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PREPARATION AND CHARACTERIZATION OF CURCUMIN-LOADED IRON OXIDE NANOPARTICLES FOR BREAST CYTOTOXIC EFFECT

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Magnetic iron oxide nanoparticles have been widely used for drug delivery. In this study, we prepared Curcumin loaded magnetic iron oxide nanoparticlesand evaluated its cytotoxic effect on MCF-7 (Michigan Cancer Foundation-7) cell lines. Magnetic Nanoparticles (MNPs) were characterized using transmission electron microscopy (TEM), Ultraviolet-Visible (UV) Spectroscopy.

KEYWORDS: Breast cancer, magnetic nanoparticles, Curcumin, drug delivery.

1. INTRODUCTION

Magnetic iron oxides nanoparticles (namely Fe_3O_4 NPs) (IONPs) have a great attention for their excellent magnetic, biocompatible and potentially non-toxic properties [1, 2]. IONPs have received tremendous attention in biomedicine applications such as drug delivery, magnetic hyperthermia, magnetic resonance imaging (MRI)[3].Breast cancer is a significant dangerous disease among women [4]. The most common methods of treatment that patients receive are surgery, chemotherapy and radiotherapy. but researchers have reported almost 50% of cancer patients die as a result of inaccurate diagnosis or incomplete treatment [5, 6].Chemotherapeutic medicines are most effective to kill rapidly dividing cancerous cells and tumors but also affect the physiological activity of normal cells. Delivering therapeutic compound to the target site is a major problem in treatment of many diseases. A conventional application of drugs is characterized by limited effectiveness, poor biodistribution, and lack of selectivity [7]. In

controlled drug delivery systems (DDS) the drug is transported to cancer cell, so it can minimize the undesirable side effects.In addition, DDS protects the drug from rapid degradation or clearance and enhances drug concentration in target tissues, therefore, lower doses of drug are required and fewer side effects [7].Curcumin (diferuloylmethane) is a polyphenol natural product isolated from turmeric, a powder produced from the rhizome of the plant Curcuma longa [8]. Curcumin has great benefits in the human body and has proven to be a powerful therapeutic agent against many human disease processes [8]. In recent years, many studies have been reported that curcumin as a promising anticancer agent against various types of cancer such as breast cancer [9],prostate cancer [10], colon cancer [11], and hepatocellular carcinoma [12].Curcumin can affect and deactivate virtually every major stage of carcinogenesis, including cell proliferation, growth, survival, angiogenesis and metastasis. The molecular mechanisms of the action that curcumin employs to affect these processes are numerous and varied, depending on the cancer cell type [8]. Curcumin does not have any toxic effect on normal human cells. I n addition, it can penetrate the cancer cells by overcoming issues related to its solubility, degradation in physiological medium, and rapid metabolism [13] and this can be achieved through advanced drug delivery systems [13, 14]. Curcuminnano-formulations based on conjugate, emulsion, lipid, polymer, and gel nanoparticles have been proposed for improving therapeutic benefits and sensitization for chemotherapy and radiation [14, 15].In the present study, we have prepared and characterized the curcumin coated iron oxide magnetic nanoparticles, thenwe evaluated its cytotoxic effect on MCF7 cell lines through MTT assay. Cur-IONP showed a promising cytotoxic effect as compared to the curcumin which highlights the importance of loading the anticancer drugs on novel nanomaterial to enhance its effectiveness.

2. MATERIAL AND METHODS

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$)(99.99%,270.3g/mol), iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$)(99.99%,198.81g/mol),curcumin (≥94% curcuminoidcotent), MTT stain and dimethyl sulfoxide (DMSO) were obtained from Sigma (St Louis, MO, USA). DMEM media, fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Gibco, USA.

2.1 Synthesis of Curcumin-coated iron oxide nanoparticles (Cur-IONPs)

Curcumin coated iron oxide nanoparticles were prepared using a simple co-precipitation method. $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ (1:1 W/W) were dissolved in 40.0 ml of water. The reaction was heated and 20 mg of curcumin (dissolved in DMSO) was added dropwise to the reaction system. The pH was adjusted to highly alkaline (pH 11) to form black precipitate[16].The reaction was then allowed to stir over hot plate stirrer (85ºC) for about one hour. The solution was centrifuged at 14000 rpm for 30 min. The final pellet of Cur-IONPs was washed and re-suspended and kept at 4 °C. The concentration of free (unloaded) Curcumin was calculated from the Curcumin calibration curve that was performed at 498 nm using anUltraViolet–Vis spectrophotometer (Jenway 6405, Barloworld Scientific, Essex, UK). The drug encapsulation efficiency (EE) was obtained from the following equation:

$$EE\% = \frac{total\ drug\ conc. - free\ drug\ conc.}{total\ drug\ conc.}\ 100\%$$

2.2Physicochemical characterization of the prepared nanoparticles

Cur-IONPs were visualized by high-resolution transmission electron microscope (TEM) (JEM 1230 electron microscope. Jeol, Tokyo, Japan). A drop of prepared nanoparticles solution was applied to a carbon grid coated with copper then the excess sample was drawn off with filter paper. The grid was left 5 minutes. to dry at room temperature prior to the beginning of the examination. The absorption spectra of
Curumin was measured using a UV-VIS spectrophotometer(Jenway UV-6420; Barloworld scientific,Essex, UK) Curcumin 0.23mg/ml solution was scanned in a UV spectrophotometer in the range of 300-800nm. Water and DMSO solution was used as baseline.

2.3 Cell Viability Assay

Human breast cancer cells MCF-7 were maintained in DMEM cell culture media containing 10% FBS and 1% penicillin/streptomycin. Cells (5000 cells/ well) were grown at 37^oC in a 5% CO_2 humidified atmosphere and left for 24 h to attach to the plate. To assess the potent therapeutic effects of the prepared nano-formulation, cells were divided into two groups: curumin group, cells treated with curumin (the concentration of curumin was calculated to be equivalent for that

loaded on nanocarriers); Cur-IONPs group, cells treated with Cur-IONPs (0.1, 0.15, 0.25 and 0.30 mg/ml) only. The cells incubated for 24 h before measuring the cell viability using MTT Cell viability assay. After that, 5 µl of MTT solution (2 mg/ml in PBS) was added to each well and incubated for 4 h. After careful removal of the media, 150 µl of DMSO was added to each well, and then after shaking the absorbance was read at 570 nm using an ELISA microplate reader (Molecular Devices, Sunnyvale, USA).

3. RESULTS AND DISCUSSION

3.1 Physical characterization of nanoparticles

The morphology and size of the MNPs were investigated by TEM (Figure 1). The TEM image of the MGNPs revealed that the prepared NPs were well dispersed, spherical in shape, with an average diameter of about 10-13 nm. An Ultraviolet-visible spectroscopy (UV) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. The UV Visible spectrum of Curcumin is shown in (Figure 2. Wavelength corresponding to maximum absorbance of curcumin in water and DMSO was observed at 455 nm.

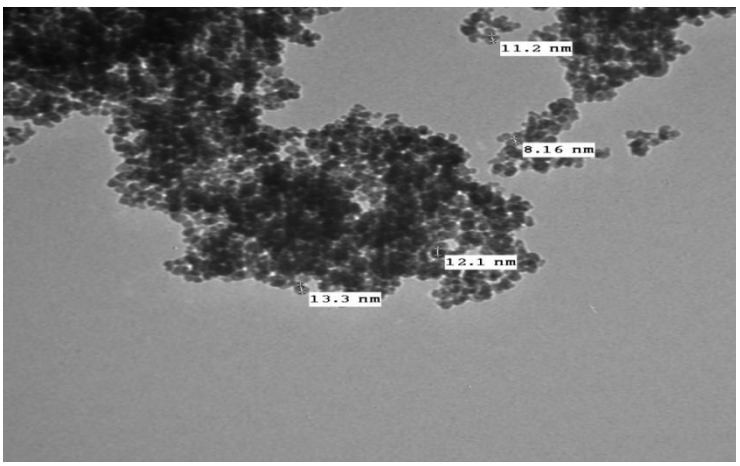

Figure 1**:** TEM images of synthesized Cur-IONPs.

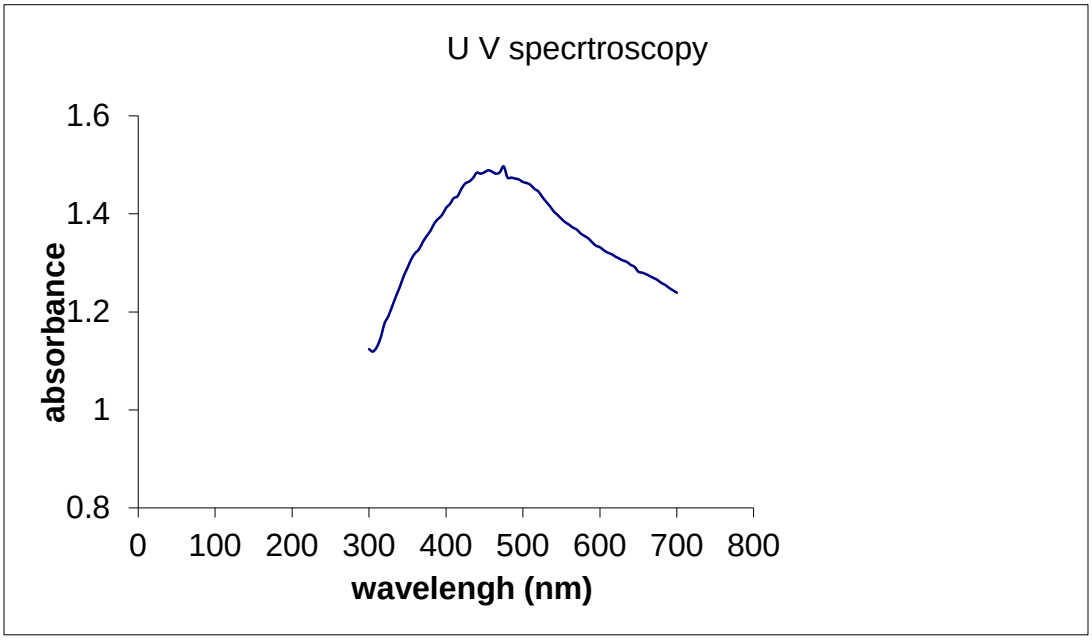

Figure 2:UV-visible absorption spectra Curcumin.

3.2 Preparation of standard calibration curve

The standard calibration curve of curcumin(Figure 3) was obtained by measuring the absorbance of curcumin solution in concentration range (0.23-.02mg/ml) prepared from stock solutions in water and DMSO at 455 nm. The Calibration curve of curcumin was then plotted with the absorbance on y-axis and curcumin concentration on the x-axis. From calibration curve we find that the concentration of free Curcumin is 0.032 mg/ml. The total concentration of curcumin is 0.487 mg/ml whilethe drug encapsulation efficiency was obtained from the following equation:

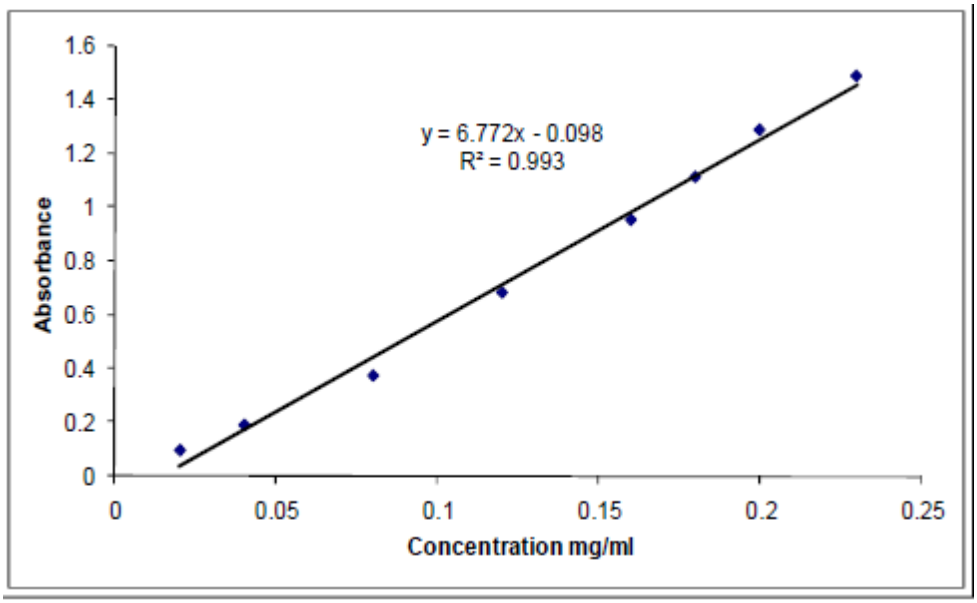

Figure 3: Calibration curve of Curcumin.

$$EE\% = \frac{initial\ amount\ of\ CUR - free\ amount\ of\ CUR}{intial\ amont\ of\ CUR}\ 100\%$$

Encapsulation efficiency is defined as the percentage of curcumin loaded to nanocarriers(iron oxide nanoparticles) relative to the total amount of curcumin [17].The encapsulation efficiency results show that a high loading of curcumin was achieved (93%). With these physiochemical parameters, the prepared nano-formulations could be used as drug delivery vehicles. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist [18]. IC_{50} values can be used to compare the potency of two antagonists. For MCF-7 cell lines, the IC_{50} valueswere 0.61 and 0.41 mg/ml for curcumin and Cur-IONPs, respectively as shown in (figure 4).

3.2 In vitro toxicity study on cancer cells

To check the biocompatibility of curcumin and curcumin loaded magnetic nanoparticles, MCF-7 cell lines were incubated for 24 h in the concentration range from 0.1-0.3 mg/mL. The cell viability of the cells after treatment with free curcumin and curcumin loaded nanoparticles was found to decrease with the increase in concentration. However the cytotoxicity of curcumin loaded

nanoparticles was significantly higher than that of the free curcumin at all concentrations as shown in (table 1 and figure 5). This might be attributed to the better dispersibility of curcumin when curcumin coated iron oxide magnetic nanoparticles [19] .The images of the cellular uptake studies with Cur and Cur-IONPs (drug)showed good cell internalization as shown in (figure 6).

4. CONCLUSION

The authors assumed that Cur-IONPs undergoes endocytosis but were not physically bound to the cancer cell surface and proposed that the entrapped Cur-IONPs will demonstrate its anticancer properties by delivering curcumin more efficiently to cancer cells[20]. Cancer cells treated with Cur-IONPs showed an obvious features of apoptosis such as ultra-structural changes, including shrinkage in nucleus, vesicle, and vacuole formation[21, 22].In summary, we developed a safe and biocompatible drug for cancer therapy. The current study showed that these as-prepared nanoparticles provided a suitable and appropriate method for in vitro delivery of curcumin on MCF-7 cell lines.

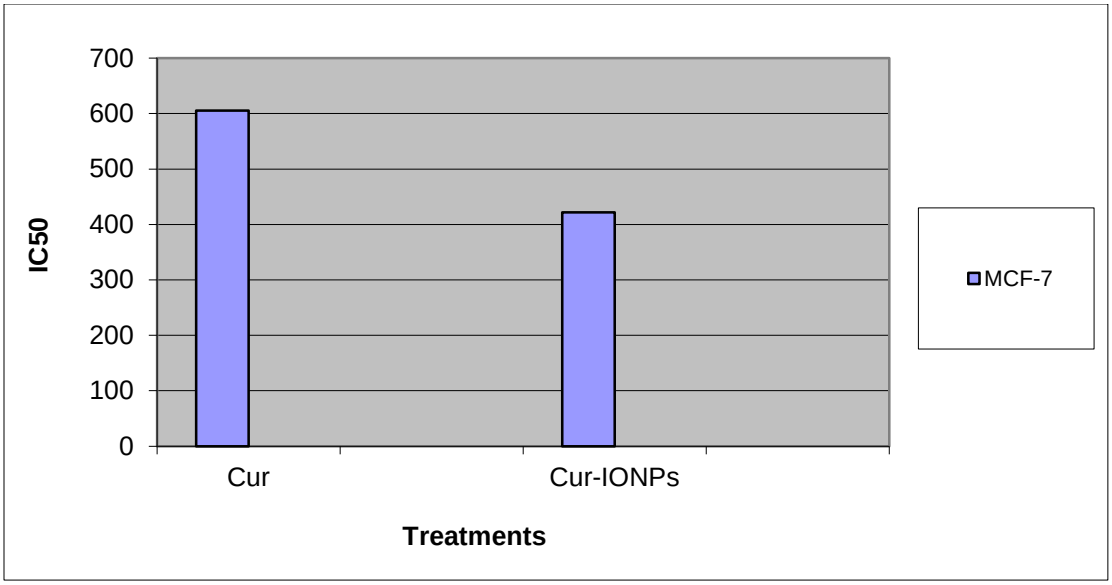

Figure 4: IC_{50} of Cur and Cur-IONPs on MCF-7 cell lines

Groups / Treatments	Cell Viability		Differences (%)
	Cur	Cur-IONPs	
Control	100	100	
0.1 mg/ml	91.25±3.13	74.28±1.87	18.6
0.15 mg/ml	85.58±3.75	71.11±2.03	16.9
0.25 mg/ml	80.85±1.05	69.47±2.96	14.1
0.3 mg/ml	79.57±2.89	66.07±3.31	17.0

Table 1: Cell viability and the percentage change between Cur and Cur-IONPs at different doses of treatment in MCF-7 cells.

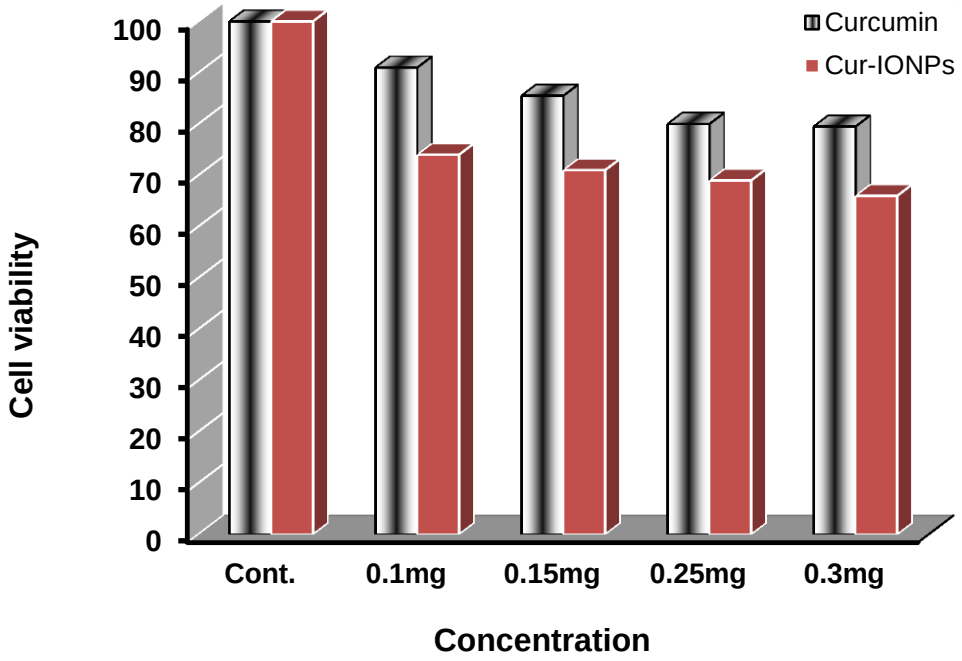

Figure 5:Percent of cell viability in MCF-7 cells after incubation with 0.1, 0.15, 0.25, 0.3 mg of curcumin (Cur) and Cur-IONPs for 24 h.

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تحضير وتشخيص جزيئات أكسيد الحديد المحمل عليها الكركومين لعلاج خلايا الثدي المسرطنة.

جزيئات أكسيد الحديد المغناطيسي تستخدم بشكل كبير كوسيله توصيل الدواء . الكركومين هو جزئ ضد السرطان (معالج للسرطان) . جزيئات أكسيد الحديد المحمل عليها الكركومين تستخدم بشكل كبير لتحسين كفاءه علاج السرطان .أوضحت النتائج علي ان جزيئات أكسيد الحديد المحمل عليها الكركومين لها تأثير أفضل علي علاج الخلايا السرطانية من استخدام الكركومين بمفرده . يتم تشخيص جزيئات أكسيد الحديد المغناطيسي بواسطة المجهر الالكتروني النافذ و الأشعه فوق البنفسجية