In vitro cytotoxicity and anti-cancer drug release behavior of methionine-coated magnetite nanoparticles as carriers

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Abstract
A novel and specific drug delivery for in vitro cancer targeted are developed successfully by a simple one-step method. A CoFe2O4@Methionine core–shell nanoparticle was prepared by the reflux assay which amino acid in the surface makes ferrite biocompatible, enhances its chemical stability, and improves the drug-loading capacity. The synthesized nanoparticles were characterized using FTIR, TGA, XRD, SEM, TEM, and VSM which coating amino acid on the surface of CoFe2O4 was confirmed by XRD and TGA. The appearance of a new peak for C≡N confirms the formation of Letrozole-loaded carrier in the FTIR. The vibrating sample magnetometer of both bare CoFe2O4 and Methionine-coated CoFe2O4 nanoparticles exhibited room-temperature superparamagnetic behavior with a saturation value of 46 emu/g and 16.8 emu/g, respectively. The morphology and size of samples were characterized by SEM and TEM that the average size of the particle was around 28–29 nm. The loading of Letrozole and the effect of pH (5, 7.4) on the release behavior of the carrier was studied. The result of the drug release in pH is equal to 5 was about 88% which higher than pH is equal to 7.4. Also, the preparation had been evaluated for determining its cytotoxicity using MCF-7, MDA-MB-231, and MCF10A cells as an in vitro model, and the result vitro experiments showed that CoFe2O4@Methionine could significantly reduce cancer in cells model. These results demonstrate that core–shell nanoparticle was prepared is biocompatible and have potential use as drug delivery.
Keywords Drug delivery · Letrozole · CoFe2O4 nanoparticles · Methionine · Cytotoxicity · Cell line

Introduction

The number of cancers overtaken patients is increasing day by day [1–6]. One of the most common types of cancer, especially in women is breast cancer, and, unfortunately, its cancer-suffering patients are increasing each year [7–14]. It is widely studied and accepted that breast cancers are hormone dependent and that is estrogen which is a key mediator in the progression and metastasis of breast cancer [15]. Letrozole is one of the most effective third-generation non-steroidal aromatase inhibitors (AIs) that can inhibit excess estrogen biosynthesis in the body [4, 6, 8, 14]. Letrozole uses as positive drug to treat breast cancers and highly potent drugs due to its estrogen receptor [16–19].

Some strong steps for the treatment of cancer are required to develop new technology. One of the ways to kill the cancer cells by using targeted drug delivery in which the word “targeted” is referred to kill only cancerous cells without any harm of healthy cells. Recently, spinel ferrites have attracted much attention for their potential use in biomedical applications like controlled drug delivery, cell separation, magnetic resonance imaging (MRI), and localized hyperthermia. [9–13, 20–23]. Among them, Cobalt ferrite (CoFe2O4) shows notable results for the high coercivity, moderate saturation magnetization, high Curie temperature, large magneto crystalline anisotropy, high mechanical hardness, and remarkable chemical stability, also appropriates biocompatibility and low toxicity [24–26]. Furthermore, they can be directly injected into cancer cells and delivered by magnetic field gradient or delivered by other efficient drug delivery systems to release their drugs [9–13, 27].

Acknowledging several robust steps in cancer therapy needs advance guard. Commonly used method in cancer treatment is targeted drug delivery where they are targeted to destroy only cancerous cell by doing no harm to healthy cells. Recent studies on spinel ferrites draw attention to its potency of drug delivery, cell separation, magnetic resonance imaging (MRI), localized hyperthermia, and several other benefits towards biomedical [9–13, 20–23]. Out of all, Cobalt ferrite (CoFe2O4) shows notable results for the high coercivity, moderate saturation magnetization, high Curie temperature, large magneto crystalline anisotropy, high mechanical hardness, and outstanding chemical stability, also suitable biocompatibility, and relatively low toxicity [24–26]. Additionally due to the importance in cancer treatment, they can be administered straight into tumor cells either by magnetic field gradient or delivered by other efficient drug delivery systems to release their drugs [9–13, 27].

So far, various magnetic nanoparticles with different formulations have been synthesized for cancer therapy which to improve biomedical applications, surface modification
is necessary to coat them with stimuli responsive [28–30]. Methionine is one of the most crucial and primary bio-compatible amino acids in the human body, which has specialized in vivo physiological purposes. Three activated functional groups of Methionine (-COOH, -NH₂, and -SH) could be simply applied for the conjugation of metal atoms(CoFe₂O₄) which can use as the surface of a carrier to examine the loading and release behaviors that have not yet been reported[31, 32]. In 2018 Guangzhou Wang, Fei Zhou et al. revealed a facile synthesis of Cobalt Ferrite (CoFe₂O₄ nanoparticles) in the presence of L-cysteine (Lys) which can serve as a great carrier to cancer therapy [9–13, 33]. Also Amoli-Diva et al. reported that FeMn₂O₄ nanoparticles coated with (TEOS) and modified with 3-mercaptopropionic acid (MPA) can be a suitable candidate for site-specific and controlled anti-cancer delivery.

In this research, synthesis and application of smart core/shell CoFe₂O₄ nanoparticles were reported which coated with methionine through the reflux assay in one step and used as a carrier of an anti-cancer drug. Nanoparticles were characterized by XRD, SEM, TEM, VSM, TGA, and FTIR techniques. Letrozole was used as an anti-cancer model drug, and the drug-loading and drug release behavior of methionine-coated CoFe₂O₄ nanoparticles is reported. Furthermore, it was reported that CoFe₂O₄@Methionine in vitro cytotoxicity was evaluated by MTT assays with varying concentrations on two cancer cell lines and normal cell line at 24, 48, and 72 h.

Materials and methodology

Materials

Iron (III) chloride hydrate (FeCl₃.6H₂O), Cobalt (II) chloride hydrate (CoCl₂.6H₂O), Sodium hydroxide (NaOH), and Methionine (C₅H₁₁NO₂S) are used as precursors. Methanol (CH₃OH) and deionized water are used as the solvent, and ethanol was used as the rinse solvent. Trypsin–EDTA, purple formazan crystals, Medium RPMI-1640, DMSO, PBS, FBS, MTT, and Penicillin/Streptomycin 100X were purchased from Gibco, USA. Letrozole was purchased from Daroo-Pakhsh Co. MCF-7, MAD-MB-231, and MCF10A cell lines were obtained from Pasteur cell bank, Iran. All the chemicals were purchased from Merck, Germany without further purification.

Preparation of methionine-coated CoFe₂O₄ nanoparticles

Cobalt ferrite nanoparticles were synthesized by the coprecipitation method. In this experiment, 1.42 g of CoCl₂.6H₂O and 3.24 g of FeCl₃.6H₂O with molar ratio 1:2 were dissolved in 180 ml deionized water and stirred for 30 min under N₂ atmosphere and then pH raised to 12 with adding NaOH (1.5 M). Then 1 g of Methionine is dissolved in deionized water, added to the mixture. The mixture was heated to 70–80 °C and refluxed for 3 h; the final formed brown precipitate was collected by magnetic separation and washed with deionized water and ethanol. The procedure is shown in Fig. 1.

Characterization

X-ray diffraction (XRD) analysis of the samples is recorded by an STOE STADI-P with Cu Kα radiation (λ = 1.54060 Å) and with the 20 range of 10–80° at room temperature. Field emission Scanning Electron Microscopy (FESEM) (model Zeiss-EHT-10.00 kV Germany) and transmission electron microscope (TEM) (model Zeiss-EM10C-100 kV Germany) were used for size and morphology measurement of the methionine-CoFe₂O₄ nanoparticles. Fourier transform infrared spectroscopy (FTIR) data were taken in the spectral range from 400 to 4000 cm⁻¹ by using a model nexus 870 spectroscopy. The amount of adsorbed and released drug is monitored as functions of soaking time by Ultraviolet–visible (UV–Vis) spectra were obtained with a Shimadzu UVS-1700 at 239 nm. The thermal properties (TGA) were performed by a Shimadzu TA Q600 (USA) system from 25 to 800 °C in the nitrogen atmosphere at a constant heating rate. The magnetic properties of the synthesized Methionine-coated CoFe₂O₄ nanoparticles and magnetic nanoparticles CoFe₂O₄ were measured at room temperature by a Quantum Design MPMS-XL-7 superconducting quantum interference device (SQUID) with an external magnetic field ranging from −15 to + 15 kOe.

Loading capacity of letrozole

0.0016 g Letrozole was dissolved in 20 ml methanol, and then 0.04 mg of Methionine-CoFe₂O₄ nanoparticles was added to this solution. This mixture was stirred for 24 h at room temperature to load drug molecules. Then the dispersion of the sample was centrifuged at 6,000 rpm for 12 min to collect the Letrozole-loaded nanoparticles and kept the supernatant for calculating the drug-loading content. The Letrozole-loaded nanoparticles which collect dried at room temperature and supernatants were collected measured by UV–Vis spectroscopy at a wavelength of 239 nm, and the loading capacity was calculated according to the standard curve with different known drug concentrations. The amount of loaded Letrozole was calculated according to Eq. (1).

\[
\text{Drug content (mg/mg sample) = } \frac{C_0V_0 - C_1V_\alpha}{w} \tag{1}
\]

where \( C_0 \) is the initial concentration of Letrozole, \( C_1 \) is the concentration of the drug which calculated by standard
In vitro release study and kinetic modeling

For the in vitro release kinetics of the Letrozole, 15 mg Letrozole-loaded Methionine-CoFe₂O₄ sample into 15 ml PBS (phosphate-buffered saline) with varied pH values (5 and 7.4) in the dark and constant shaking (100 r/min) at a constant temperature (37 °C) investigated. The supernatant (2 ml) is extracted at different time intervals and exchanged with the same volume of fresh PBS with the same pH value. Percentage of released Letrozole was calculated by UV–Vis technique at a wavelength of 239 nm according to Eq. (2).

\[
\text{Drug release (\%)} = \frac{C_e \times V}{W} \times 100 \tag{2}
\]

where \(C_e\) (mg/ml) is the concentration of Letrozole in the supernatant, \(V\) (ml) is the volume of buffer solution, and \(W\) (mg) is the amount of drug loading.

The drug release data were analyzed mathematically according to the models fitted in kinetic models’ equations for the release kinetic studies and to investigate the release mechanism. The linear form diagrams which used for models are zero-order kinetics (cumulative % drug released vs. time), first-order kinetics (log % drug retained vs. time), Higuchi model (cumulative % drug released vs. square root of time), and Korsmeyer–Peppas equation (log amount of drug released vs. log time). The correlation coefficient \((r)\) values for the linear curve were calculated obtained by regression of the above plots.

In vitro cytotoxicity

To investigate the cytotoxicity effects of the Methionine-CoFe₂O₄ nanoparticles on the cancer cell lines (MCF-7, MAD-MB-231) and normal cell line (MCF10A), MTT assay was used. The cells in the 96-well plate seeded at a density of \(2 \times 10^4\) cells per well and cultivated in a medium containing 1% penicillin/streptomycin and fetal bovine serum (FBS, 10%) at 37 °C in a humidified incubator with 5% CO₂. After 24 h of incubation, the suspensions of Methionine-CoFe₂O₄ with various concentrations (0–80 μg/ml) were added to the medium and continuously incubated for 24 h, 48 h, and 72 h, respectively. Then, the contents of the 96-well plates were removed, and 0.05 ml of MTT solution was added to each well following another 4 h of incubation in a 5% CO₂ atmosphere and at 37 °C. The medium was then replaced with 0.05 ml of dimethyl sulfoxide (DMSO) that was added to each well to dissolve the purple formazan crystals [34, 35]. Finally, the absorbance of each well was measured using a microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT) at a wavenumber of 570 nm. Also, half-maximal inhibitory concentration \((IC_{50})\) was calculated and the rate of cytotoxicity was calculated according to Eq. (3).

\[
\text{Cell survival rate} = \frac{\text{absorbance of control cells}}{\text{absorbance of treated cells}} \times 100 \tag{3}
\]
Results and discussion

XRD analysis

The structure of nanoparticles was characterized by X-ray diffraction. Figure 2 shows the XRD patterns of CoFe₂O₄ and Methionine@CoFe₂O₄ nanoparticles. The diffraction peaks at 20 values of a Crystal spinel CoFe₂O₄ nanoparticles (JCPDS No. 98-001-6669), 18.72°, 30.35°, 35.70°, 43.33°, 53.71°, 57.23°, and 62.84° were observed and 20 values of Methionine@CoFe₂O₄ nanoparticles (JCPDS No. 98–001-6669) are 18.39°, 30.24°, 35.69°, 43.17°, 53.64°, 57.23°, and 62.84° that decreasing of the 2θ values indicates the entry of methionine in the network cavities and increasing of the network space. The characteristic diffraction peaks are corresponded to (111), (022), (113), (004), (224), (115), and (044) crystal planes, respectively. According to the Debye–Scherrer’s equation (Eq. 4), average size of nanoparticles was 23 nm:

$$D = \frac{K\lambda}{\beta \cos \theta}$$

where D denotes the crystalline size, β is the full width at half maximum, K is the shape factor, θ represents the Bragg angle corresponding to the peak, and λ is the wavelength of the X rays.

Morphologic studies of methionine@CoFe₂O₄ nanoparticles

FESEM micrographs of the synthesized Methionine@CoFe₂O₄ nanoparticles have been shown in Fig. 3. As observed in Fig. 3a and b, the spherical shapes with nearly uniform sizes of the Methionine@CoFe₂O₄ nanoparticles are exhibited from the SEM images in which the average size of the spheres is around 28–29 nm. Figure 3c and d shows the TEM micrographs of Methionine@CoFe₂O₄ nanoparticles with slight agglomeration which may be due to the strong magnetic interactions between nanoparticles.

Magnetic studies

The magnetic hysteresis loops of the prepared core/shell Methionine@CoFe₂O₄ nanoparticles and bare CoFe₂O₄ were measured at room temperature by SQUID in an external magnetic field ranging from −15 to +15 kOe as depicted in Fig. 4. The magnetization curves of bare CoFe₂O₄ show that they have a negligible hysteresis loop due to its approximately superparamagnetic behavior. The saturation magnetization values at room temperature for the CoFe₂O₄ and Methionine@CoFe₂O₄ nanoparticles are 46 emu/g and 16.8 emu/g, respectively, which value of Ms in bare CoFe₂O₄ is more than magnetization value after coating with Methionine in the sample. Ms reduction attributed to nonmagnetic Methionine shell around the magnetite nanoparticles.

TGA analysis

As shown in Fig. 5, existence of Methionine on the CoFe₂O₄ nanoparticles was further examined by thermal analysis that demonstrates TGA curves of the bare CoFe₂O₄ and Methionine-coated CoFe₂O₄ nanoparticles. The weight of bare CoFe₂O₄, initial weight loss from room temperature up to 150 °C, is probably due to the removal of surface hydroxyls or physically adsorbed water but by increasing the temperature to 800 °C due to the high stability of structure, the curve is almost constant. The occurrence of this report was also found in the synthesis of L-cysteine-coated cobalt ferrite nanoparticles [33]. In the second sample, which Methionine coated cobalt ferrite nanoparticles; the TGA curve shows that the weight loss of 13.83% is observed at 400 °C which is related to thermal decomposition of surface-treated CoFe₂O₄.
Fig. 3  SEM images of Methionine@CoFe$_2$O$_4$ nanoparticle (a, b) and TEM images of Methionine@CoFe$_2$O$_4$ nanoparticles (c, d)

Fig. 4  M–H curves of CoFe$_2$O$_4$ nanoparticles (a) and Methionine@CoFe$_2$O$_4$ nanoparticles (b)

Fig. 5  TGA curves of bare CoFe$_2$O$_4$ (a), Methionine@CoFe$_2$O$_4$ nanoparticles (b)
with additions of Methionine molecules. So weight loss of Methionine@CoFe$_2$O$_4$ has occurred in a range of 400 °C, which was related to degradation of Methionine molecules.

**FTIR analysis**

Figure 6 shows the FTIR spectra of CoFe$_2$O$_4$, Methionine-coated CoFe$_2$O$_4$ before and after Letrozole loading. In the spectrum of the Methionine amino acid, due to the nature of their dipole ions, their spectra are a combination of carboxylate salts and the first-type amine. The two absorption bands at 1517 and 1630 cm$^{-1}$ are ascribed to the symmetric and asymmetric N–H bonding, respectively. Also symmetric and asymmetrical stretching COO$^-$ bands are assigned at 1419 and 1600 cm$^{-1}$. Peaks of the 1232–1330 cm$^{-1}$ region refer to the C–O band. The absorption bands around 400 and 600 cm$^{-1}$ in the spectrum of CoFe$_2$O$_4$ are related to the intrinsic vibration of tetrahedral and octahedral metal–oxygen complexes, respectively, which are mainly depend on Fe–O distances. The peaks of Methionine which is determined by the spot chain in the spectrum were similar with peaks of Methionine@CoFe$_2$O$_4$ which clearly shows the presence of the Methionine on the surface of CoFe$_2$O$_4$. The absorption bands in Letrozole spectrum around 671 and 1007 cm$^{-1}$ are caused by bonding of $\equiv$C–H and spectra around 1143 and 1262 cm$^{-1}$ related to C–O. The peak in region 1447—1500 cm$^{-1}$ is attributed to aromatic ring and peaks in the 1417, 1640–1670, and 2240 cm$^{-1}$ region refer to the C=C, C=N, and C≡N stretching, respectively. Also peaks at 3114 cm$^{-1}$ are attributed to CH sp$^2$-hybridized stretching. However, the capacity of the loading drug can be attributed to the shell, which could hold Letrozole molecules. The C≡N band at 2240 cm$^{-1}$ present in Methionine@CoFe$_2$O$_4$ appears after Letrozole loading, and it suggests that the hydrogen bond has formed between the carboxylic group of Methionine and Letrozole molecules, also aromatic ring bands are shifted from 1447 to lower 1361 cm$^{-1}$ when bonding are formed between sure face of carrier and the Letrozole. On the other hand, the entire band which relates to Methionine present in the Methionine@CoFe$_2$O$_4$ almost diminishing after Letrozole loading which can be verified by the FTIR.

**In vitro Loading capacity and release of Letrozole**

To calculate the Letrozole loading capacity of the sample at 239 nm wavelength UV–Vis spectroscopy was used. To determine the loading capacity of Letrozole on the Methionine@CoFe$_2$O$_4$ with different initial Letrozole concentrations, the amount of Methionine@CoFe$_2$O$_4$ was transferred to 20 ml of different initial Letrozole concentrations. The maximum loading capacity of the Methionine@CoFe$_2$O$_4$ when the initial drug concentration is 0.08 mg/ml can reach 0.025 mg/mg which is in another word obvious that in 1 mg of nanoparticle, 0.62 mg of the drug is loaded. The result loading capacity in this study strongly depends on the initial drug concentrations which is shown in Fig. 7.

Afterward, to evaluate the drug release behaviors, Letrozole-loaded Methionine@CoFe$_2$O$_4$ nanoparticles are suspended in a PBS buffer media with various pH values for the simulated environment of tumors, which are pH5 and pH7.4 which are a physiological pH of the body at temperature (37 °C) over 72 h measured. Figure 8 shows the cumulative drug release of Letrozole from Methionine@CoFe$_2$O$_4$ at both pHs. As can be seen, drug release at acid solution conditions (pH 5) in 72 h shows higher release than neutral conditions (pH 7.4). Also, this study observed that the release of Letrozole from the carrier in the beginning 8 h occurs in a rapid manner after which the process gradually slows down up to 72 h. The reason for the rapid manner
can be related to the immediate dissolution of Letrozole on the surface of Methionine @CoFe$_2$O$_4$ nanoparticles. After that, the slower release of the Letrozole takes place in the structure of these nanocarriers seems due to the physical and chemical interactions between Letrozole and Methionine@CoFe$_2$O$_4$.

As reported in the literature, the model delivery system is sensitive to pH, which is very important and useful for drug delivery because in the neutral conditions (pH 7.4), the low release rate of drug reduces relieves the side effects of antitumor medications to healthy cells and the losing of drugs in the blood transportation system, and at the same time intracellular lysosomes, endosomes, or cancerous tissues are tuned with acidic (pH 5) setting corresponding to facilitate anti-cancer drug active release [26]. To govern the release behavior of letrozole letrozole-loaded Methionine@CoFe$_2$O$_4$ nanoparticles, mathematical models were cast-off with higher linear regression coefficient for each (closer to 1), to designate the kinetic model of the ideal sample release. Table 1 attends to the coefficient of determination ($R^2$) for each model at different pH values (5 and 7.4). According to which, the release data for pH values follow the Korsmeyer–Peppas model, and the obtained $n$ values ($n<0.45$) in Korsmeyer–Peppas model for these two conditions suggest that the Fickian diffusion mechanism determines the release of letrozole molecules from Methionine@CoFe$_2$O$_4$ nanoparticles.

### In vitro cytotoxicity test

The cytotoxicity of magnetic nanoparticles as known generally depends on some factors, such as aggregation degree, surface area, hydrophobicity, surface coating, and particle size [36]. As shown in cytotoxicity studies there’s no toxicity of the magnetic nanoparticles in human breast cancer cells (MCF-7, MDA-MB-231) and normal cell (MCF10A), by MTT assay. Cells are incubated with free letrozole, Methionine@CoFe$_2$O$_4$ nanoparticles, and letrozole loaded on Methionine@CoFe$_2$O$_4$ nanoparticles in different concentrations (0–40 μg/ml), for 24, 48, and 72 h, respectively. The results demonstrated that the Methionine@CoFe$_2$O$_4$ exhibited almost no toxicity to human breast cancer cells (MCF-7, MDA-MB-231) and normal cell (MCF10A).

### Table 1

| Release model       | Equation            | $R^2$ pH 5  | $R^2$ pH 7.4 |
|---------------------|---------------------|------------|-------------|
| Zero Order         | $C_t = C_0 + K_0 t$ | $R^2 = 0.8984$ | $R^2 = 0.7340$ |
| Korsmeyer–Peppas   | $M/M = K_1^n$       | $R^2 = 0.9904$ | $R^2 = 0.8875$ |
|                     |                     | $n = 0.4365$ | $n = 0.3969$ |
| First Order        | $\log C = \log C_0 + K_2 t$ | $R^2 = 0.9528$ | $R^2 = 0.7574$ |
| Higuchi            | $Q = k_{H} \sqrt{t}$ | $R^2 = 0.9764$ | $R^2 = 0.8746$ |
cancer cells while letrozole-loaded Methionine@CoFe₂O₄ is more cytotoxic even more than the free drug which shows that the letrozole-Methionine@CoFe₂O₄ nanoparticles are more readily internalized through the receptor-mediated endocytosis mechanism, while free letrozole is transported into cells by a passive diffusion mechanism [37]. It is also demonstrated that letrozole-Methionine @CoFe₂O₄ exhibited more cytotoxic effects on the MCF-7 cells, compared to MDA-MB-231 cells. Besides, normal MCF10A cells were treated with the same concentrations of Methionine @CoFe₂O₄ and letrozole-Methionine@ CoFe₂O₄. Results indicated that Methionine@CoFe₂O₄ and letrozole-Methionine@CoFe₂O₄ had no significant toxicity on MCF10A cells after 72 h treatment, indicating they have enough biocompatibility to use as a drug delivery system. This result demonstrated that the loading of the drug on carrier synergistically improved the growth inhibition effect on cancer cells and the potentials of using Methionine@CoFe₂O₄ for therapy. The results of the cell viability of MCF-7 in Fig. 9, MDA-MB-231 in Fig. 10 for 24, 48, and 72 h are shown. The IC50 values of the free drug and Letrozole loaded on Methionine@CoFe₂O₄ formulations to MCF-7 and MDA-MB-231 cells are summarized in Table 2.

Conclusions

In this study, we synthesized Methionine@CoFe₂O₄ nanoparticles and study drug delivery and in vitro cytotoxicity. The methionine coating improved the colloid stability and biocompatibility of magnetic nanoparticles. The in vitro drug delivery ability of Methionine@CoFe₂O₄ nanoparticles is confirmed by using letrozole as a model drug at body temperature (37 °C) which showed a pH-sensitive release behavior. It was found that the effectiveness and selectivity of the drug carrier system can be beneficial for the inhibition of quick release for the anti-cancer drugs in neutral blood systems but the acceleration of drug release at acidic tumor cells. The results of MTT assays for Methionine@CoFe₂O₄ as model carrier exhibit low cytotoxicity even at a high concentration after 72 h treatment and for the Letrozole-Methionine@CoFe₂O₄ that demonstrated high cytotoxicity in both types of cancer cells. Thus, the Methionine@

![Fig. 9](image_url)

Concentration-dependent survival curves of MCF-7 cells treated by Letrozole-Methionine @CoFe₂O₄ nanoparticles and Letrozole for a 24, b 48, and c 72 h. Data are expressed as mean ± SD (n = 5)
CoFe$_2$O$_4$ Nanocarrier is expected to be a promising drug-releasing agent and could be used in therapy.

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**Table 2** IC50 values of Letrozole and Letrozole-Methionine@CoFe$_2$O$_4$ after 24, 48 h, and 72 h in MCF-7 and MDA-MB-231 cells

| Cell lines    | Incubation time (h) | IC50 (μg/ml) Letrozole | Free Letrozole | Letrozole loaded on Methionine@CoFe$_2$O$_4$ |
|---------------|---------------------|-------------------------|----------------|--------------------------------------------|
| MCF-7         | 24                  | 63.52 ± 1.91            | 48.43 ± 1.75   |                                            |
|               | 48                  | 42.36 ± 1.33            | 26.63 ± 1.44   |                                            |
|               | 72                  | 33.90 ± 1.34            | 15.10 ± 0.81   |                                            |
| MDA-MB-231    | 24                  | 81.10 ± 1.52            | 69.04 ± 0.93   |                                            |
|               | 48                  | 61.08 ± 0.92            | 38.32 ± 1.50   |                                            |
|               | 72                  | 44.14 ± 0.80            | 17.47 ± 0.59   |                                            |

**Data availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare no conflict of interest.

**Informed consent** Not applicable.

**Institutional review board** Not applicable.

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