical conditions. Then, 10 µL of MTT reagent was introduced to each sample, followed by a five-hour incubation period at 37 °C. Absorbance measurement was performed at a wavelength of 570 nm [33].

Assay for hemolysis using MgO nanoparticles

The cytotoxicity test of prepared nano magnesium oxide was conducted at different concentrations (µg/mL, 50, 250, 500) to determine the toxicity of the prepared compound towards red blood cells. The cytotoxicity of the prepared compound was estimated by taking a blood sample from a healthy donor under the supervision of a specialist in the laboratory using a sterile medical

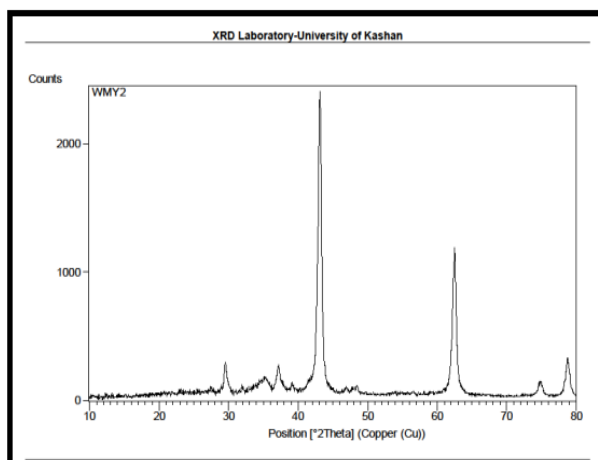


Fig. 2. XRD pattern of compound MgO NPs.

syringe and transferring it to an EDTA Tube. The blood sample was examined under a microscope at 100x magnification to ensure that the platelets were not broken. The EDTA tube was placed in a centrifuge for 10 minutes at a speed of 1000 cycles/min to separate the cells from the blood plasma. The plasma was withdrawn from the blood cells gently and neglected. The blood cells were washed three times using sterile phosphate buffered saline (PBS) and using a centrifuge for

two minutes at a speed of 1000 cycles/min. Red blood cell suspension was prepared by taking 1 ml of washed cells and adding 9 ml of PBS buffer. 300 μ L of cell suspension was withdrawn and 1200 μ L of nano oxide solution was added to obtain the final volume (1.5 mL) and the tubes were left in the incubator for 2 hours. Control treatments were used (a test tube containing cell suspension and deionized water only as a positive control, and a test tube containing cell suspension and PBS buffer

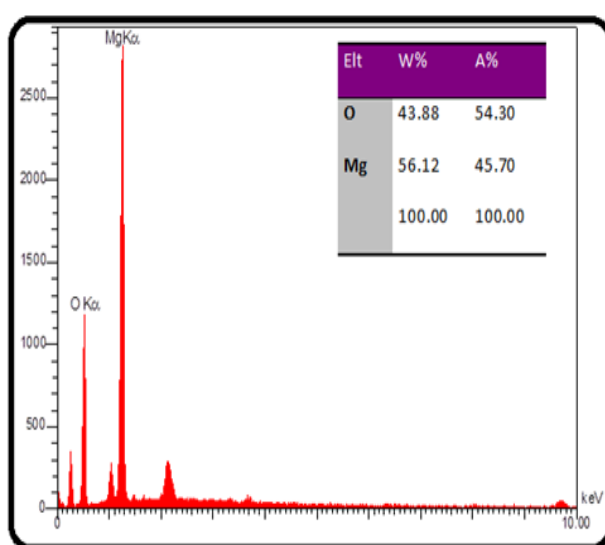


Fig. 3. EDS spectrum of the compound MgO NPs.

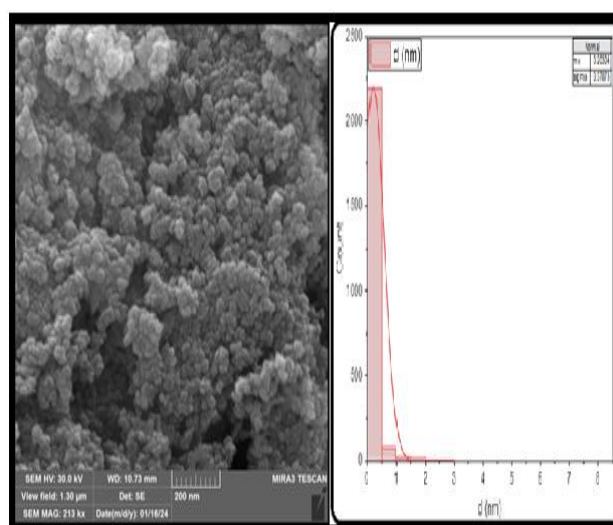


Fig. 4. SEM image of the compound MgO NPs.

as a negative control) to observe the difference in hemolysis. After the incubation period, separation was performed using a centrifuge at a speed of 1000 cycle/min for 5 minutes. The test result is read by observing the presence of red blood cell dissolution in the upper layer after separation, and the result is considered positive (+) (i.e. indicating the presence of toxicity of the chemical compound), while the option (-) indicates if the upper layer remains clear and colorless (indicating the absence of toxicity of the chemical compound) [34-35]

RESULTS AND DISCUSSION

Characterization of MgO nanoparticles by FT-IR

The prepared MgO NPs were characterized by green chemistry method by FT-IR spectroscopy using salt tablets of KBr salt in the range of 400-4000. The results showed in the Fig. 1, the appearance of the frequency band U (470) is due to the stretching of the (Mg-O) bond, and this is consistent with the literature [36].

Characterization of MgO by X-ray Diffraction (XRD)

The crystalline structure and purity of MgO nanoparticles prepared via the green chemistry method were characterized using X-ray diffraction. The X-ray diffraction spectrum of the prepared MgO nanoparticles matched well with the standard spectrum of MgO, as shown in Fig. 2, along with International Centre for Diffraction Data (ICDD) card no: 01-075-0447 [37]. The average crystallite size was calculated to be 25.38 nm.

Characterization of MgO by Energy Dispersive X-rays

The elemental composition of prepared magnesium oxide nanoparticles was determined by green chemistry using energy dispersive X-ray diffraction, and the results showed the presence of oxygen at 43.88%, and magnesium at 56.12%, indicating a high degree of purity of the compound, as shown in the Fig. 3.

Characterization of MgO by SEM

The morphological and structural compositions of MgO nanoparticles, synthesized using environmentally friendly methods, were analyzed using a scanning electron microscope (SEM). Fig. 4 demonstrates that the particles were created at the nanoscale scale. The scanning electron microscopy (SEM) scans revealed that the majority of the nanoparticles were well dispersed. However, a portion of them were observed in an agglomerated state. The formation of this cluster is a result of electrostatic forces, and the average size of these particles is approximately 0.203 nm.

Anticancer Activity of MgO-NPs

This study used MTT assay to measure the cytotoxicity of MgO nanoparticles on PC3 prostate cancer cells. Concentrations of 20, 40, 80, 160, and 320 parts per million (ppm) were added after 48 h, and the concentration at which half the inhibitory effect (IC₅₀) was observed was determined. The viability of PC3 cells was evaluated after incubation with MgO NPs synthesized using green chemistry

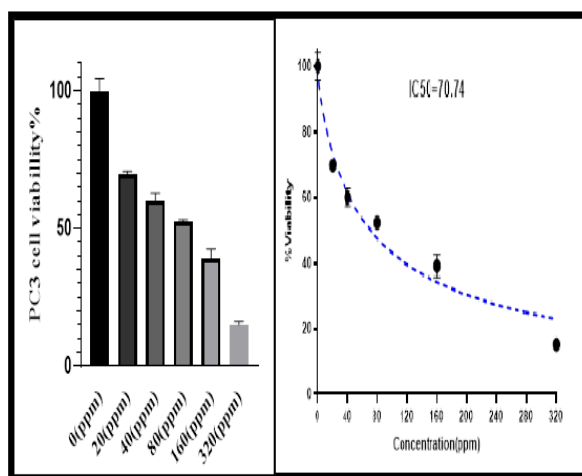


Fig. 5. Effect of MgO NPs on PC3 cell survival and IC₅₀ value at 48 h.

compared to the blank control. After 48 h of culture at a concentration of 20 $\mu\text{g/ml}$, the cancer cell death rate was determined to be 30.18%, indicating the effect of MgO NPs on cancer cells. The cell death rates at doses of 40, 80, 160, and 320 $\mu\text{g/ml}$ were 39.97%, 47.73%, 60.88%, and 84.9%, respectively. These results indicate that increasing time and more significant concentrations had a greater effect on cell death [22]. The figure shows that the IC₅₀ value at 48 h was 70.74%. The effect of flutamide on the viability of PC3 prostate cancer cells was studied compared with the blank control group after 48 h incubation at a concentration of 20 $\mu\text{g/mL}$. The cancer cell death rate was 18.69%, indicating the effect of flutamide on cancer cells. At concentrations of 40, 80, 160 and 320 ppm, the cell death rates were 26.79%, 39.27%, 45.27% and 68.29%, respectively, indicating an increase in the effect with higher concentrations. The results indicated an IC₅₀ value of 149.9% after 48 h, as shown in the figure. The metastatic nature of prostate cancer is characterized by increased expression of fatty acids (FA) and lipid synthesis. Overexpression of FAS on the cell surface, a death receptor known as FAS, is derived from tumor tissues and cell lines. It indicates an advanced stage of cancer cell metastasis to other parts of the body. As a result, it inhibits fatty acid synthesis. Bioactive compounds that pharmacologically induce apoptosis are essential for suppressing cancer cell growth. Therefore, our understanding of the effects of oregano-based nanoemulsion for cancer prevention is currently limited. Therefore, our current study aims to evaluate the effect

of oregano on lipid metabolism and promote apoptosis activation in PC3 cells. Our results suggest that oregano has the potential to be an effective therapeutic option because it can induce apoptosis in prostate cancer cells [23].

Anticancer Activity of MgO-NPs

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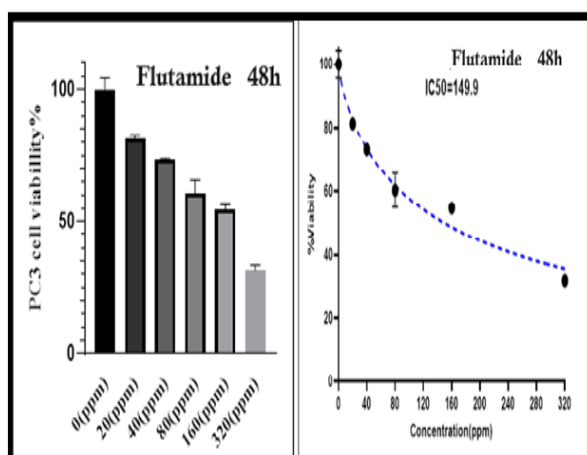


Fig. 6. Effect of Flutamide on PC3 cell survival and IC₅₀ value at 48 h.

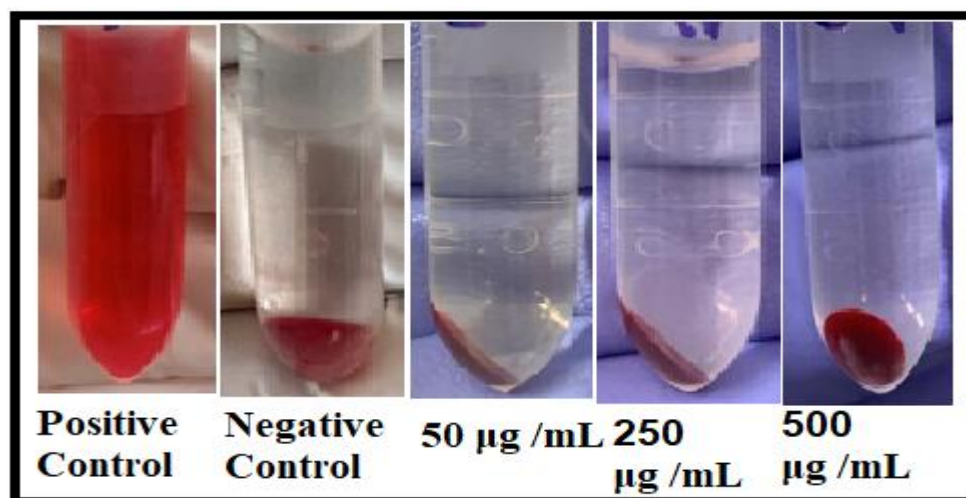


Fig. 7. Hemolysis test for MgO NPs.

results indicated an IC₅₀ value of 149.9% after 48 h, as shown in the Fig. 6. The metastatic nature of prostate cancer is characterized by increased expression of fatty acids (FA) and lipid synthesis. Overexpression of FAS on the cell surface, a death receptor known as FAS, is derived from tumor tissues and cell lines. It indicates an advanced stage of cancer cell metastasis to other parts of the body. As a result, it inhibits fatty acid synthesis. Bioactive compounds that pharmacologically induce apoptosis are essential for suppressing cancer cell growth. Therefore, our understanding of the effects of oregano-based nanoemulsion for cancer prevention is currently limited. Therefore, our current study aims to evaluate the effect of oregano on lipid metabolism and promote apoptosis activation in PC3 cells. Our results suggest that oregano has the potential to be an effective therapeutic option because it can induce apoptosis in prostate cancer cells [39].

Toxicity Test MgO NPs on Blood Cells

The cytotoxicity of MgO NPs was tested at concentrations of (50, 250, 500) µg/mL. The test results showed the safety of the compound (non-toxic) for all concentrations, as shown in the Fig. 7.

CONCLUSION

In this study, MgO NPs nanoparticles were prepared using oregano extract in a novel, simple and inexpensive procedure involving co-precipitation and green chemistry techniques. The following methods were used to characterize

the produced nanoparticles: XRD, FT-IR, EDX and SEM. To determine their structural properties. MgO nanoparticles show promise as therapeutic materials due to their ability to gradually release heavy metal ions. This makes them an anticancer agent. MgO NPs nanoparticles were demonstrated in a cell viability assay to inhibit the PC3 cell lineage. However, our understanding of the effects of oregano basic nanoemulsion on cancer prevention is currently limited. Therefore, our current study aims to evaluate the effect of the synthesis metabolism and promote apoptosis activation in PC3 cells. Our results suggest that MgO NPs have the potential to be an effective therapeutic option because they can induce apoptosis in prostate cancer cells.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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