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Anticancer activities of franklinite $ZnFe_2O_4$nanoparticles made from Origanum vulgare leaf extract

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ABSTRACT
In this study, a new, easy and cheap method was used to prepare ZnFe2O4 nanoparticles using Origanum vulgare leaf extract, where three methods were combined into one method: green chemistry, co-precipitation, and ultrasound to prepare ZnFe2O4 nanoparticles. The prepared nanoparticles were characterized using the following techniques: XRD, FT-IR, EDX, SEM, and DLS. The average size of the particles in the SEM was 134.48 nm, while the average size of the particles in the XRD was 41.88 nm. At the same time, the average size of the particles in the DLS was 381.5 nm. The effectiveness of the prepared ZnFe2O4 nanoparticles was compared with the drug Tamoxifen used in Iraq in treating breast cancer on the MCF-7 cell line. The results showed the excellent effectiveness of the prepared nanoparticles and their superiority over the drug used. They were also characterized by their lack of cytotoxicity compared to the toxicity of the drug on red blood cells in the toxicity screening test if the results showed cell killing for ZnFe2O4 (32.5%, 54.44%, and 76.7%. 89.37%, and 97.59%) while the drug Tamoxifen cell killing results are (8.01%, 17.14%, 28.96%, 43.56%.and 64.05%).

Keywords: franklinite, ZnFe2O4, nanoparticles,Origanum,Tamoxifen,MCF-7 cell

INTRODUCTION

Generally speaking, in developed and developing countries, breast cancer (BC) is the most common gynaecological cancer in terms of incidence and death(Siegel et al., 2018) [1]. Even though the prognosis and quality of life for patients with BC have significantly improved due to the development of medical therapeutic techniques such as surgery, targeted therapies, and chemotherapy and radiotherapy, drug resistance continues to be a major obstacle to the cure of BC(Harbeck and Gnant, 2017) [2]. The result of abnormal and unchecked cell proliferation of malignant cells in the breast tissue is. BC is the third most prevalent cause of death worldwide and the second most common malignancy in women(Ganesan and Xu, 2020) [3]. Surgery, radiation, chemotherapy, and other adjuvant and neoadjuvant therapies are all part of the multidisciplinary approach used in BC therapy(Tampaki et al., 2018) [4]. Chemotherapy is a method that uses chemical substances to kill cancer cells. Despite being the most successful method of cancer treatment, these chemotherapy drugs have several adverse effects due to their cytotoxic properties (Fisusi and Akala, 2019)[5]. Additionally, radiation therapy lowers the chance of cancer death and recurrence. However, it usually entails radiation exposure to nearby organs, raising the possibility of lung and cardiac conditions. These treatments may raise the risk of leukaemia, particularly when used with specific adjuvant chemotherapy classes(Taylor and Kirby, 2015) [6]. On the other hand, because of their detrimental effects on healthy tissues and organs, these therapeutic approaches frequently fail to treat BC(Zhu et al., 2021) [7].The failure of therapeutic medicines, which operate not only on the tumour sites but also cause severe adverse effects on healthy tissues and organs, producing toxicity to humans, is the primary cause of these bad effects and the death rate. BC is classified according to histological categories and is a very diverse and heterogeneous illness. Invasive ductal carcinoma is the most prevalent kind of BC, while other less frequent subtypes are notable for their severity and clinical presentation(Wu et al., 2017) [8].The tumour's stage is the next main issue. Cancer develops in two stages: the first is a primary tumour that starts inside the breast tissue and grows quickly to other tissues and lymph nodes (stages 2-3) or distant organs such as the lung, bone, liver, or brain (metastasis, or stage 4)(Sinn and Kreipe, 2013) [9].Most common medications lose much of their potency after the cancer spreads. Therefore, developing new, safe, and effective treatments for this deadly malignant illness is essential. To target cancerous tissues, it is imperative to find highly effective therapies that can discriminate between benign and malignant cells and cross natural barriers. These drugs "smartly" respond to the intricate tumour microenvironment to release the correct dosage range when needed(Peer et al., 2020, Ganesan et al., 2019) [10, 11]. Cancer diagnosis and treatment could be modernized

with the help of tumour nanotechnology. Protein engineering and material science advancements have aided in the creation of creative nanoscale targeting techniques, giving BC sufferers new hope. By thoroughly penetrating tumours, nanoparticles (NPs), recognized as pharmaceutical carriers, offer a novel avenue for drug delivery to cancer cells with a high degree of specificity to the targeted cancer cells(Khoobchandani et al., 2020, Alsagaby et al., 2020, Xiao et al., 2021, Ke et al., 2019) [12–15]. Moreover, NP therapy reduces harmful effects on organs and healthy tissues(Mamnoon et al., 2020) [16]. The National Cancer Institute has authorized nanotechnology, believing it to be a superb paradigm-shifting method for enhancing BC detection and treatment(Chen et al., 2021) [17]. With encouraging clinical results, several therapeutic NPs have already received approval and are widely used as BC adjuvant therapy(Montero et al., 2011, Zhao and Astruc, 2012) [18,19]. Regarding the size, shape, and makeup of the biomaterials containing the drugs, NP-based drug delivery systems offer a variety of viable designs that improve drug solubility, stability, circulatory half-life, biodistribution, and release rate while lowering immunogenicity, toxicity, and side effects(Ghafari et al., 2020) [20]. Researchers are increasingly using plant extracts to create medications; phytotherapy is a field of medicine that uses plants to cure or heal diseases. This type of treatment is used in a wide range of situations, including as a post-chemotherapy measure, to address respiratory infections, to treat cold and flu symptoms, to reduce inflammation, to treat neuropathic pain, to treat depression, and to treat a variety of chronic illnesses(Efferth et al., 2017) [21]. The majority of the plants in the Lamiaceae family are utilized as therapeutic agents in alternative medicine to treat a variety of illnesses. These plants or their extract are employed in numerous medical specialities. The Lamiaceae family comprises 4000 species and 200 genera worldwide. Because of its organoleptic qualities, the Thymus, Satureja, and Origanum genera are added to meals. They are also frequently drunk as herbal teas to alleviate rheumatism, indigestion, infectious disorders, cramps, diarrhoea, and muscle soreness. Many species in the Origanum genus have demonstrated their pharmacological potential as anti-inflammatory, Renoprotective, Vaso protective, cardioprotective, antinociceptive, insecticidal, hepatoprotective, antidiabetic, antihyperlipidemic, and anti-cancer. These findings are based on multiple preclinical studies(Sharifi-Rad et al., 2021) [22]. Scientific studies have shown that NPs have the potential to be therapeutic agents and that they function as a medication delivery mechanism (Hu and Du, 2020)[23]. NPs participate in a variety of biological processes, including proliferation, metastasis, immunosurveillance, and drug sensitivity, and they can target several types of cancer cells, including cancer cells and cancer stem cells(Ruenraroengsak et al., 2019) [24]. Additionally, NPs demonstrated great effectiveness as a cytotoxic agent when aimed at drug-resistant cancer cells(Liu et al., 2016, Hammadi, 2022a) [25, 26]. In this study, we present a worksheet to prepare for the novel design and development of franklinite ZnFe2O4 nanoparticles made from Origanum vulgare leaf extract, which represents a tailored and promising tool for the treatment of BC. And comparing the results with the drug tamoxifen, which is used to treat malignant and benign tumours of breast cancer in Iraq Materials and Methods

Items of Research

$ZnCl_2$, Molecular Weight=136.28 from India (CDH) Purity=98%, $FeCl_3$, Molecular Weight=162.21 from India (CDH) Purity=99%, Deionized waterfrom Iraq Babylon Purity=99%, NaOH, Molecular Weight=40.00 from India (SDS) Purity=98%, $NaBH_4$ Molecular Weight=37.83 from Sigma-Aldrich Purity=99%, CH_3CH_2OH Molecular Weight=46. 07 from England (BDH) Purity=99%, Origanum vulgare from Iraq Market, Tamoxifentablets 20mgfrom Saudia Arabia.

Preparation of Origanum vulgare leaf extract

Take 50g of Origanum vulgare leaves, add 500ml of deionized water in a ratio of 10:1, and place it on a magnetic stirrer for an hour at a temperature of 50°C then filter the mixture and store the filtrate in a cool place(Hammadi, 2022b)[27].

Preparation of Franklinite ZnFe2O4 nanoparticles by using green chemistry

Prepare 0.5 molar of FeCl3 salts by dissolving 2.01gm in 25ml of Origanum vulgare leaf extract. Prepare 0.5 molar of ZnCl2 by dissolving 1.7gm in 25ml of the extract. The two solutions were mixed and placed on the magnetic stirrer for half an hour, 350 cycles at a temperature of 40°C after that. The mixture was placed in an ultrasonic device for 10 minutes, after which the NaOH solution was slowly added at a concentration of 2 molars, the pH was adjusted to 7, and it was left on the magnetic stirrer. Then NaBH4 was added to it slowly at a concentration of 1 molarity. After that, the precipitate was filtered and washed using ethanol twice and deionized water three times. The residue was dried at 180°C. For 4 hours, then the residue was burned at 650 C for 4 hours. Note: All concentrations were prepared using Origanum vulgare leaf extract.

Approaches for characterization

Numerous techniques, such as X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy, were used to analyze the $ZnFe_2O_4$ nanoparticles (SEM). XRD was used to

determine the crystallite size of the nanoparticles (Shimadzu, Kyoto, Japan). The samples' FTIR spectra were obtained using Shimadzu (Tokyo, Japan). SEM analysis was performed using a 200 kV Zeiss SEM (Germany). Malvern Panalytical - particle analyzer - Zetasizer Ultra. Litesizer DLS 100 Anton Paar.

MTT test for $ZnFe_2O_4$ nanoparticles

For the ZnFe2O4 nanoparticle MTT assay, 10 mg/ml of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide was utilized as the MTT dye. ZnFe2O4 nanoparticle samples were dissolved in 0.2% DMSO to produce gradients of concentration measured in ppm at 20, 40, 80, 160, and 320. An RPMI media-prepared 200 µl sample of suspended cells (1 × 104 cells/well) was distributed. The cells were grown in 5% CO2 for 24 hours at 37 C. After the cell cultures were treated with 20 µl of ZnFe2O4 -NPs, they were let to incubate for a further twenty-four hours under the same conditions. After that, 10 µl of MTT reagent was added to each sample, and it was incubated for five hours at 37°C. The absorbance was measured at 570 nm(Hammadi, 2022a)[26].

Assay for hemolysis using $ZnFe_2O_4$ nanoparticles

The hemolysis assay was used to screen for ZnFe2O4 at various levels (50, 250, and 500 ppm) to identify hazardous or non-toxic compounds. The blood sample was extracted from the lab, put in an EDTA tube, viewed under a microscope at a magnification of (100), and then examined on a slide. After separating the blood cells and plasma in an EDTA tube, the mixture was centrifuged for ten minutes. Following the removal of the cells' plasma layer, the cells underwent ten minutes of centrifugation cycle repetition while being repeatedly rinsed with PBS and supplemented with 1ML of PBS.The cells were removed from the PBS after two minutes. Once the blood cells underwent many rounds of washing, 1ML and 9ML PBS were combined to generate the blood cell suspension. Each tube has a volume of 1200 µL for the antagonist, which is introduced in different concentrations. The final volume (1.5 ml) is then filled with 300 µL of the cell suspension. After two hours of incubation, each tube is spun apart for five minutes at a speed of 1000 cycles per minute.The difference in hemolysis was then measured using the Heh control settings (test tubes containing blood and PBS, test tubes containing blood and deionized water only). The compound's toxicity in combination with blood components is shown by the (+) option following centrifugation. The (-) option indicates that the blood components were not mixed after centrifugation, indicating that the medication was safe(Wayne, 2011)[28].

RESULTS AND DISCUSSION

Characterization of $ZnFe_2O_4$ nanoparticles by FTIR

Description Sample functional groups are analyzed by FTIR characterization. The FTIR spectrum was used to examine the ZnFe2O4 nanoparticles' chemical bonding, with a wavenumber ranging from 400 to 4000 cm^{-1}. Absorption peaks are visible in Figure 1, including one at wavenumber 578 cm^{-1}, which corresponds to the metal's vibration mode on a tetrahedral lattice (Zn−O). According to theory, the interaction of the metal with oxygen on a tetrahedral lattice occurs in the wavenumber ranges of 550–750 cm^{-1} [(Li et al., 2011, Li et al., 2017)29,30]. At wave number 470-694 cm^{-1}, the octahedral vibration mode (FeO) was detected(Lakay, 2013, Husain et al., 2021) [31,32].

Characterization of $ZnFe_2O_4$ nanoparticles by dynamic light scattering (DLS)

The granular size of ZnFe2O4 was measured to determine the extent of stability of the nanoparticles in colloidal solutions and how they are affected by the solvent. Ionic water was used as a solvent, and the granular size of ZnFe2O4 was 381.5 nm. as shown in Figure 2

Characterization of ZnFe2O4 nanoparticles by (XRD) and (EDX)

Using XRD, ZnFe2O4 nanoparticles were measured. In Figure 3, the XRD data are displayed. There are ten primary peaks from the multiple known peaks at 2θ; seven of these peaks are sharp and intense, indicating a high degree of ZnFe2O4 crystallinity. The peaks, which are 30.45, 35.74, 37.35, 43.34, 53.62, 57.62, 62.65, 66.82, and 70.97. According to the ICCD card standard No. (96-900-6896) and the Crystal system cubic data, the relative intensity data of the peaks on the XRD diagram compare favourably with the properties of ZnFe2O4(Levy et al., 2000)[33]. Using the Debye-Scherer equation, the average crystalline size was determined to be 41.88 nm. The percentage of elements present in nano-ZnFe2O4 prepared with green chemistry was determined using energy dispersive X-rays (EDX), as shown in Figure 4. The results showed the presence of iron96.59%, oxygen2.62% and zinc 1.01%which indicates a good degree of purity(Kumar et al., 2016) [34].

Characterization of $ZnFe_2O_4$nanoparticles by SEM

The morphological and structural compositions of ZnFe2O5 nanoparticles prepared with green chemistry were studied using a scanning electron microscope (SEM). Figure 5 shows that the particles were prepared in the nanometer range. The SEM images indicated that most of the nanoparticles were well separated, while some

were present in an agglomerated form. This agglomeration is due to electrostatic effects, and the rate of the diameter of these particles is about 134.48nm(Shetty et al., 2017) [35].

Inhibition of $ZnFe_2O_4$ nanoparticles for MCF-7 cells

A study was made of the effect of ZnFe2O4 nanoparticles prepared with green chemistry on the vitality of MCF-7 cell lines compared with the drug Tamoxifen after a 24-hour incubation period at a concentration of 20, 40, 80, 160, and 320 ppm (the percentage of killing cancer cells, respectively) was 32.5%, 54.44%, and 76.7%. 89.37% and 97.59%, which indicates increased killing effectiveness of ZnFe2O4 on cancer cells with increasing concentration, as shown in Figure 6. The results in 24 hours showed an IC50 value = 34.42, as in Figure 7, while the results of Tamoxifen after a 24-hour incubation period at a ppm concentration of 20, 40, 80, 160, and 320 were, respectively, the percentage of killing cancer cells (8.01%, 17.14%, 28.96%, 43.56%). 64.05%. It can be seen that the killing effectiveness of ZnFe2O4 nanoparticles is much greater than that of Tamoxifen when using the same concentrations and the same conditions, as shown in Figure 8. The results showed an IC50 value of 192.2, as shown in Figure 9.

Toxicity test of ZnFe2O4 nanoparticles on blood cells

The cytotoxicity of ZnFe2O4 nanoparticles was tested on blood cells at concentrations of 500, 250, and 50 ppm, and the results were compared with the drug Tamoxifen. The results showed no toxicity of ZnFe2O4 nanoparticles, as shown in Figure 10. In contrast, the toxicity of the drug was observed at the same concentrations as the control group, as shown in Figure 11. increases redness with increased toxicitySeveral investigations have also revealed that cellular absorption efficiency, concentrations, exposure duration, and nanoparticle size may all have an impact on the degree to which NP-mediated cell death occurs(Foldbjerg et al., 2009, Asare et al., 2012) [36, 37]. Recent research in the relevant literature indicates that nanoparticles can kill tumour cells while having little to no influence on the cell death of normal cells(Taccola et al., 2011) [38].Determination of Apoptotic Evaluations Notably, as mitochondria are thought to be essential signalling nodes in the apoptotic pathway, several apoptosis regulators can be inhibited or cause damage to mitochondria during apoptosis. Anti-apoptotic proteins (Bcl-2) and Bcl-2 family proteins (Bak, Bax) can also play a main role in mitochondrial-mediated apoptosis(Green and Walczak, 2013, Whitaker and Placzek, 2019) [39, 40]. Moreover, a great deal of research has examined the ability of NPs to induce oxidative stress and then produce ROS through the use of nanoparticles that can destroy tumour cells(Pati et al., 2016, Yang et al., 2009) [41, 42].

Conclusions

ZnFe2O4NPs demonstrated positive cytotoxicity against breast malignant cells. Treated cells can trigger apoptotic pathways. Furthermore, ZnFe2O4NPs raised the mRNA expression of the tumour suppressor gene p53. The newly produced ZnFe2O4NPs may serve as a significant starting point for further research on cancer treatment. Therefore, there is hope for halting the spread of breast cancer from the results of the current study, unlike chemotherapy.

Author contribution

Heba Jassim Younis, Mustafa Hammadi .analyzed data, interpreted results, prepared manuscript, conceptualized and collected data.

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Competing financial interests

The authors have no conflict of interest.

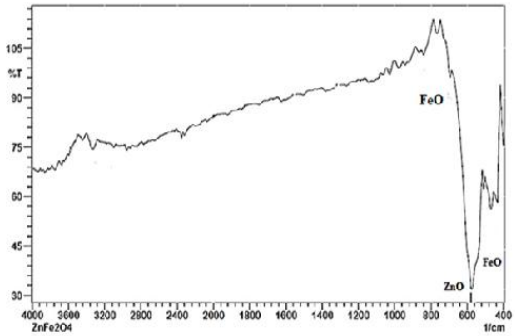


Figure 1: ZnFe2O4 nanoparticles' FTIR spectra

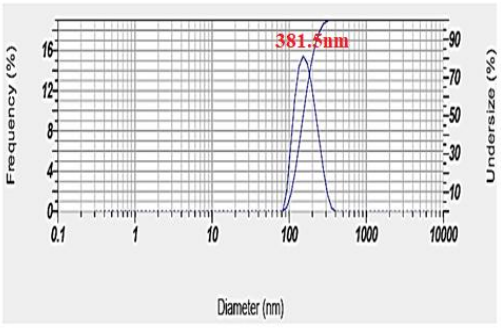


Figure 2: ZnFe2O4 nanoparticles dynamic light scattering (DLS)

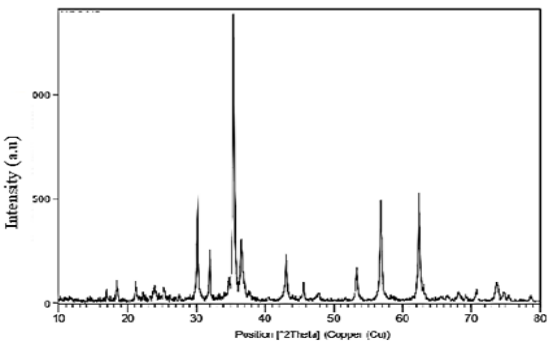
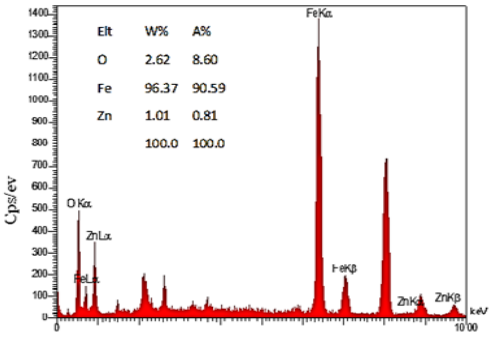
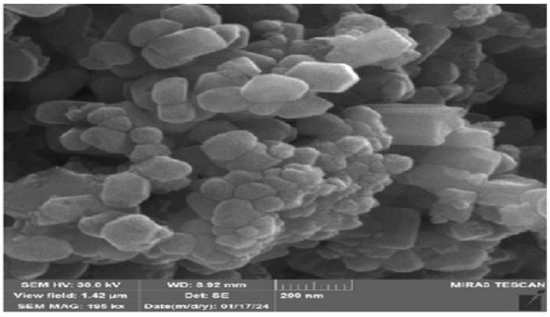


Figure 3 :X-ray diffraction spectrum of ZnFe2O4 nanoparticles



Figur.4 : Energy-dispersive X-rays of ZnFe2O4 nanoparticles



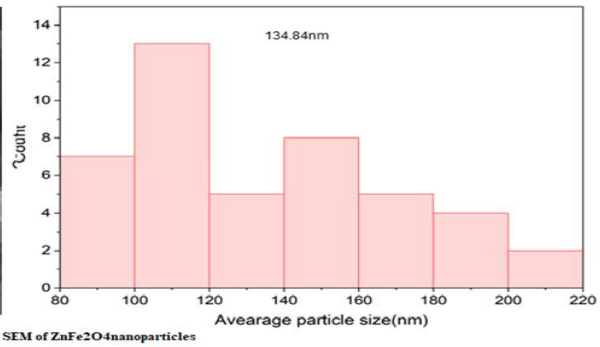


Figure. 5 : SEM of ZnFe2O4nanoparticles

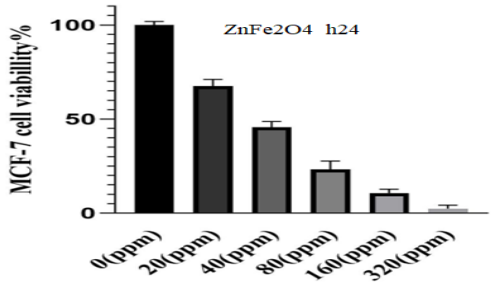


Figure 6: Inhibition of ZnFe2O4nanoparticles for MCF-7 in 24 h

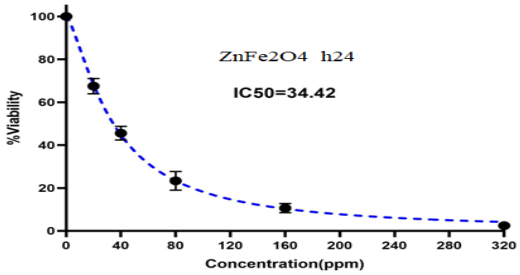


Figure 7: IC50 of ZnFe2O4 nanoparticles for MCF-7in 24h

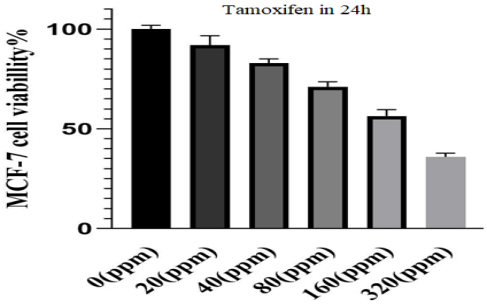


Figure8: Inhibition of Tamoxifen for MCF-7 in 24 h

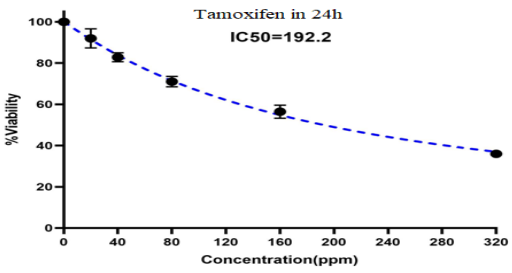


Figure9: IC50 of Tamoxifen for MCF-7 in 24 h

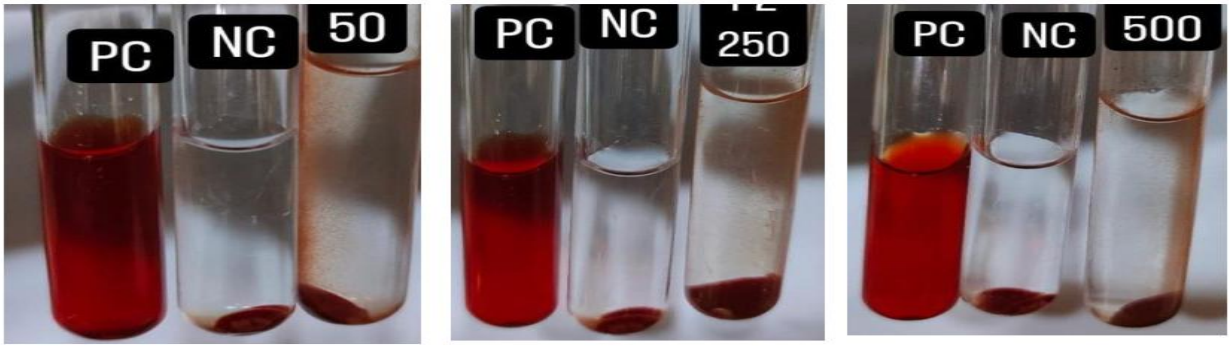


Figure 10: Hemolysis test for ZnFe2O4 nanoparticle

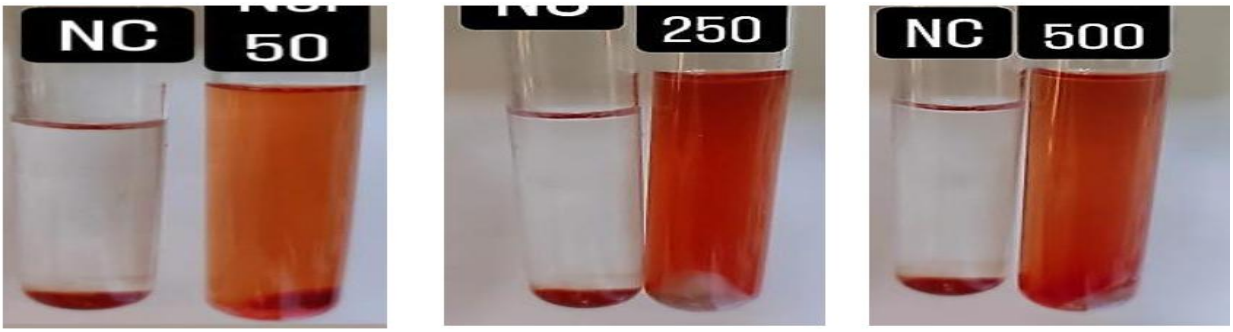


Figure 11: Hemolysis test for Tamoxifen nanoparticle

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