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RESEARCH ARTICLE

Cytotoxic effect of cobalt ferrite nanoparticles on NIH-3T3 and malignant cancer cell lines CaCo2 and CAL27

Amirhossein Baharvand[1], Shirin Shafiei Lialestani[1,2*]

[1] Student Research Committee, Arak University of Medical Sciences, Arak, Iran

[2] Faculty of Dentistry, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

The focus of many investments was pulled towards magnetic nanoparticles and their extending range of implementations in medicine and biology that particularly include drug delivery, magnetic resonance imaging, and cancer treatment through hyperthermia, as well as other objectives such as pollutants degradation and compounds separation. This work attempted to arrange cobalt ferrite nanoparticles ($CoFe_2O_4$ NPs) by taking advantage of starch aqueous solution combined with a calcination process at 500, 600, and 700 °C. The acquired prepared NPs, which were muti-dimensional shape, were configured by the employment of X-rày Diffraction (XRD), Vibràting Sample Màgnetometer (VSM) and Energy Dispersive X-rày (EDX), Field-emission Scànning Electron Microscope (FESEM), and Ràman spectroscopy. The outcomes of VSM analysis were indicative of a ferrimagnetic attitude, while the performance of MTT assay helped in assessing the cytotoxicity of $CoFe_2O_4$ NPs on colon càncer cell (CaCo2), oral squamous cell carcinoma (OSCC) CAL27 and mouse embryò fibròblast cell (NIH-3T3) lines, which resulted to be non-toxic towards cancer and normal cell lines. Considering these observations, we can approve the ideal applicability of our synthesized $CoFe_2O_4$ NPs in medical implementations similar to drug delivery and non-medical utilizations.

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INTRODUCTION

Nowadays, nanotechnology has been brought into the service of many varying industrial and medicinal fields owing to its facilitation of consequential alterations in the physical and chemical qualities of materials[1]. More particularly, magnetic nanoparticles succeeded in gathering an extending rate of attention from biomedical arena by exhibiting magnetic features and the potential to form biological interactions throughout cellular and molecular tissues [2, 3]. Among the examples of medical and pharmaceutical implementations of magnetic nanoparticles, one can refer to their employment in drug delivery, magnetic resonance imaging, and thermotherapy of cancer cells [4-6], which provides the localization of heat therapy to heighten the temperature of cancer cells up to above 22 degrees Celsius and lead to their annihilation. Considering the escalating rate of investments in magnetic heat therapy as a result of its low side effects, it was predictable to witness an expansion in conducted researches on the application of specific substances similar to Spinelite ferrites due to containing the topmost thermal efficiency[7].

With the chemical formula of MFe_2O_4 (M: Fe, Co, Mg, Mn, Ni), Spinelite ferrites are included in magnetic products category, which proved to be quite convenient for medical utilization by exhibiting fascinating traits that include being chemically stable, containing a comparatively high magnetization, and the ability to generate heat throughout magnetic fields with high frequency. As a magnetic substance, Cobalt ferrite ($CoFe_2O_4$)

* Corresponding Author Email: *shirin.shafiei1995@gmail.com*

is consisted of high blind temperature (520 °C), high coercive field (5.4 kOe), medium saturation magnetism (80 emu/g), and high anisotropy constant (~ 10^6 erg/cm^3), which is also known to be mechanically hard, electrically resistance, and chemically stable. The combination of these features suggest cobalt ferrite as a an appropriate product for pharmaceutical implementations similar to drug delivery[8, 9] .

Particle size is an essential factor that can impact the obtained thermal efficiency since varying sizes contain their own thermal efficiencies[10]. The Neel and Brown relaxation of magnetic fields with particular frequencies rely on the size of nanoparticles and in cases with smaller particles, the dominance of Neel relaxation and its powerful effect on the generated heat is quite evident [11, 12]. There are several synthesizing approaches for cobalt ferrite nanoparticles including hydrothermal, co-precipitation, sol-gel, microemulsion and sono chemical procedures[13-16]. However, a considerable number of these options proved to be environmentally dangerous and therefore, greener synthesizing methods can stand as an interesting proposition. In particular, the focus of many was attracted to the exertion of harmless and low toxic secondary metabolites from plants in the roles of reducing and inhibiting agents [17, 18]. In contrast to other regular chemical and physical procedures, nanoparticles with varying morphologies can be achieved through a cost-effective manner by the benefits of using greener precursory choices, which include less complicated, inexpensive, and unchallenging fabrication process and a lower rate of waste production.

The manufacturing of cobalt ferrite nanoparticles in differing sizes and magnetic features can be facilitated by the employment of environmentally friendly extracts. These materials are capable of affecting the obtained physical qualities as stated in the work of D. Gingasu *et al,* which reported the synthesis of $CoFe_2O_4$ by the employment of hibiscus flower and leaf extracts through the self-combustion and wet ferritization reactions. In this investigation, the plants flower/ leaf extracts were applied in the form of gelling and reducing agents. The obtained $CoFe_2O_4$ NPs through the self-combustion approach resulted in a complex porous nanostructure with the crystal size range of 10 to 18 nm, whereas the average crystalline size of synthesized samples achieved by the wet ferritization reaction was reported to be 18.8 nm[19]. According to another assessment, the attachment of cobalt ferrite nanoparticles was facilitated by the exertion of ginger root and cardamom seeds aqueous extracts in the form of reducing agents with the mean sizes of 12.4 and 14.7 nm, respectively. Both of the samples resulted in exhibiting astonishing magnetic features in their Mössbauer spectra[20].

The synthesized products of green routes can display an efficient performance in biomedical applications. The morphology, particle size, size distribution, and magnetic features of synthesized NPs are firmly reliant on the applied green sources (plant leaf extracts, plant roots, fruits, seeds, etc.). Biocompatibility and low toxicity are among the offered advantages of these NP that can be utilized throughout pharmaceutical and medical implementations[21-23] . This project attempted to arrange $CoFe_2O_4$ nanoparticles by the exertion of *starch in order to* study the photocatalytic and cytotoxic functionality of the obtained product towards colòn cancer cell (CaCo2), oral squamous cell carcinoma (OSCC) CAL27 and mòuse embryò fibroblast (NIH-3T3) cell lines through the results of MTT assay.

MARTIALS AND METHOD

Synthesis of $CoFe_2O_4$ nanoparticles

Shortly, an Erlenmeyer flask was prepared to hold 20 mL of iron nitrate ($Fe(NO_3)_2.9H_2O$) and 10 mL of cobalt nitrate solutions (weight ratio of 2:1) to undergo a paraffin bath at the temperature of 100 ˚C up to the point of reaching a homogeneous mixture. Thereafter, it was required to append 40 mL of starch (0.1 g/mL) aqueous suspension. Once the solvent was withdrawn, a calcination process was done on the eventuated dark precipitate by exerting a furnace at the temperatures of 500, 600, and 700 ˚C, respectively. The closing black powder of cobalt ferrite nanoparticles ($CoFe_2O_4$ NPs) was labeled as 500-CF, 600-CF, and 700-CF in terms of their calcination temperature.

Characterization

The Powder X-ray diffraction (PXRD) spectra of our product was configured by utilizing an X-ray diffraction mòdel X'Pert PRO MPD PANalytical Company (Netherlands Formation). Moreover, the identification of existing framework called for the exertion of scánning electron micròscopy (SEM), TESCAN model MIRA3, whereas the raman spectra were recorded by a Raman Takram

P50C0R10 at the wavelength of 532 nm. As the last part, we settled the electronic adsorption through the employment of an UV-Vis spectrophotometer model 1800, made by Shimadzo Japan.

Cytotoxic test

Cell culture

This section implicated the culturing of procured colon cancer cell (CaCo2), CAL27 and mouse embryo fibroblast cell (NIH-3T3) cell lines from the Iranian Biological Resource Center (IBRC, Tehran, Iran) within 25 mL flasks, which were consisted of DMEM, 10% (v/v) FBS, and 10 µL/mL penicillin-streptomycin antibiotics. In the following, the incubation of culture medium at the conditions of 5% CO_2 at 37 °C was required for the proliferation and growth of the cells.

MTT Assay

The seeding of cell lines (CaCo2, CAL27 and NIH-3T3) was individually completed in 96 multi-well plates with a density of 10×10^3 cells per well at the degree of 37 °C throughout 24 h up to the point of obtaining confluences. Thereafter, several doses of $CoFe_2O_4$ NPs (1–3000 µg/mL) were applied to the cells for the duration of 24 hours, while doxorubicin was put in the stance of positive control. To continue the procedure, it was needed to append 10 µL of MTT reagent to the wells for conducting incubation for an extra 4 h at 37 °C within dark conditions. Subsequent to the detachment of medium, the addition of 100 µL DMSO was considered for every well. Next, we recorded the generated formazan through the identification of absorbance at the wavelength of 570 nm, which required the employment of a microplate reader (Bio-Tek ELX800, USA). The data of viable cell percentages were provided in terms of the vehicle-treated group.

RESULTS AND DISCUSSION

XRD analysis

Fig. 1 illustrates the XRD spectra of obtained $CoFe_2O_4$ NPs by the performed synthesis through the usage of starch and calcination procedure at 500, 600 and 700 °C. The detected Bragg indexes of (220), (311), (400), (511), and (440) expressed the existence of a cubic spinel-kind of framework (JCPDS no. 79-1744) [24]. The outcomes of Scherrer equation [25] helped in approximating

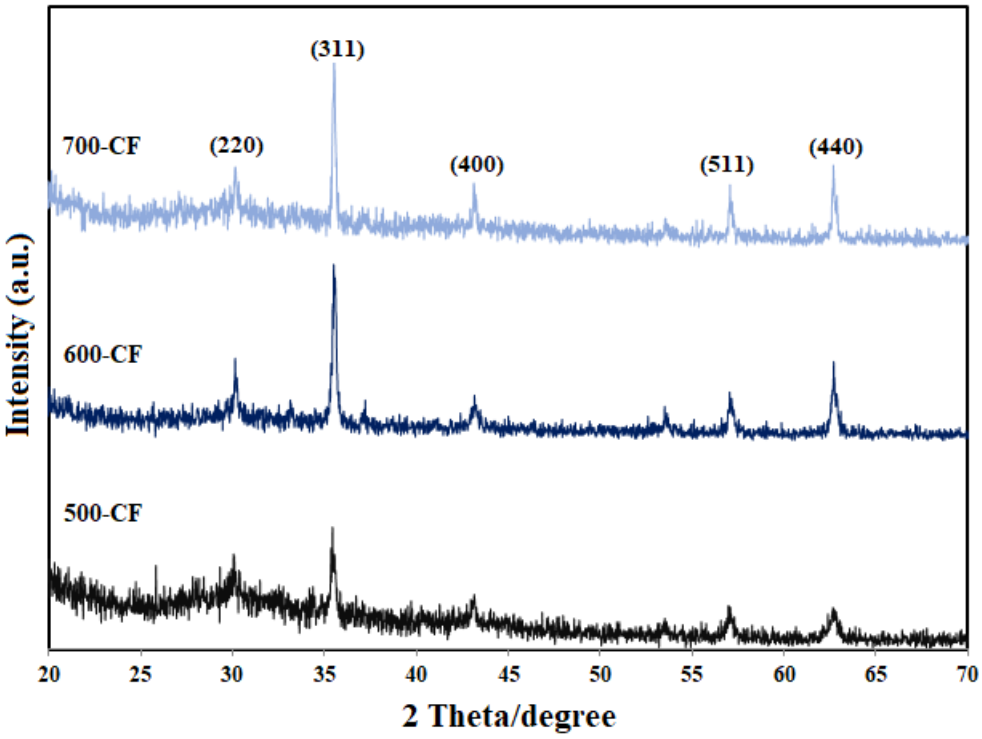

Fig. 1. XRD spectra of prepared $CoFe_2O_4$ nanoparticles using starch.

the crystallize size of synthesized products at 500, 600 and 700 ˚C temperatures to be 25.98, 43.59 and 55.48 nm, respectively, which also provided data for selecting the average width of highest intensity peak (311). As denoted by the observations, a heightening in the applied temperature of calcination led to the enlargement of particles sizes. Apparently, a larger number of active sites can be achieved in the case of small crystals, which would result in the absorbent of more photons and also fabricate a larger number of electrons-holes.

FESEM and EDX analysis

Fig. 2 demonstrates the FESEM images of $CoFe_2O_4$ NPs throughout three varying degrees of temperature, which exhibit the uniformity and multi-dimensional shapes of the particles in the course of heightening the exerted temperature of calcination. These data are in complete agreement with the XRD assessments as they mentioned the existence of a powerful connection among the simultaneous growth of particles upon the heightening of calcination temperature. The

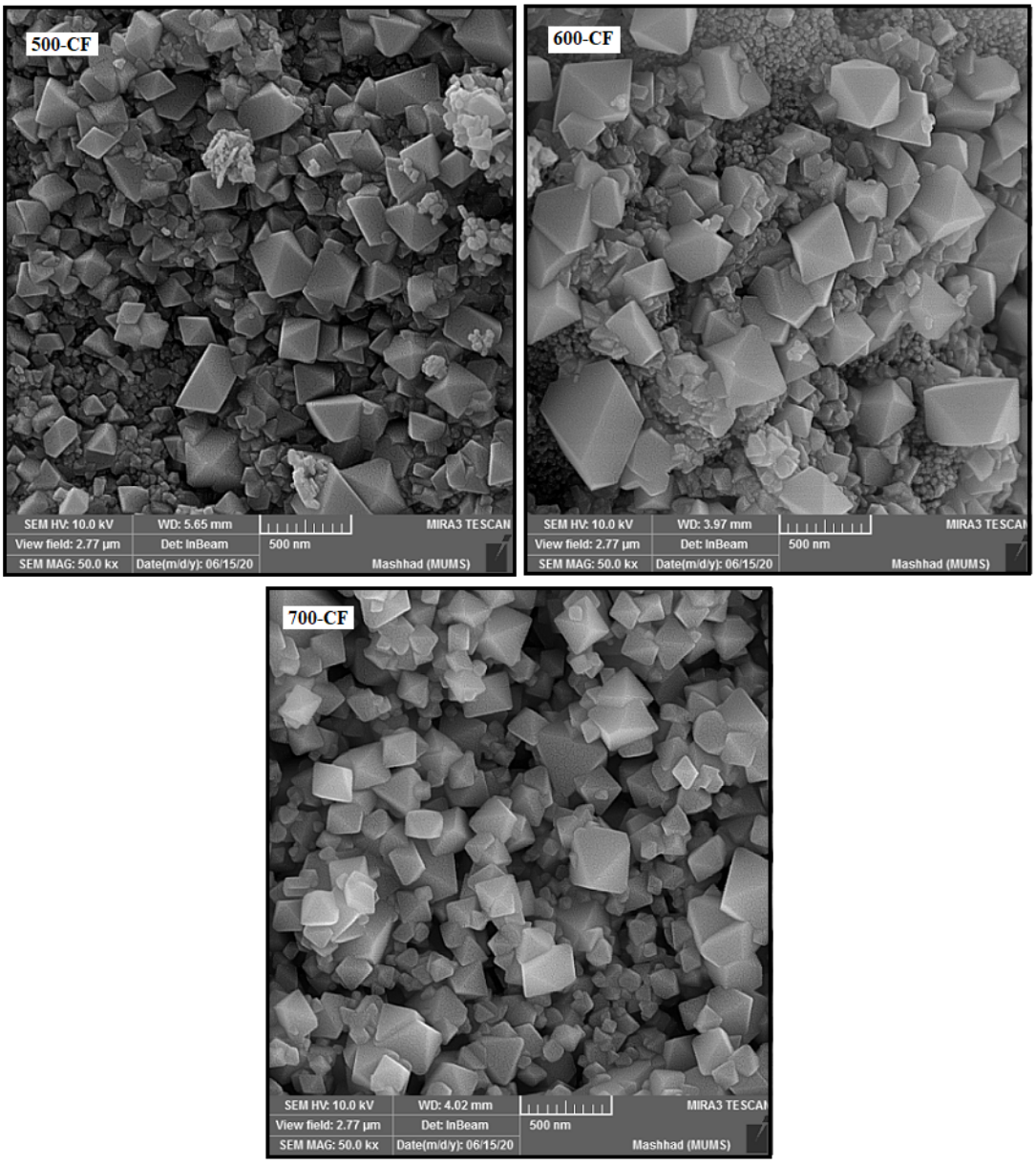

Fig. 2. FESEM images of prepared $CoFe_2O_4$ nanoparticles using starch.

acquired EDX graph was indicative of our products purity by exhibiting the solo appearance of iron, oxygen, and cobalt (Fig. 3).

VSM analysis

We inquired into the magnetic features of $CoFe_2O_4$ NPs through the implementation of

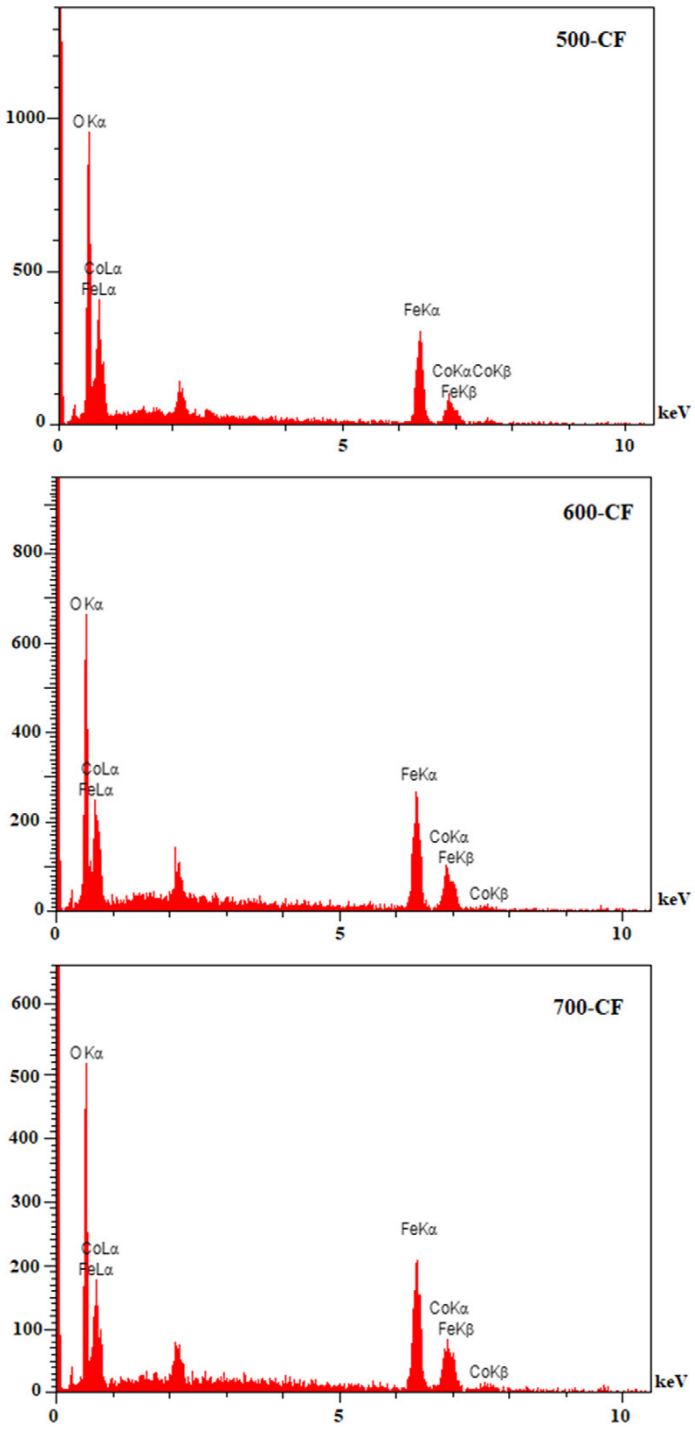

Fig. 3. EDX spectra of prepared $CoFe_2O_4$ nanoparticles using starch.

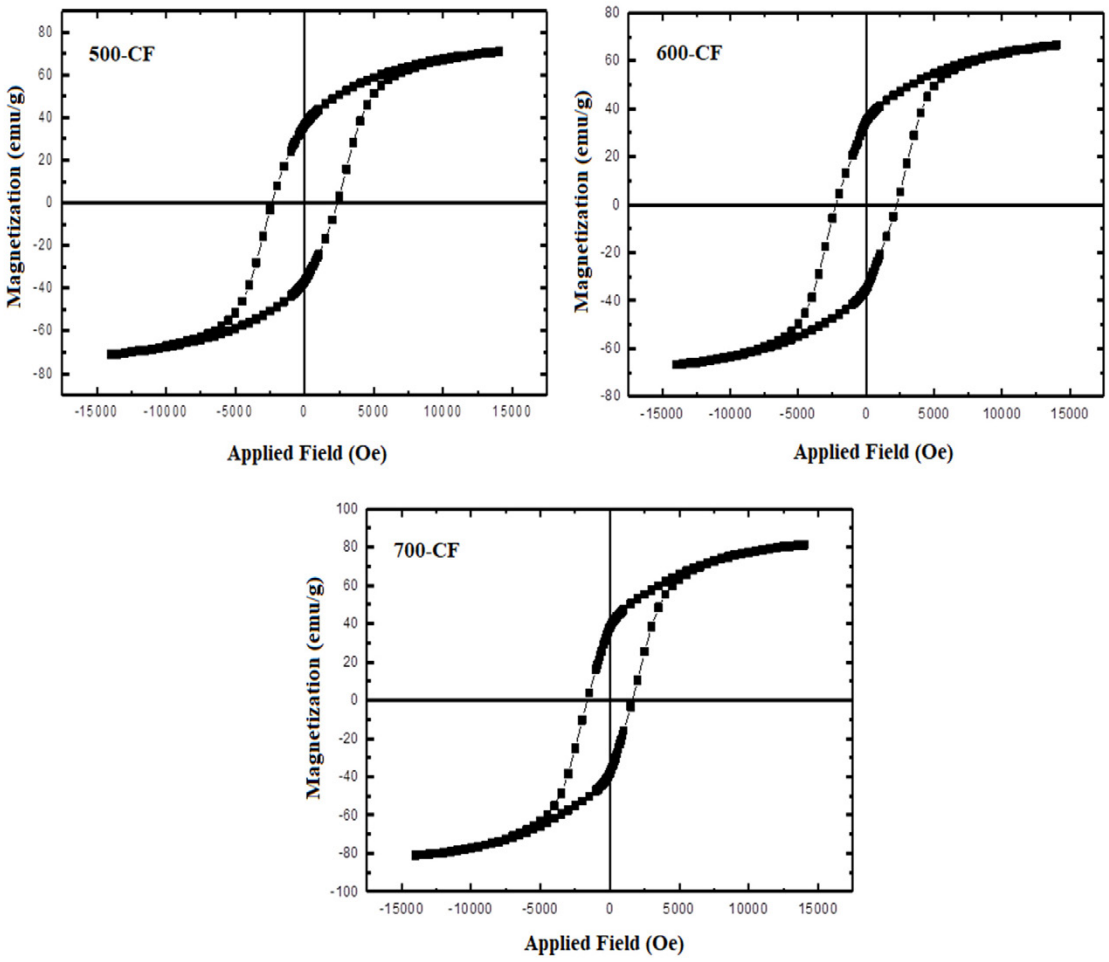

Fig. 4. VSM graph of prepared $CoFe_2O_4$ nanoparticles using starch.

vibrational sample magnetization at maximum magnetic. In coordination with Fig. 4, certain ferrimagnetic peculiarities were exhibited for the synthesized products by the hysteresis loops of $CoFe_2O_4$ NPs. The saturation magnetization (Ms) of $CoFe_2O_4$ NPs at 500, 600, and 700 ˚C were configured to be 65.2, 72.7 and 83.2 emu/g, respectively. As an assumption, magnetocrystalline anisotropy can be accountable for the existing relationship of calcination temperature with achieving a higher rate of Ms. A lower rate of saturation magnetism was perceived from the case of samples when compared to that of $CoFe_2O_4$ bulk (80.8 emu/g)[25], which may be ascribable to the spin rotation throughout the surface of nanoparticles. There is a presumption for the accountability of single domain (s) regarding the extension of nanoparticles coercive (from 65.2 to 83.2 Oe) upon the heightening of calcination temperature. Fig. 4 reveals the inducement of an increase in the magnetism subsequent to intensifying the ratio of surface-to-volume, which also expanded the volumetric magnetic effects of crystal and the saturated magnetism.

Cytotoxic performance

The attained data from MTT Assay as an uncomplicated, non-radioactive, and high-throughput approach can help in configuring the proliferation, viability, and cytotoxicity of cells, while being contingent on the transformation of water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into an insoluble formazan. Although the active metabolism of viable cells provides the reformation of MTT to formazan, however, there are no signals produced

by annihilated cells due to losing this feature. In this regard, the parameter of color formation can fulfill the function of a proficient marker for only the cases of viable cells. The quantified absorbance at OD 590 nm was equivalent to the amount of viable cells. The metabolic functionality of cells can be scrutinized through the conduction of MTT assay. Apparently, a sample can induce stronger signals upon the existence of higher metabolic functionality.

We attempted to survey the cytotoxicity of 500-CF nanoparticles towards colon cancer cells (CaCo2), CAL27 and mouse embryo fibroblast cell (NIH-3T3) line by the employment of MTT test, and the gathered outcomes are demonstrated in Fig. 5. Meanwhile, Table 1 presents the configured values of IC_{50} for 500-CF and the doxorubicin, which took the stance of control. The material of Figure 5 denote the non-toxicity of 500-CF nanoparticles in the cases of CaCo2, CAL27and NIT-3T3 cell lines, while the assays of IC_{50} were perceived to be 1086, 1290 and 2412 µg/mL for CaCo2, CAL27 and NIT-3T3 cell lines, respectively (Table 1). Our observations implied and approved

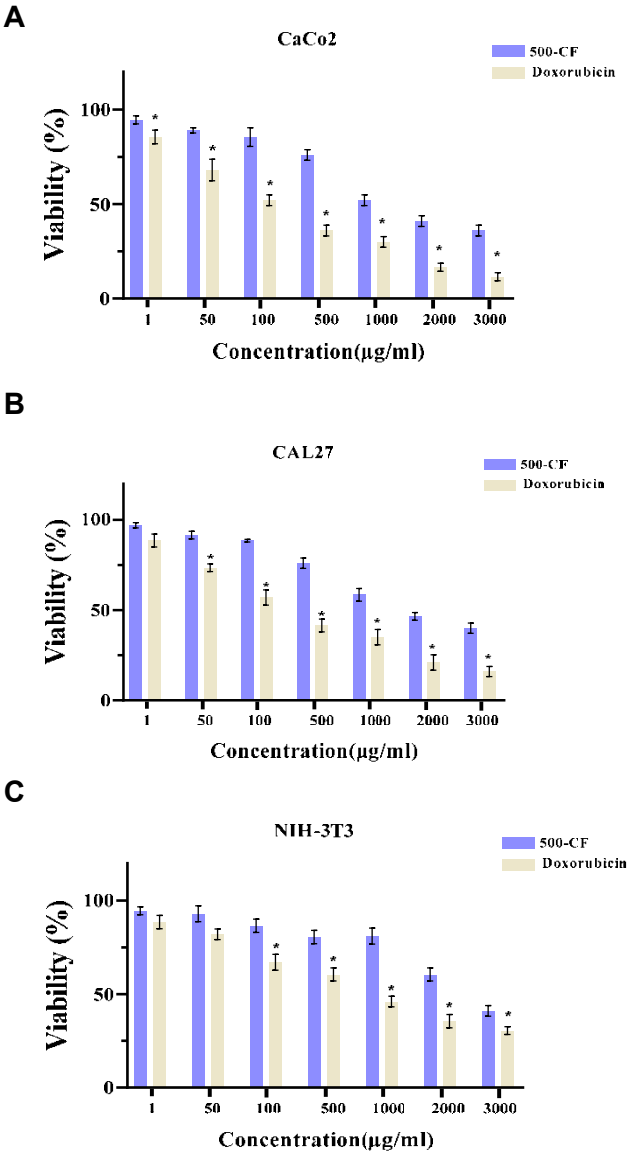

Fig. 5. Cell viability of 500-CF nanoparticles on CaCo2, CAL27 and NIH-3T3 cells after 24 h incubation.

Table 1. IC_{50} values of synthesized 500-CF nanoparticles using starch

Cell line	IC_{50} (µg/mL)	Control
	CF-500	Doxorubicin
CaCo2	1086	120.1
CAL27	1290	234.3
NIH-3T3	2412	856

the biocompatibility of green synthesized nanoparticles.

The relative non-toxicity of green nanoparticles affirms their secure utilization in biomedical implementations. To state an example, the assay of Rasouli *et al* implicated the manufacturing of green synthesized magnetite nanoparticles (Fe_3O_4-NPs) within an aqueous solution by the exertion of ferric and ferrous chloride along with company of several loads of organic honey (0.5, 1.0, 3.0, and 5.0 percent w/v). They performed an in vitro survey on the Fe_3O_4-NPs to check the viability through the implication of MTT assay on WEHI164 cells, while there was a lack of any evident toxic effects throughout larger volumes up to 140.0 ppm. These observations legitimate the utilization of this product in biological implementations similar to drug delivery[26] . Moreover, Hou et al. reported a description of utilizing *Ziziphora clinopodioides* Lam's aqueous extract to manage the green synthesis of cobalt nanoparticles (Co NPs). Their cytotoxicity assay revealed the non-toxicity of employed tactic due to prevailing the reliance of their products' cell survival on the applied dosage[27].

CONCLUSION

An expeditious and inexpensive approach was trialed to execute the synthesizing process of Cobalt ferrite nanoparticles ($CoFe_2O_4$ NPs) by the exertion of starch. In coordination with the FESEM images and PXRD assay, the particle size, crystallinity, and crystal size of the samples were enlarged as a result of heightening the degree of calcination. Furthermore, next to perceiving a ferrimagnetic attitude as the magnetic feature of our product, we also noticed an extension in saturation magnetization subsequent to heightening the degree of calcination. In the following sections, the toxicity of 500-CF towards CaCo2, CAL27and NIH-3T3 cell lines was inquired by MTT assay, which disclosed the non-toxic manner of 500-CF nanoparticles throughout the three cell lines. On the account of these observations, the deployment of this product in comparison to other formulations can be approved for cancer therapy and associated biological implementations similar to drug delivery.

CONFLICT OF INTEREST

There is no conflict of interest

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