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Synthesis, Green Chemistry Method Characterization of CuFe5O8 Nanocomposite and Assessment of Its Prostate Cancer-Preventive Effects

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Abstract

In this work, $CuFe_5O_8$ nanoparticles were prepared to utilize Origanum vulgare leaf extract in a novel, simple, and inexpensive procedure that incorporated three techniques: co-precipitation, ultrasound, and green chemistry. The following methods were used to characterize the produced nanoparticles: XRD, FT-IR, EDX, SEM, and DLS. The average particle size measured in the XRD was 45.101 nm, but the average particle size in the SEM was 64.17 nm. The DLS had particles with an average size of 924.4 nm simultaneously. The efficacy of the synthesised $CuFe_5O_8$ nanoparticles was evaluated against that of the medication Flutamide, which is administered to treat prostate cancer in Iraq using the PC3 cell line. The results demonstrated the nanoparticles' outstanding efficacy and superiority over the medication. In addition, they were distinguished by their lack of cytotoxicity when measured against the drug's toxicity on red blood cells in the toxicity screening test. If the results indicated that the drug Flutamide had a cell killing value of 6.51, 10.55, 27.69, 32.48, and 52.53) at 24 hours, as well as for CuFe5O8, respectively, the cell killing results were 33.69, 38.53, 46.42, 56.11, and 69.59)% as well value IC50 = 88.46.

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1. INTRODUCTION

With its cutting-edge techniques for identifying, treating, and monitoring a variety of malignancies, including prostate cancer, nanotechnology has demonstrated considerable promise in the field of cancer therapies[1]. Among the many nanomaterials being studied for their potential in cancer therapy, nanometal oxides, especially iron and copper, have attracted much attention due to their distinct physicochemical properties and biocompatibility [2]. Mainly, inorganic metal oxide nanoparticles (NPs) constitute a significant component in clinical developments in the detection and management of cancer[3]. Examples include titanium peroxide in the pancreas, platinum in the lung, graphene in non-small cell lung cancer, iron oxide NPs in gliomas, graphite for the prostate, gold in the cervical region, selenium, ruthenium, and copper in the breasts. In addition, inorganic ferrite magnetic nanoparticles, such as zinc, nickel, cobalt, and superparamagnetic iron oxide, exhibit strong anticancer properties. Furthermore, the nanoparticles created specifically aim at prostate cancer by utilizing an aptamer, antibodies, and the surface marker prostate-specific membrane antigen[8].

Throughout human history, the utilization of natural substances in conventional medicine has revealed that the advancement of novel treatments depends on bioactivity. Natural products will play a leading role in discovering new medicinal molecules for treating human ailments. Furthermore, starting in 2004, the World Health Organization officially sanctioned alternative medicines as supplementary treatments based on solid proof of their advantages. This signifies an apparent resurgence of interest in herbal therapy and its advancement. Phytochemical compounds with novel properties that can fight against cancer have been widely acknowledged in cancer treatment [9]. Various Origanum species have demonstrated cytotoxic effects on distinct cancer cell lines. For instance, the ethanol and ethyl acetate extracts of O. vulgare exhibited significant cytotoxicity against breast cancer cell lines. Moreover, the ethanolic extract of O. compactum exhibited antiproliferative action, explicitly targeting breast cancer cells. Furthermore, a study has revealed an anti-cancer impact on lung cells in SMMC-7721 hepatoma cells [10]. Prostate cancer (PC) is the predominant form of cancer and remains the leading cause of mortality in men despite the advancements in therapies and diagnostic techniques. Based on the most recent research completed by Globocan and IARC (International Agency for Research on Cancer), the projected number of new cancer cases worldwide is 10,065,305. Specifics regarding the projected number of cancer cases in 2023 amounte to a total of cases[11]. In this study, we introduce a method for the preparation of new ferrite $CuFe_5O_8$ nanoparticles using Origanum vulgare leaf extract. These nanoparticles show great potential as a customized tool for treating prostate cancer. Furthermore, the results were compared with those obtained from the administration of flutamide a medication commonly prescribed in Iraq for the treatment of both malignant and benign tumors associated with prostate cancer**.**

2. Materials and Methods

The chemical compounds $CuCl_2.2H_2O$, $FeCl_3$, H_2O, NaOH, $NaBH_4$, and CH_3CH_2OH are sourced from various countries and manufacturers. $CuCl_2.2H_2O$ has a molecular weight of 170.48 and is obtained from ALPHA CHEMICAL in India with a purity of 99%. $FeCl_3$ has a molecular weight of 162.21 and is sourced from CDH in India with a purity of 99%. H2O, with a molecular weight of 18.015, is obtained from Babylon in Iraq with a purity of 99%. NaOH has a molecular weight of 40.00 and is sourced from SDS in India with a purity of 98%. Sodium tetrahydridoborate is obtained from Sigma-Aldrich with a purity of 99% Ethanol is sourced from BDH in England with a purity of 99%. Origanum leaves are obtained from the market in Iraq, and Flutamide tablets 250 mg are sourced from Germany.

Oiganum leaf extract

Take 50 grams of Origanum leaves and mix them with 500 ml of deionized water in a ratio of 10 parts water to 1 part leaves. Place the combination on a magnetic stirrer and let it stir for one hour at a temperature of 50°C. Afterwards, filter the mixture and store the resulting liquid in a cool location[12].

Synthesis and Characterization of $CuFe_5O_8$ Nanoparticles

Prepare 0.5 molars of $FeCl_3$ using oregano leaf extract as a solvent. Also, prepare 0.5 molar of $CuCl_2.2H_2O$ in the same way. Mix the first solution with the second with a magnetic stirrer for 30 minutes at a temperature of 40 C. Then, use an ultrasound device for 10 minutes. Slowly add 2 M from the NaOH solution until the pH becomes 7, and leave the mixture on a magnetic stirrer. Later, 10 ml with one molar concentration of NaBH4 are added dropwise and left for half an hour at 40°C. Finally, it is filtered, washed three times in alcohol and twice in deionized water, and dried at 180 °C. Finally, it is burned at 650 °C for four hours.

MTT Assay for $CuFe_5O_8$-NPs

The MTT test for CuFe5O8 nanoparticles utilized a 10 mg/ml concentration of 3 [4,5 dimethylthiazole two yl] 2,5 diphenyltetrazolium bromide as the MTT dye. The $CuFe_5O_8$ nanoparticle samples were dissolved in a solution with 0.2% DMSO to establish concentration levels. The concentrations were measured in parts per million (ppm) at 20, 40, 80, 160 and 320. A 200 µl sample of suspended cells (1 × 104 cells/well) prepared in RPMI medium was dispersed. The cells were cultured in an environment containing 5% carbon dioxide for 24 hours at 37 degrees Celsius. After adding 20 µl of $CuFe_5O_8$-NPs to the cell cultures, they were incubated for twenty-four hours under identical conditions. Subsequently, 10 µl of MTT reagent was introduced to each sample, followed by an incubation period of five hours at a temperature of 37°C. The absorbance measurement was conducted at a wavelength of 570 nm[13].

Assay for hemolysis using $CuFe_5O_8$ nanoparticles

The hemolysis assay was employed to evaluate the presence of $CuFe_5O_8$ at different concentrations (50, 250, and 500 ppm) to determine the compounds' toxicity. The blood sample was obtained from the laboratory, transferred into an EDTA tube, observed under a microscope at a magnification 100, and subsequently analyzed on a slide. After separating blood cells and plasma in an EDTA tube, the combination underwent

centrifugation for 10 minutes. After removing the plasma layer of the cells, they were subjected to ten minutes of repeated centrifugation cycles, rinsed multiple times with PBS and supplemented with 1 mL of PBS. The cells were extracted from the PBS solution after two minutes. After multiple rounds of washing, the blood cells were mixed with 1 mL and 9 mL of PBS to create the blood cell suspension. Each tube contains 1200 µL of the antagonist, introduced in varying quantities. The remaining volume of 1.5 ml is then supplemented with 300 µL of the cell suspension. Following a two-hour incubation period, each tube is centrifuged for five minutes at 1000 revolutions per minute. The disparity in hemolysis was further assessed utilizing the Heh control parameters, which involved test tubes containing blood and PBS and test tubes containing blood and deionized water only. The positive result observed after centrifugation demonstrates the compound's toxicity when combined with blood components. The presence of the (-) option suggests that the blood components were not combined upon centrifugation, implying the safety of the medicine [14,15].

3. Results and discussion

Characterization of $CuFe_5O_8$ nanoparticles by FT-IR

The chemical bonding of $CuFe_5O_8$ nanoparticles was analyzed using the FTIR spectrum, which covered a wavenumber range of 400 to 4000 cm^{-1}. Salt tablets made of KBr were employed for this purpose. Figure 1 shows distinct absorption peaks, one observed at a wavenumber of 570 cm^{-1}. This particular peak corresponds to the vibration mode of the metal in copper oxide (Cu-O). A band was observed at a frequency of 470 cm^{-1}, which can be attributed to the vibration of the iron oxide (Fe-O) bond. These findings are in agreement with previous studies[16].

Characterization of $CuFe_5O_8$ by X-ray Diffraction (XRD)

The crystalline structure and purity of CuFe5O8 nanoparticles prepared via the green chemistry method were characterized using X-ray diffraction. The X-ray diffraction spectrum of the prepared CuFe5O8 nanoparticles matched well with the standard spectrum of **$CuFe_5O_8$**, as shown in Figure 2, along with International Centre for Diffraction Data (ICDD) card no: 96-153-8388 [17]. The average crystallite size was calculated to be 45.10 nm. The crystal shape was found to be cubic.

Characterization of $CuFe_5O_8$ by Zeta Potential (ZP)

The surface charge of iron-copper nanocomposites was characterized to indicate the stability or instability of nanoparticle dispersion. The potential values of Zeta potential (mV) suggested significant instability at (±0-10) mV, relative stability at (±10-20) mV, somewhat stability at (±20-30) mV, and high stability at ±30 mV, providing sufficient repulsive force to achieve better physical colloidal stability. Overcoming van der Waals forces involves electrostatic repulsion forces. The surface charge of prepared **$CuFe_5O_8$** nanoparticles via green chemistry was measured to be (20.6 mV) [18], as shown in Figure 3.

Characterization of CuFe5O8 by dynamic light scattering (DLS)

The DLS technique, along with assessing the stability of nanoparticle dispersion in colloidal solutions, is influenced by the solvent used. The solvent should not dissolve the nanoparticles and should have low viscosity to prevent nanoparticle agglomeration. Hence, water was utilized as a solvent for CuFe5O8 nanoparticles of low density, implying non-agglomeration [19]. The CuFe5O8 nanoparticles' granular size was measured, and the Z-average was 924.4 nm, as depicted in Figure 4.

Characterization of CuFe5O8 by Energy Dispersive X-rays

The elemental composition of $CuFe_5O_8$ nanoparticles prepared via green chemistry was characterized using energy-dispersive X-rays, as illustrated in Figure 26.3. The results showed the presence of iron at 47.8%, oxygen at 9.4%, and copper at 42.7%, indicating a high degree of purity [20], as depicted in Figure 5.

Characterization of $CuFe_5O_8$ by SEM

The morphological and structural compositions of CuFe5O8 nanoparticles, synthesized using environmentally friendly methods, were analyzed using a scanning electron microscope (SEM). Figure 6 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 64.17 nm [21].

Anticancer Activity of CuFe5O8-NPs

This study employed the MTT test to measure the cellular toxicity of metal oxide nanoparticle composites in PC3 prostate cancer cells. Concentrations of 20, 40, 80, 160, and 320 parts per million (ppm) were added

after 24 hours, and the concentration at which half of the inhibitory effect was seen (IC50) was determined. The vitality of PC3 cells was evaluated following incubation with CuFe5O8 nanoparticles synthesized using green chemistry compared to the blank control. Following 24 hours of cultivation at a concentration of 20 µg/ml, the cancer cell mortality rate was determined to be 33.69%, demonstrating the impact of CuFe5O8 on cancer cells. The percentages of cell death at doses of 40, 80, 160, and 320 µg/ml were 38.53%, 46.42%, 56.11%, and 69.59%, respectively. These results suggest that more significant concentrations had a greater influence on cell death[22]. Figure 7 demonstrates that the IC50 value at 24 hours was 88.46%. The effect of flutamide on the viability of PC3 prostate cancer cell lines was studied compared to the blank control after a 24-hour incubation period at a concentration of 20 µg/ml. The percentage of cancer cell death was 6.51%, indicating an effect of Flutamide on cancer cells. At concentrations of 40, 80, 160, and 320 ppm, the percentages of cell death were 10.55%, 27.69%, 32.48%, and 52.53%, respectively, indicating an increased effect with higher concentrations. The results indicated an IC50 value of 296.8% at 24 hours, as shown in Figure 8. The metastatic nature of prostate cancer is marked by the increased expression of fatty acids (FA) and lipid synthesis. The overproduction of cell surface FAS, a death receptor known as FAS, is derived from tumor tissues and cell lines. Indicates an advanced phase of the spread of cancerous cells to other parts of the body. As a result, it hinders the synthesis of fatty acids. Pharmacologically triggering programmed cell death Biologically active compounds are crucial for suppressing the growth of cancer cells. However, our understanding of the effects of oregano essential oil-based nanoemulsion (OENE) on cancer prevention is currently restricted. Therefore, our current study aimed to assess the influence of OENE on lipid biosynthesis metabolism and enhance the activation of apoptosis in PC3 cells. Our findings indicate that OENE has the potential to be an effective therapy option since it can induce apoptosis in prostate cancer cells[23].

Toxicity Test of CuFe5O8 NPs on Blood Cells

The cellular toxicity of metal oxide nanoparticle composites on blood cells was tested at 50, 250, and 500 µg/mL concentrations. The test results, as shown in Figure 9, demonstrated the compounds' non-toxic nature at all concentrations.

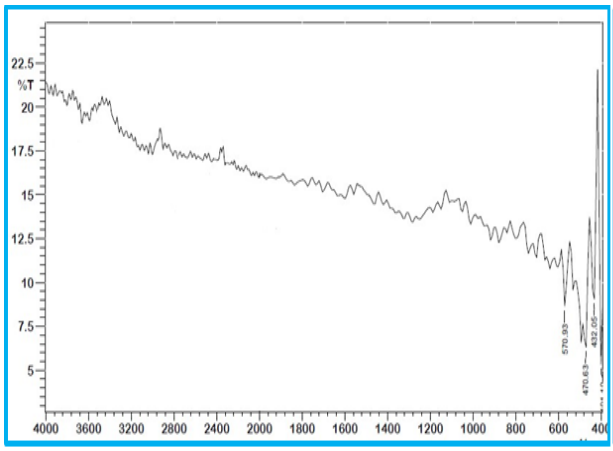

Figure 1: FT-IR spectrum of the compound ($CuFe_5O_8$).

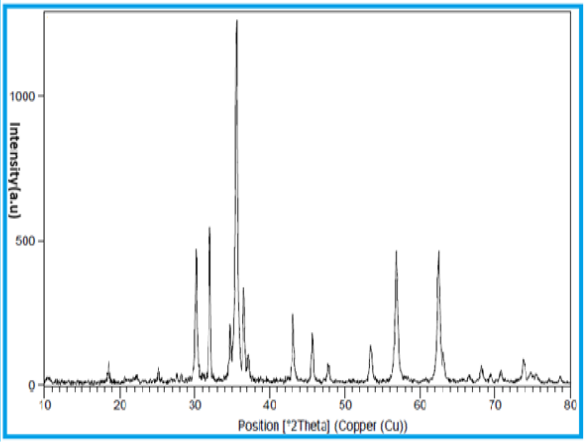

Figure (2): XRD spectrum of the compound ($CuFe_5O_8$).

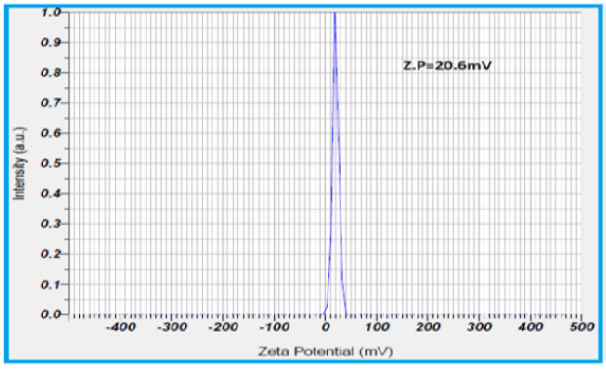

Figure 3: zeta potential of the compound ($CuFe_5O_8$).

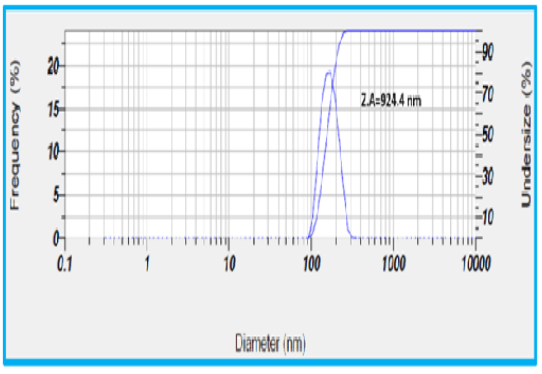

Figure 4: Zeta potential of $CuFe_5O_8$ nanoparticles

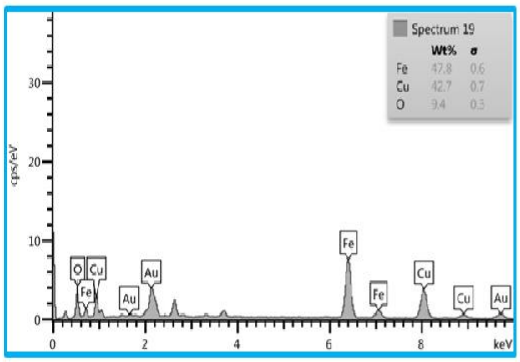

Figure 5: Energy Dispersive X-ray spectrum of the compound ($CuFe_5O_8$

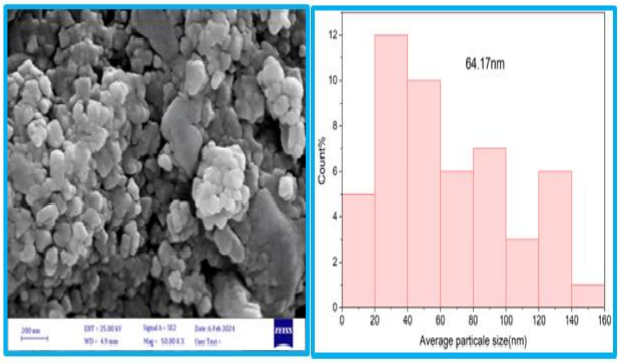

Figure 6: SEM $CuFe_5O_8$ nanoparticles

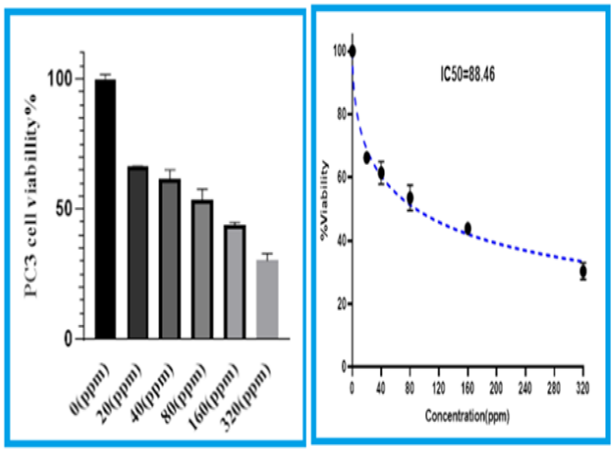

Figure 7: Effect of nano $CuFe_5O_8$ on PC_3 cells in 24 hours

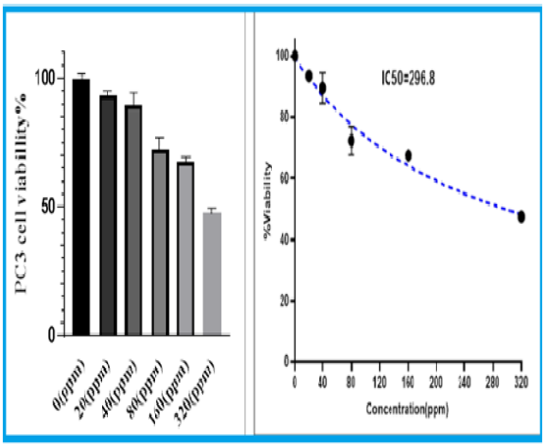

Figure 8: Effect of Flutamide on PC_3 cells at 24 hours

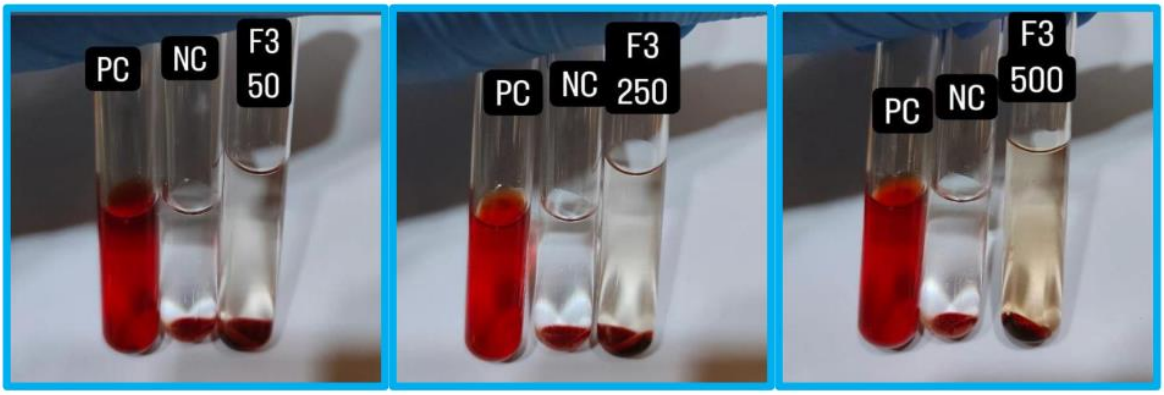

Figure 9: Hemolysis test for $CuFe_5O_8$ nanoparticle

4. CONCLUSION

In this study, CuFe5O8 nanoparticles were prepared by utilizing Origanum vulgare leaf extract in a novel, simple, and inexpensive procedure that incorporated three techniques: co-precipitation, ultrasound, and green chemistry. The following methods were used to characterize the produced nanoparticles: XRD, FT-IR, EDX, SEM, and DLS. to ascertain their structural properties. $CuFe_5O_8$-NPs show promise as a therapeutic material because of their ability to release heavy metal ions gradually.

This makes them an anticancer agent. $CuFe_5O_8$-NPs were demonstrated in a cell viability assay to inhibit the PC3 cell line. However, our understanding of the effects of oregano essential nanoemulsion on cancer prevention is currently restricted. Therefore, our current study aimed to assess the influence of biosynthesis

metabolism and enhance the activation of apoptosis in PC3 cells. Our findings indicate that $CuFe_5O_8$-NPs have the potential to be an effective therapy option since they can induce apoptosis in prostate cancer cells

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