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RESEARCH PAPER

Green Synthesis of $CuCr_2O_4$ Nano Composite from Rosemary Extract and Evaluation of Its Anti-Breast Cancer Properties

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ABSTRACT

In this study, $CuCr_2O_4$ nanoparticles were prepared by utilizing rosemary 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 Zeta Potential. The average particle size measured in the XRD was 40.65nm. Still, the energy dispersive X-ray spectroscopy analysis showed that the weight percentages of the $CuCr_2O_4$ nanoparticles prepared by the green method are Cr 47.8%, Cu 42.7%, and O 9.4%. The Zeta Potential ZP was 5.6 mV. The average particle size in the SEM was 41.51 nm. The efficacy of the prepared nanoparticles was studied on the MCF-7 breast cancer cell line and compared with the drug Nolvadex-D used in the treatment of breast cancer in Iraq. 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 Nolvadex-D had a cell killing value of (12.29%, 25.29%, 41.01%, and 54.59%) respectively at 24 hours, as well as for $CuCr_2O_4$, respectively at 24 hours, the cell killing results were (28.96%, 60.55%, 43.56%, and 64.01%) as well value IC50 (247.7ppm).

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INTRODUCTION

Recent advancements in synthesizing nanomaterials via green and sustainable methods have attracted significant interest, attributed to their environmental benefits, economic viability, and capacity for large-scale production [1]. Green synthesis utilizes natural resources, including plant extracts, which act as reducing and stabilizing agents in nanomaterials. Rosemary (Rosmarinus officinalis) is notable among plant extracts for its abundant bioactive components, including polyphenols, flavonoids, and terpenoids, which have significant antioxidant and anticancer effects. Copper chromite ($CuCr_2O_4$) is a substantial material in nanotechnology, distinguished by its exceptional physicochemical features, including excellent thermal stability, electrical conductivity, and catalytic activity. Furthermore, $CuCr_2O_4$ has demonstrated considerable anticancer efficacy, which can be augmented when synthesized using environmentally friendly processes utilizing bioactive plant extracts. The nanoparticles specifically aim for breast cancer using an aptamer, antibodies, and the surface marker breast-

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specific membrane antigen [4]. The synthesized nanoparticles precisely target breast cancer by employing an aptamer, antibodies, and the breast-specific membrane antigen as a surface marker. The World Health Organisation formally endorsed alternative medicines as adjunctive therapies based on substantial evidence of their benefits. This indicates a noticeable revival of interest in herbal medicine and its development. Phytochemical substances exhibiting unique capabilities that combat cancer have been extensively recognized in cancer therapy [5]. MCF-7 is an adhesive epithelial cell line recognized for significantly contributing to breast cancer research, particularly in studying estrogen receptor (ER) alpha. This cell line is among the few that express considerable levels of ER, resembling the majority of invasive human breast cancers that also express ER[6]. Nanoparticles generated using this environmentally friendly method improve stability, minimize deformation and aggregation of nanoparticles, and promote phytochemical adsorption from the nanoparticle surface, increasing reaction velocity [7]. Research indicates that plant-derived Cu/CrO nanoparticles possess antitumor efficacy against colon, breast, leukaemia, liver, cervical, ovarian, skin (epithelioma), lung, and gastric malignancies [8]. This paper presents a method for synthesizing novel copper $CuCr_2O_4$ nanoparticles utilizing Rosmarinus officinalis leaf extract. These nanoparticles exhibit significant potential as a tailored instrument for treating breast cancer. Additionally, the outcomes were juxtaposed with those derived from the administration of Nolvadex-D, a medicine frequently prescribed in Iraq for the treatment of both malignant and benign tumours related to breast cancer.

MATERIALS AND METHODS

Internationally sourced chemicals are utilized; rosemary vulgare is procured from the Iraqi market, and Nolvadex-D tablets with a potency of 250 mg are obtained from ALIUD PHARMA in Germany.

Preparation of rosemary leaf extract

Fifty grams of rosemary vulgare leaves should be measured out and mixed with 500 milliliters of deionized water at a 10:1 ratio. After placing the mixture on a magnetic stirrer, let it stir at 50°C for an hour. Filter the mix after that, and keep the liquid that results in a cool place [9].

Synthesis and Characterization of $CuCr_2O_4$ Nanoparticles

Dissolve 2.01 grams of $CrCl_3.6H_2O$ in 25 ml of rosemary vulgare leaf extract to get a 0.5 molar solution of $CrCl_3.6H_2O$ salts. Dissolve 1.7 grams of $CuCl_2.2H_2O$ in 25 ml of the extract to make a 0.5 M solution. The two solutions were combined and subjected to magnetic stirring for 30 minutes, undergoing 350 cycles at a temperature of 40 °C after that. The mixture was subjected to ultra-sonication for 10 minutes. Subsequently, a NaOH solution with a concentration of 2 M was gradually introduced. The pH was then adjusted to 7, leaving the mixture on a magnetic stirrer. Subsequently, $NaBH_4$ was progressively introduced to it at a concentration of 1 M. Subsequently, the solid residue was subjected to filtration and rinsed with ethanol on two occasions and deionized water on three occasions. The remaining substance was subjected to a drying process at a temperature of 180 °C. The residue was subjected to combustion at 650 °C for 4 hours.

Characterization Techniques of $CuCr_2O_4$ NPs

X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) were used to analyze the $CuCr_2O_4$ nanoparticles. Using a Shimadzu device from Kyoto, Japan, an X-ray diffraction (XRD) study was used to estimate the crystallite size of the nanoparticles. The samples' FTIR spectra were obtained with a Shimadzu device from Tokyo, Japan. A 200 kV Zeiss SEM from Germany and a Zetasizer Ultra particle analyzer from Malvern Panalytical.

MTT Assay for $CuCr_2O_4$ NPs

The MTT assay for $CuCr_2O_4$ nanoparticles employed a 10 mg/ml concentration of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide as the MTT dye. The $CuCr_2O_4$ nanoparticle samples were dissolved in a solution containing 0.2% DMSO to create concentration gradients. The concentrations were determined 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 $CuCr_2O_4$-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 [10].

Assay for hemolysis using $CuCr_2O_4$ NPs

The hemolysis assay was employed to evaluate the presence of $CuCr_2O_4$ 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 of 100, and subsequently analyzed on a slide. Following 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 [11].

RESULTS AND DISCUSSION

Characterization of $CuCr_2O_4$ nanoparticles by FT-IR

The chemical bonding of $CuCr_2O_4$ 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. Fig. 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 880 cm^{-1}, which can be attributed to the vibration of the Chrome oxide (Cr-O) bond. These findings are in agreement with previous studies [12].

Characterization of $CuCr_2O_4$ by X-ray Diffraction (XRD)

The crystalline structure and purity of $CuCr_2O_4$ nanoparticles synthesized by the green chemistry approach were analyzed using X-ray diffraction.

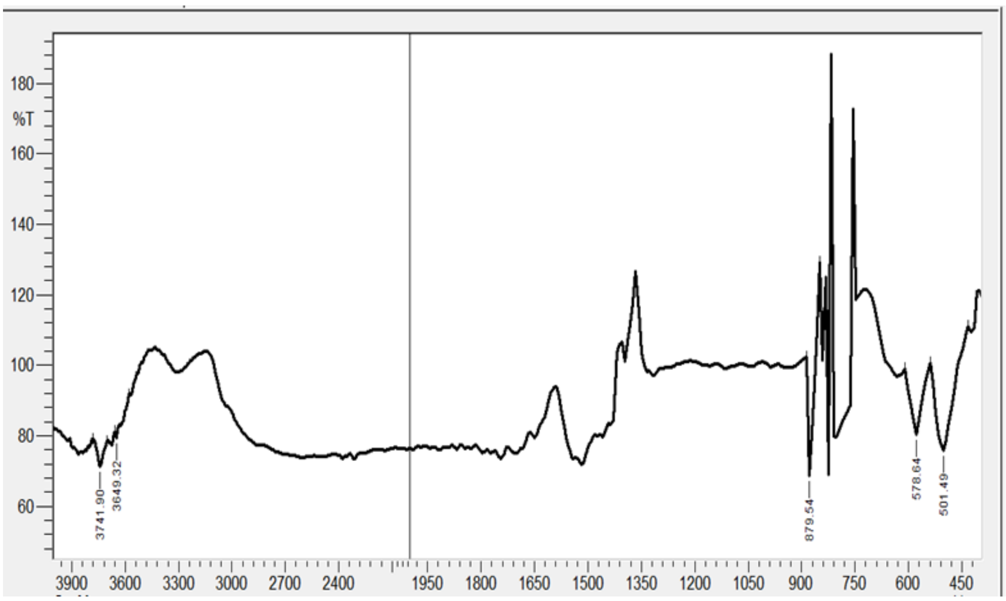

Fig. 1. FT-IR spectrum of the compound $CuCr_2O_4$

The X-ray diffraction spectrum of the synthesized $CuCr_2O_4$ nanoparticles closely corresponded with the standard spectrum of $CuCr_2O_4$, as illustrated in Fig. 2, in conjunction with International Centre for Diffraction Data (ICDD) card no: 96-231-0654 [13]. The mean crystallite size was determined to be 40.65 nm. The crystal morphology was determined to be tetragonal.

Characterization of $CuCr_2O_4$ by Zeta Potential (ZP)

The surface charge of chrome-copper nanocomposites was analyzed to assess the stability of nanoparticle dispersion. The values of Zeta potential (mV) indicate significant instability at (±0-10) mV, relative stability at (±10-20) mV, some stability at (±20-30) mV, and high stability at ±30 mV, which offers adequate repulsive force for improved physical colloidal stability. Overcoming van der Waals forces requires the presence of electrostatic repulsion forces [14]. The surface charge of $CuCr_2O_4$ nanoparticles synthesized through green chemistry was measured at 5.6 mV, as illustrated in Fig. 3.

Characterization of $CuCr_2O_4$ by Energy Dispersive X-rays

Energy-dispersive X-rays were used to determine the elemental makeup of $CuCr_2O_4$

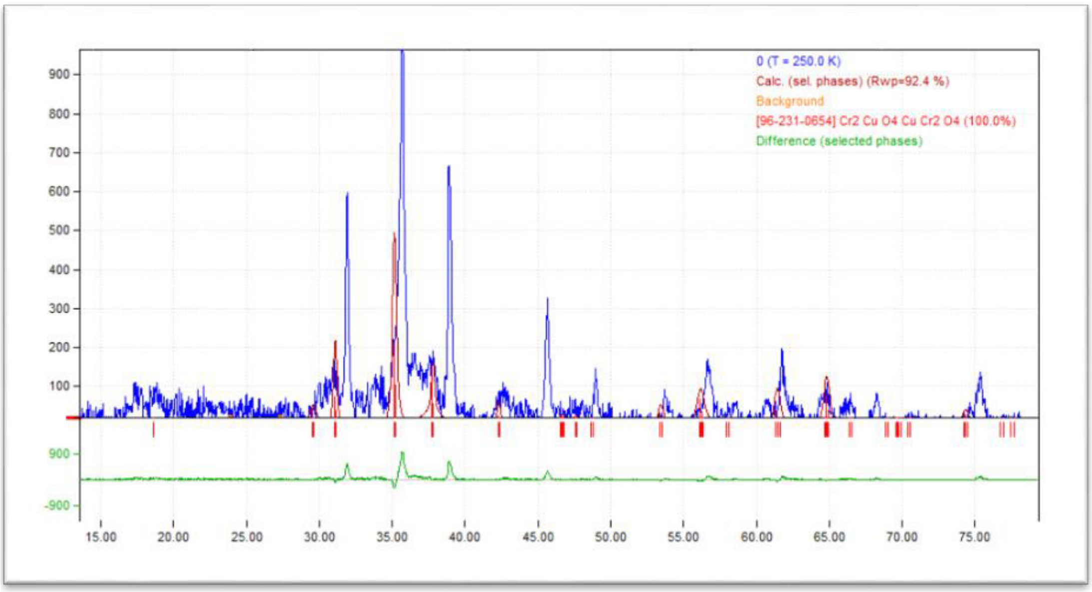

Fig. 2. XRD spectrum of the compound $CuCr_2O_4$

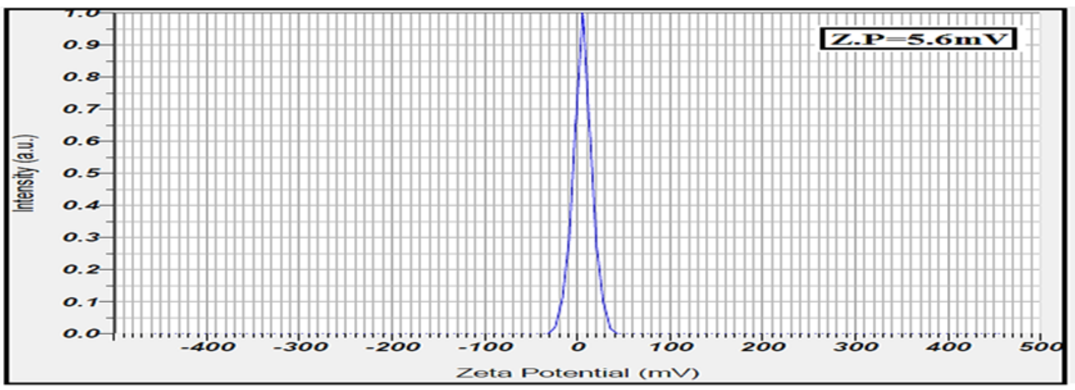

Fig. 3. Zeta Potential of the compound $CuCr_2O_4$ nanoparticle

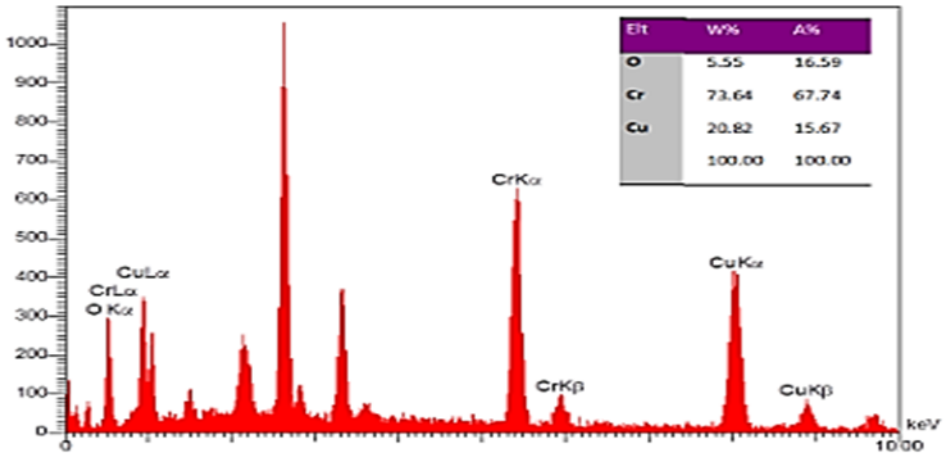

Fig. 4. X-rays of the compound $CuCr_2O_4$ nanoparticle

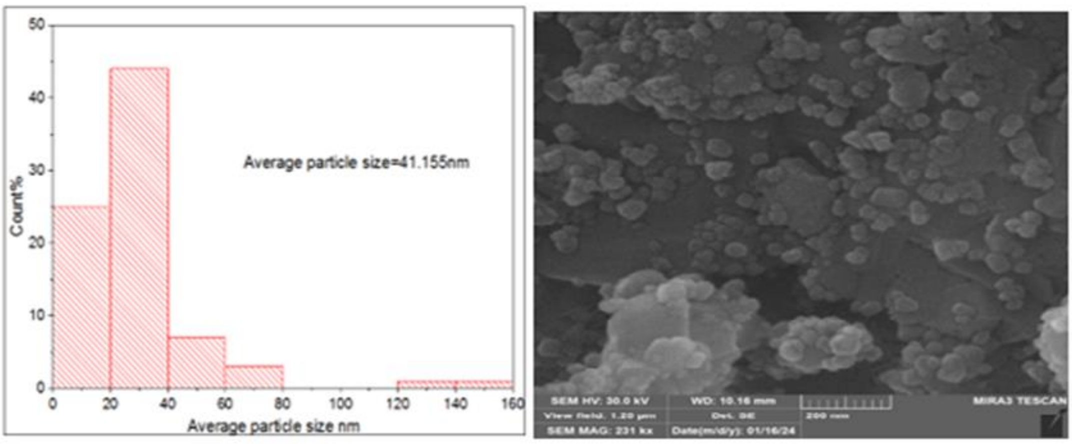

Fig. 5. SEM of the compound $CuCr_2O_4$ nanoparticle

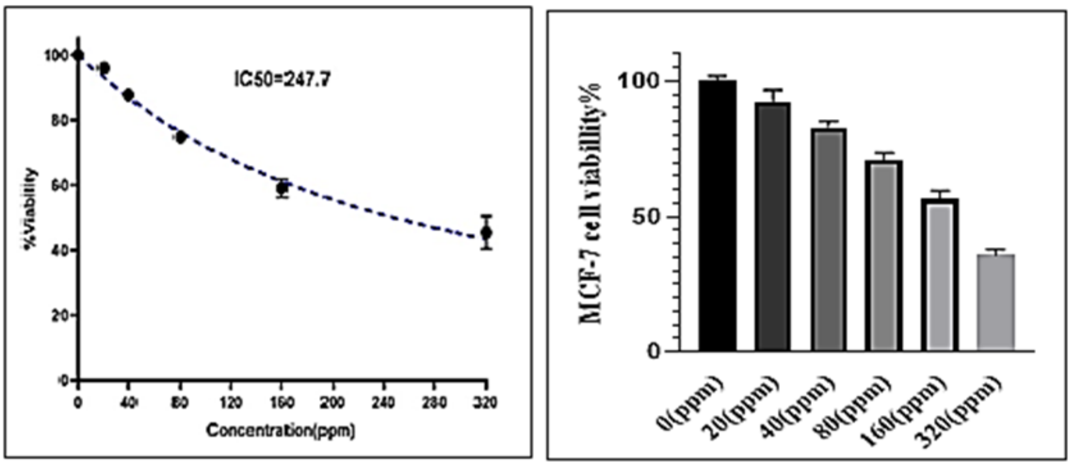

Fig. 6. Effect of nano $CuCr_2O_4$ onMCF-7 cell in 24hours

nanoparticles made using green chemistry, as shown in Fig. 4. The results indicated a high level of purity, with copper present at 42.7%, oxygen at 9.4%, and chrome at 47.8%.

Characterization of $CuCr_2O_4$ by SEM

Using a scanning electron microscope (SEM), the morphological and structural makeup of $CuCr_2O_4$ nanoparticles produced in an eco-friendly manner were examined. The particles were produced at the nanoscale scale, as seen in Fig. 5. According to the scanning electron microscopy (SEM) scans, the majority of the nanoparticles were widely distributed. Nonetheless, several of them were seen to be clumped together. Electrostatic forces caused this cluster to develop, and these particles had an average size of about 41.51 nm [15].

Anticancer Activity of $CuCr_2O_4$ NPs

The cellular toxicity of $CuCr_2O_4$ nanoparticle composites in MCF-7 cells was assessed in this study using the MTT test. To determine the concentration at which half of the inhibitory effect was observed (IC50), 20, 40, 80, 160, and 320 (ppm) concentrations were added after 24 hours. After being incubated with green chemistry-synthesized $CuCr_2O_4$ nanoparticles, the viability of MCF-7 cells was assessed compared to the blank control. Fig. 6 shows the cell-killing results after 24 hours, which were 28.96%, 60.55%, 43.56%, and 64.01%, along with the IC50 value of 247.7 ppm. The impact of Nolvadex-D on the vitality of MCF-7 cancer cell

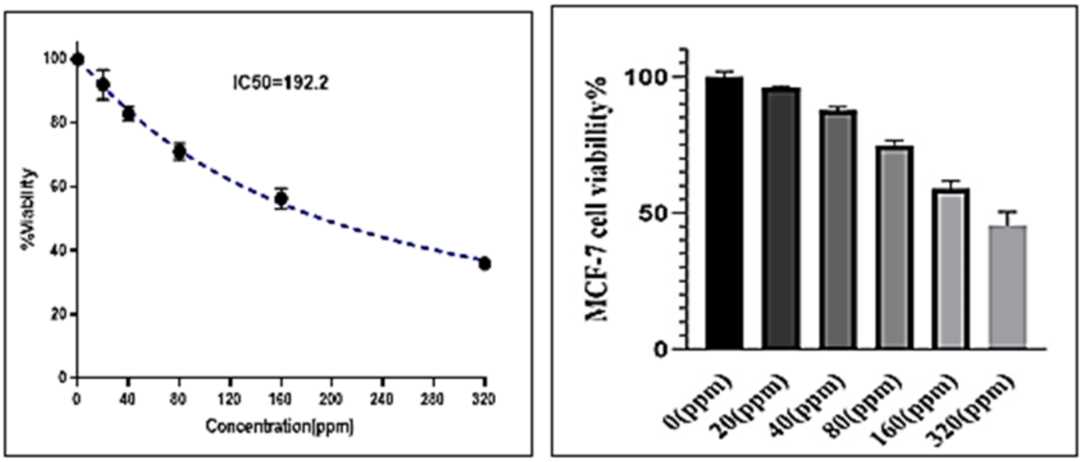

Fig. 7. Effect of Nolvadex-D on MFC-7 cell in 24hours

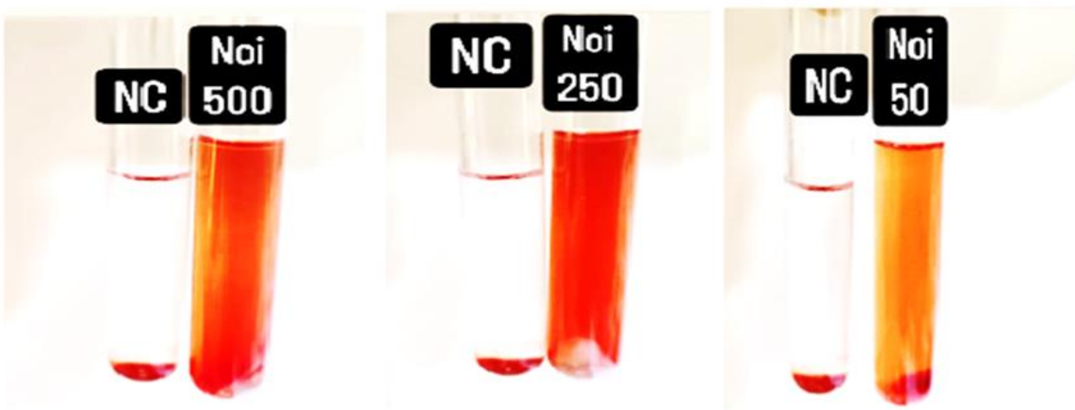

Fig. 8. Hemolysis test for the compound $CuCr_2O_4$ nanoparticle

lines was examined against the blank control during a 24-hour incubation at concentrations of 20, 40, 80, 160, and 320 ppm. The percentages of cancer cell death were 12.29%, 25.29%, 41.01%, and 54.59%, respectively, demonstrating an enhanced impact with elevated doses. The results showed an IC50 value of 192.2% at 24 hours, as illustrated in (Fig. 7). Nanoparticles exhibit toxicity by inducing oxidative stress by generating reactive oxygen species [16]. The elevated levels of reactive oxygen species (ROS) induce oxidative stress within cells, ultimately resulting in cell death via programmed mechanisms [17]. Furthermore, elevated levels of reactive oxygen species (ROS) can damage the mitochondrial membrane, ultimately triggering programmed cell death [18]. In HeLa cells, treatment with nanoparticles results in cell death attributed to reduced glutathione levels and elevated lipid peroxide levels, leading to cell death through an oxidative stress response [19]. The accumulation of silver nanoparticles in the nucleus of GBM cells results in chromosomal instability and mitotic disruption. Nanoparticles disrupt the structure of cellular actin, ultimately leading to cell death. Mitochondria-dependent apoptosis may also be induced by treatment with nanoparticles. Nanoparticles can dissociate ions, disrupting mitochondrial membranes and releasing cytochrome into the cytoplasm [11].

Toxicity Test of $CuCr_2O_4$ 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 Fig. 8 demenstruated the compounds' non-toxic nature for all concentrations.

CONCLUSION

This work used a unique, easy, and affordable process that combined three methods, co-precipitation, ultrasound, and green chemistry, to create $CuCr_2O_4$ nanoparticles using rosemary leaf extract. The generated nanoparticles were characterized using XRD, FT-IR, EDX, and SEM to determine their structural characteristics. $CuCr_2O_4$-NPs' capacity to release heavy metal ions gradually makes them a promising therapeutic material. They are an anticancer agent as a result. $CuCr_2O_4$-NPs were shown to inhibit the MCF-7 cell line in a cell viability study. However, we know little about how rosemary essential nanoemulsion affects cancer prevention. Thus, our current work sought to improve the activation of apoptosis in MCF-7 cells and evaluate the impact of biosynthetic metabolism. According to our research, $CuCr_2O_4$-NPs can cause breast cancer cells to undergo apoptosis, which suggests that they could be a practical therapeutic approach.

CONFLICT OF INTEREST

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

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