ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Preparation and Characterization of Folated Chitosan/Magnetic Nanocarrier for 5-Fluorouracil Drug Delivery and Studying its Effect in Bladder Cancer Therapy

Sharafaldin Al-Musawi[1*], Ameer Jawad Hadi[1], Suhad Jameel Hadi[2], Nada Khazal Kadhim Hindi[3]

1. *College of Biotechnology, Al Qasim Green University, Babylon, Iraq.*
2. *College of Veterinary Medicine, Al Qasim Green University, Babylon, Iraq.*
3. *College of Nursing, University of Babylon, Babylon Province, Iraq.*

***Corresponding Author: Sharafaldin Al-Musawi**

Abstract

Among the vigorous anticancer factors, 5-Fluorouracil (5-FU) has been presented fantastic capability against several types of tumor cells. Chitosan-covered super paramagnetic iron oxide nanoparticles (CS-SPION) were synthesized and applied as a nano-carrier for loading of 5-FU (CS-5-FU-SPION) through a reverse micro emulsion technique. In the final preparation process the nanosystem was folic acid functionalized (FA-CS-5-FU-SPION) for targeted therapy purposes. This nanoformulation was studied towards bladder cancer cell lines. Each size and morphology characteristic was evaluated by zeta sizer, AFM and FESEM. Fluorescence microscopy was used to determining the cell internalization rate of prepared nanosystem. Cell viability and apoptosis were surveyed by MTT assay and flowcytometry method respectively. The data indicated that the prepared FA-CS-5-FU-SPION has spherical shape with 79 ± 13 nm average diameter size and appropriate polydispersity rate. Additionally, the remarkable drug loading efficiency (~73%) was notable. This FA-CS-5-FU-SPION also demonstrated sustained release of 5-FU at 37 ◦C in both phosphate and citrate buffer solutions separately. Then the MTT assay and flow cytometry study indicated significant cell toxicity and apoptosis induction by FA-CS-5-FU-SPION. The results supported the fact that FA-CS-5-FU-SPION had become greatly represented antitumor characteristics. In the other hands no adverse outcome were reported for normal cells. Furthermore, it became proved that the FITC-labeled FA-CS-5-FU-SPION, has an effectively entrance into cancerous cell and stimulate cell death and apoptosis.

Keywords: *Nanoformulation, Chitosan, 5-Fluorouracil, Magnetic nanoparticle, Bladder cancer.*

Introduction

The cancer is a main complicated issue that contains one of the most leading causes of death worldwide. Several tactics such as chemotherapy, radiation therapy and surgery, were developed for different cancers type, but most of these methods are not officiously deadly to treated cancer cells. On the other hand, the normal cells are also influenced and subjected to damage or even death [1, 2]. Scientists make several studies to increase the effectiveness of cancer treatment by reducing harmful effects on normal cells and tissues. One of the strategies that have been considered in recent years is targeted drug delivery specifically for the tumor and by drug loading in biocompatible carriers and releases it in the target site [3, 4]. The 5-fluorouracil (5-FU) is an anti-metabolite drug that is widely used for the treatment of several types of cancer, such as rectum, breast, pancreas and stomach particularly for colorectal cancer [5, 7.]. A mechanism of action by this chemotherapic agent is interfering in thymidine synthesize by inhibiting thymidylate synthase that consequence to cell death and apoptosis induction [8]. Drug resistance of tumors against 5-FU, its negligible selectivity, low plasma half-life (8-20 min) inappropriate cell toxicity, and consequently its poor bioavailability in vivo due its water insolubility are the remarkable challenges to

efficient chemotherapy with 5-FU. Therefore, to maximize the chemotherapeutic efficiency against tumor cells and minimize its toxicity percentage in normal cells and tissues, 5-FU can be loaded into a biocompatible, pH-sensitive nano-carrier and released at a targeted site. Several strategies have been enhanced the aqueous solubility of 5-FU.

Among this methods, encapsulation of 5-FU in different materials such as lipids, proteins, natural and synthetic polymers, are the main tactics for sustained and targeted drug delivery system (DDS) [9, 11]. This material in nano scale present notable pharmacokinetic properties such as high surface to volume ratio, developed water solubility, prolonged half-life (consequence to enhancing blood circulation time), controlled drug release and specific-targeted drug delivery.

Among different nanomaterials, the natural polymeric nanocarriers have an extensive application and consequence wide importance in DDS [12]. Meanwhile some biopolymers like chitosan (CS) have been extremely used in DDS as nano-carriers. CS is a natural cationic bio poly amino saccharide with linear molecular structure that achieved due to deacetylation of chitin. CS has been received notable attention in the pharmaceutical industry due to its low toxicity, biodegradability/compatibility, and chemical structure. On the other hand pH-sensitive nature of this biopolymer leads to sustained release of loaded drugs from its structure and consequence solve the problem of low half-life of chemotherapy drugs [13, 15].

With the developing progress in nanotechnology, the super paramagnetic iron oxide nanoparticles (SPION), are extremely studied and have been demonstrated its various capability in different field such as cytotoxicity, cell metabolism and cell apoptosis study [16, 18]. SPION has become a fascinating nanoparticle in the different medical investigation comprising gene delivery system (GDS), magnetic resonance imaging (MRI) and DDS [17, 20].

The core-shell DDS based on SPION as a core and CS as a shell can be easily assisted the conduction of CS-SPION nanoformulation magnetically to specific target site duo to externally magnetic field [21]. Additionally the CS-SPION nanoformulation was leads to prolong the release of chemotherapy agent.

One of the main advantages of SPION is its capability to surface coating by different materials that supply the water solubility in order to facilitate medicine applications [22, 23]. Moreover this property simplifies the drugs loading process by means of shell part of core-shell nanoformulation [24, 26]. Accordingly, the synthesized core-shell (CS-SPION) is candidate as a noteworthy nanoformulation for tumor targeting therapy which can increase the drug circulation time and release it in controlled manner [27,28].

In order to enhance the targeting efficiency the CS-SPION was surface modified by folic acid (FA) molecule functionalization. The folic acid receptors are found manifold at the surface of the cancer cells, compared to normal cells. Nevertheless, negligible studies, investigate the 5-FU delivery system using FA-CS-SPION as drug nanocarriers [29, 32].

The aim of this study is a synthesis of 5-FU DDS with high level of circulation time, controlled release and adequately specific for target site. Accordingly, the first step was preparing a nanoformulation of FA-CS-5-FU–SPION. Then in order to determining suitability of this nanosystem for DDS purposes, the physicochemical properties were characterized. Finally the cell internalization rate and the accurate drug dosage for treatment were recognized duo to related tests. The FA-CS-5-FU–SPION effect was studied in bladder transitional cell carcinoma T24 cells as cancer cell line and human bladder epithelial cells (HBlEpC) as a normal cell line.

Materials and Methods

Materials

5-Fluorouracil, Chitosan (deacetylation degree 85%, medium molecular weight), folic acid, SPION and Fluorescein Isothiocyanate (FITC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DMEM medium, Fetal Bovine Serum (FBS) was purchased from Gibco (Tulsa, OK, USA). T24cells and HBlEpC cells were both obtained from ATCC. All other reagents were purchased from Merck Company.

Synthesize of FA-CS-5-FU-SPION

FA-CS-5-FU-SPION was prepared by reverse micro emulsion method as previous work [2]. Briefly, the SPION solution was prepared in 10 mg per ml concentration.

Then 50 mg of chitosan (CS) powder was dissolved in 1% (w/v) glacial acetic acid including SPION solution. Subsequently 10 mg of 5-FU drug was added to CS-SPION solution. The obtained solution added dropwise to 100 ml three-necked flask including 50 ml of Tween-80 at 55°C. Afterward the achieved reverse water-in-oil micro emulsion was moved to water bath and stirred at 800 rpm/min for 10 h.

Also, the 1 ml of 20% (w/w) sodium citrate solution was gently added into the three-necked flask and the reaction was continued for 30 minutes in neutral pH. Finally, the CS-5-FU-SPION was collected with a powerful magnet and rinsed triplicate with isopropanol. The collected CS-5-FU-SPION was exposed to 55°C for overnight. The bare CS-SPION (without drug) was prepared in a same method as stated above [33, 34].

Folate Decoration of CS-5-FU-SPION

25 mg of FA powder was added to 5 ml distilled deionized water (DDW). 20 µl of 10 M NaOH solution was added to the FA/DDW mixture and vortexed to obtain clear solution. 1 ml of obtained solution was added into 10 ml (1 mg per ml) of CS-5-FU-SPION solution. The solution was stirred in 500 rpm for 45 minutes at room temperature. The FA-functionalized CS-5-FU-SPION solution was centrifuged at 10000 rpm for 7 minutes. The supernatant including free FA was separated and precipitated material was rinsed and suspended again with DDW.

Characterization

Different main *FA-CS-5-FU-SPION* Nano formulation properties such as size, charge and polydispersity were achieved by zeta sizer (Malvern Instruments, UK). Additionally the size and morphology of nanosystem were studied using atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM). AFM images were achieved by (Dimension 3100, USA), at ambient temperature using a drop of fresh solution. Furthermore the size and morphological evaluation were studied by field emission scanning electron microscopy (Philips XL30) using gold coating of the FA-CS-5-FU-SPION sample.

5-FU **Encapsulation**

FA-CS-5-FU-SPION (10 mg) was dispersed in 50 ml of 1mol/L Hydrochloric acid using sonication. The solution was centrifuged at 14000 rpm for 10 minutes. Then the supernatant containing free unloaded 5-FU drug was collected and measured using fluorescence spectroscopy (Hitachi, Japan) in 280 nm absorption and 240-380 nm emission and the gap width of 10 nm. The 5-FU encapsulation efficiency in FA-CS-5-FU-SPION was acquired by the following equation:

Encapsulation efficiency (%) = [(drug fed – drug loss) / (drug fed)] × 100% Eq. (1)

5-FU Release Profile

In order to measurement of drug release value from FA-CS-SPION, different buffers such as citrate (0.01 M and pH=5.4) and phosphate (0.01 M and pH=7.4) buffers were used in 37^{oC}. 1 ml of nanoformulation solution were added in dialyze bag and placed in 100 ml citrate and phosphate buffers separately. The tween 80 was utilized as an emulsifier for inhibit the sedimentation of released drug. Release process was performed by shaking water bath. The sampling was done at 0, 4, 8, 12, 24, 48, 72 and 96 h. The 500 µl was aliquoted, freeze-dried in each sampling process and resolved in 2 ml methanol. Finally the 5-FU release was measured by fluorescence spectroscopy.

The final 5-FU release was measured using following equation:

$$R= V \Sigma^{n-i} C_i + V_0 C_n / m_{drug}$$

Where, R is the 5-FU release value (%), V is the sample volume, V_0 is the initial 5-FU volume, C_i and C_n are the 5-FU concentrations, i and n are the times each sampling, and m_{drug} is the mass of 5-FU in nanoformulation.

Cell Culture

Bladder transitional cell carcinoma T24 cells (Rockville, MD) and human bladder epithelial cells (HBlEpC) as a normal cell line were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Both cell lines were cultured in DMEM medium supplemented with 10% FBS, and were separated by 0.5 g/l trypsin and passaged several time for obtaining logarithmic phase. The cells incubation in all steps should be in 37℃ and 5% CO2 pressure.

Cell Internalization Assay

The FA-CS-5-FU-SPION was functionalized with FITC (fluorescein 5(6)-Isothiocyanate) to evaluate its cell internalization efficiency by fluorescence microscope (Nikon Eclipse TE2000-U). So the cells were treated with 20 µM FITC-FA-CS-5-FU-SPION for 3 hours. Afterward the nanoformulation included medium was throwed out and the cells washed with phosphate buffered saline (PBS). The imaging process from cells was carried out using fluorescence microscope (Nikon Eclipse TE2000-U). For studying the efficiency of FA-CS-SPION carrier for developing the solubility and bioavailability of 5-FU, one group of cells considered to treating with the same concentration of void 5-FU. Then this cells were imaged by fluorescence microscopy and compared with other one.

MTT Assay

5 mg of MTT powder was dissolved in 1 ml PBS to preparation of MTT solution. In order to MTT study, the plates with 96 well were utilized and cultured with 10^4 cells. Then the 200 µl of DMEM medium were added in each well. Afterward, left the cells to growth and proliferate at 24 h. In this step, the different dose (10-60 µM) of void drug (5-FU), nanoformulated 5-FU (FA-CS-5-FU-SPION) and the bare nanoparticle (FA-CS-SPION) that solved in DMSO, in 2% V/V compares with medium was added into cells comprised wells. Each test was carried out in triplicate wells and repeated at least two times. The MTT analysis was performed at 24 and 48 h after treatment. After 4 h incubation with MTT, the entire solutions were evacuated and then replaced with 100 µl DMSO into each well. The plates were placed in the shaker for 12 minutes then evaluated by ELISA reader (BioTek Power Wave XS).

Apoptosis Assay

In order to Apoptosis study, the plates with 6 well were applied and cultured with 10^4 cells. Different treatments of void 5-FU, FA-CS-5-FU-SPION, and FA-CS-SPION in 48 hours were considered to cell apoptosis study. Apoptosis value was estimated by the Annexin V-FITC Apoptosis Detection Kit (Beyotime, Biotechnology Co., Ltd. Nantong, China). The cells were separated after 48 hours and centrifuged at 200 g for 7 minutes. The supernatant evocated and the pellet resuspended with 500 µl of 1x binding buffer. The achieved solution was treating with 5 µl of Annexin V-FITC and 5 µl PI. Then this reaction solution mixed and incubated for 15 min at room temperature. Finally the Samples were transferred to FACS tubes and evaluated by FAC Scan Flow Cytometer (Becton Dickinson, Oxford, UK).

Statistical Analysis

SPSS 24 software was used for statistical analysis. Statistical differences between control and treatment groups were calculated by T-TEST method. The obtained data were considered statistically significant with the $P < 5\%$.

Result

Characterisation of Nanoparticles

FESEM and AFM images proved that the achieved nanosystem has a spherical shape with a good dispersity without remarkable aggregation (Fig. 1). The average diameter size of FA-CS-SPION and its dispersion rate in 25 ℃ was 79 ± 13 nm and 0.080 ± 1.5 respectively and the nanoparticle surface charge was -30 ± 0.8 mV (Fig. 2). All results exhibited that DLS, FESEM and AFM data reveal similar size (~90 nm). The spherical morphology for FA-CS-SPION was confirmed by FESEM and AFM images.

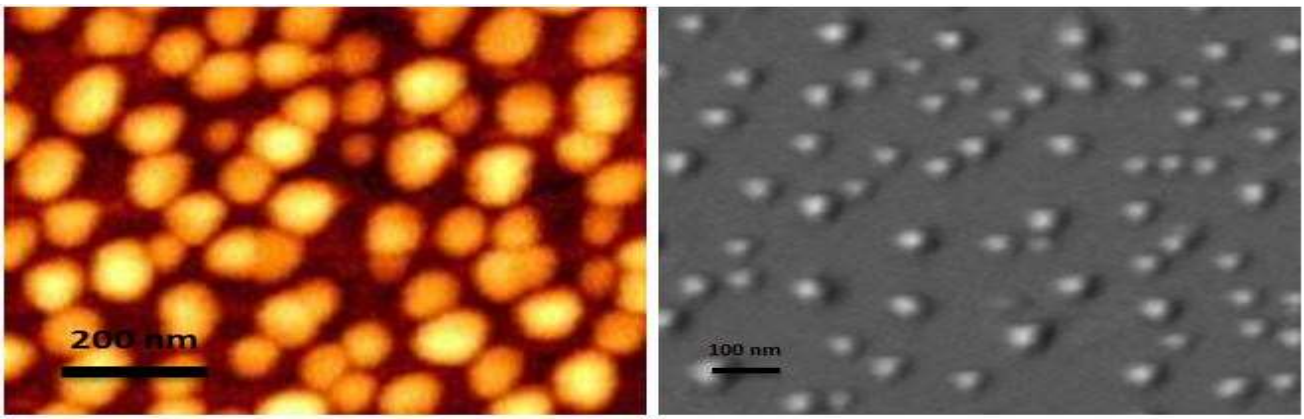

Figure 1: Morphology and size determination of FA-CS-SPION nanosystem by atomic force microscope (A) and field emission scanning electron microscope (B)

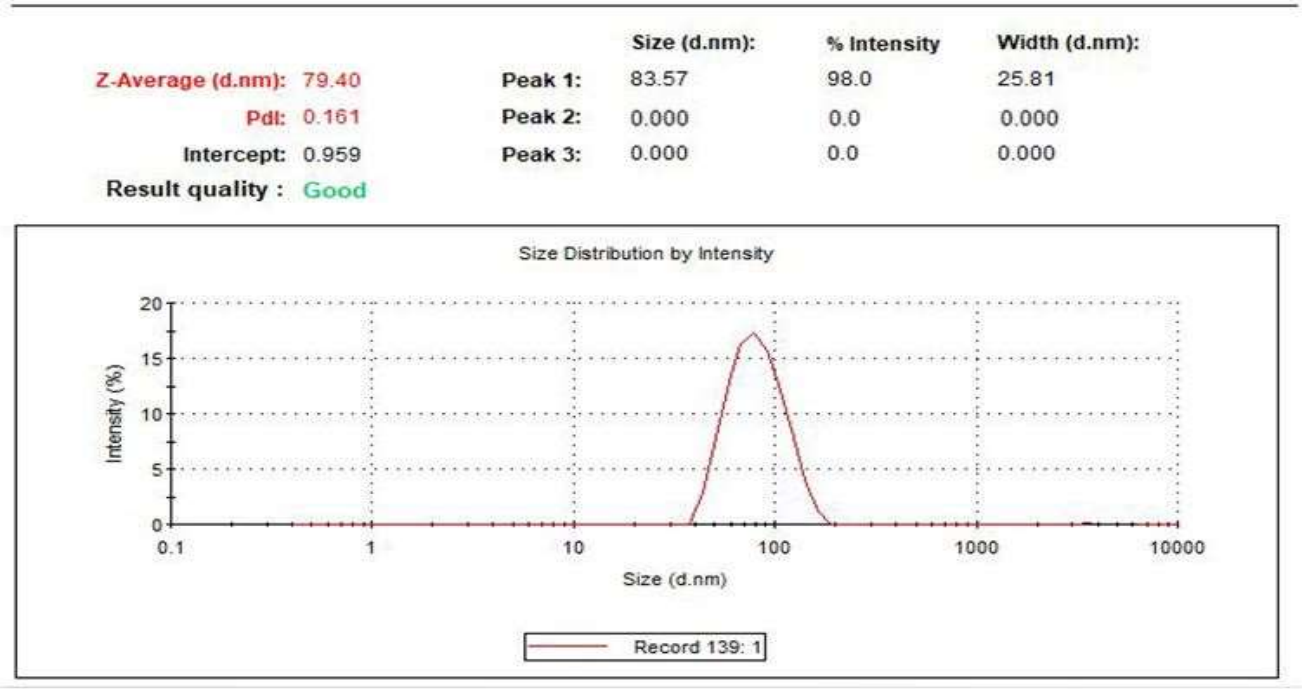

Figure 2: FA-CS- 5-FU-SPION size and polydispersity using dynamic light scattering (DLS)

Encapsulation Efficiency

The FA-CS-5-FU-SPION solution was centrifuged after synthesis and the supernatant collected. The supernatant evaluated by spectrophotometer (UV-Pharma spec, shimadzu) in 280 nm. The 5-FU encapsulation efficiency in FA-CS-SPION nanosystem was diagnosed 77 ± 0.3%. The nanoformulation exhibited remarkable colloidal stability and proper drug permanency in this period.

Release Profile

As shown in release curves of Fig. 3 the 5-FU releases time from loaded nanosystem (FA-CS-5-FU-SPION) over a 96 h period was slower at phosphate buffer with normal pH (pH 7.4) as compared to citrate buffer with acidic pH (pH 5.4). In other hand the release profiles of free 5-FU, showed similar release algorithm at pH 7.4 and 5.4. The curve results proved a faster 5-FU release rate at pH 5.4 under the same conditions compare with pH 7.4.

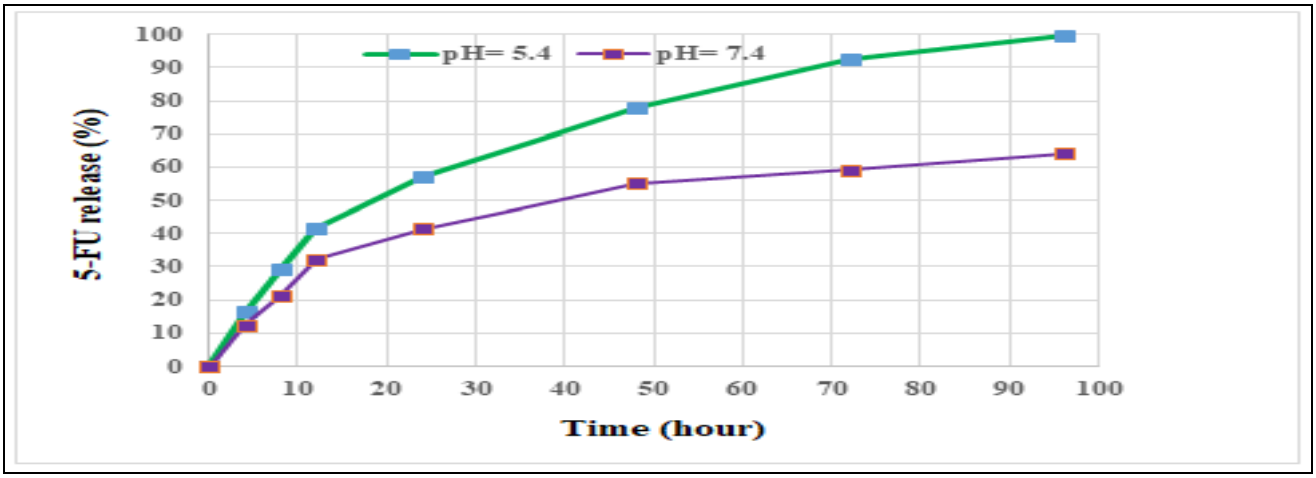

Figure 3: 5-FU release curve in two various pH of 7.4 and 5.4

Cell Internalization

Evaluation of 5-FU internalization into T24 cancer cells and by its functionalization by FITC, carried out by fluorescence imaging. As indicated in Figure 3, the treated cells with FITC functionalized nanoformulation indicated green because of FITC-FA-CS-5-FU-SPION internalization due to solubility increasing of 5-FU. While the free 5-FU treated cells, demonstrate green and star like particles in intercellular space due to 5-FU water insolubility.

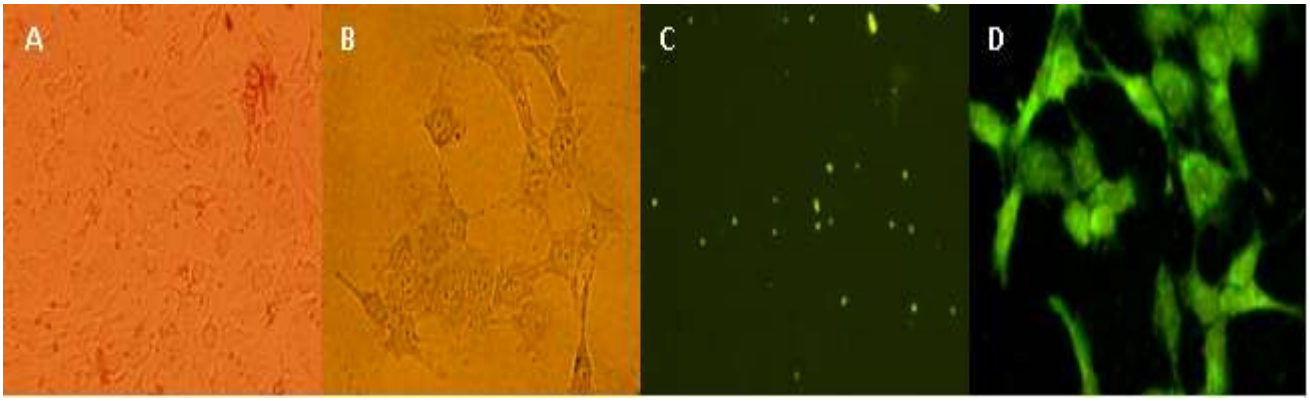

Figure 4: Cell internalization evaluation of FA-CS-5-FU-SPION in T24 cell line using fluorescence microscope (400× magnification). Optic microscopy image of 5-FU treated cells (A).Optic microscopy images of FA-CS-5-FU-SPION treated cells (B). Fluorescence microscopy image of 5-FU treated cells (C). Fluorescence microscopy image of FA-CS-5-FU-SPION treated cells (D)

Cytotoxicity Assay

The cytotoxicity study of void 5-FU, bare nanoparticle (without 5-FU) and nanoformulated 5-FU were evaluated by MTT assay on T24 cancer and HBlEpC normal cell lines. This test performed in two 24 and 48 hours periods and was indicated in curve (fig. 5). In the first step, the cells were treated with various concentration of FA-CS-5-FU-SPION (10-60 µM) for 24 and 48 hours. FA-CS-5-FU-SPION significantly ($P<0.01$) inhibited the T24 cancer cells proliferation time and dose dependent compared with bare nanoparticle and free 5-FU but didn't show any remarkable change in cell growth and proliferation after treating normal HBlEpC cell lines with mentioned treatments. The achieved IC50 data indicated that 38.13 and 27.96 µM are the IC50 concentration for 24 and 48 hours respectively. Moreover, both of bare nanoparticles and free 5-FU treatment didn't indicate any remarkable cytotoxic effect in all utilized concentration.

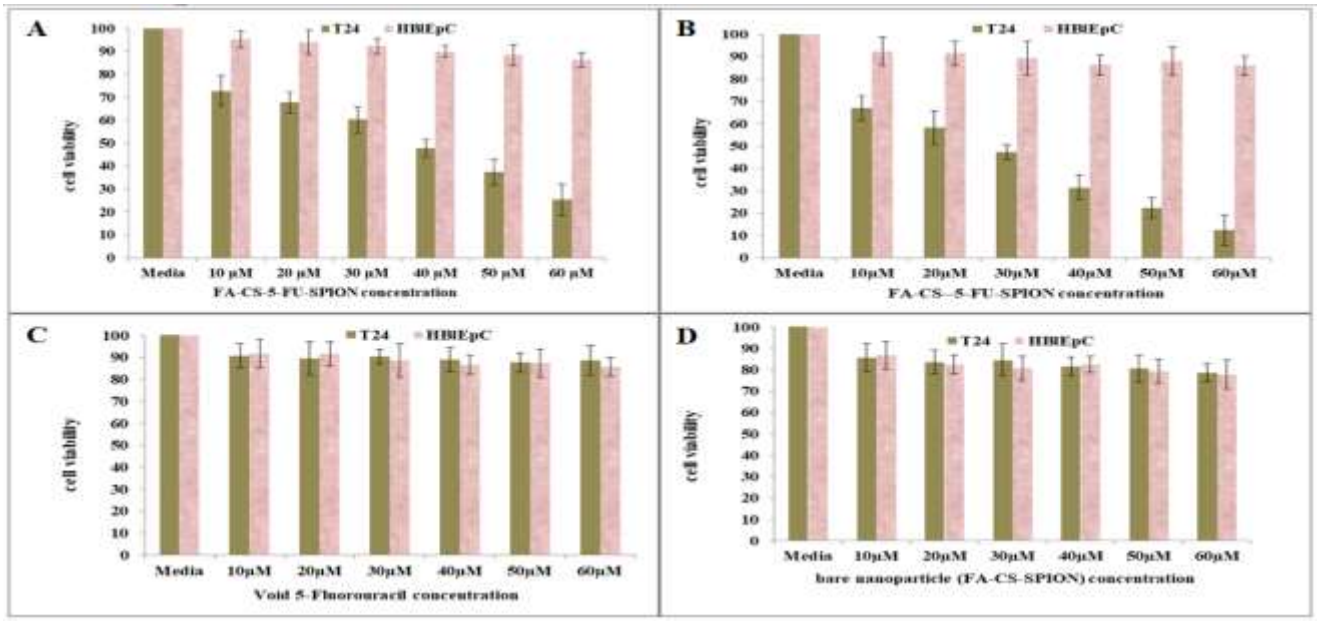

Figure 5: Cytotoxic effect of various concentrations (10-60 µM) of treated FA-CS-5-FU-SPION after 24 (A) and 48 (B). Bare FA-CS-SPION (C) and void 5-FU (D) after 48 h on T24 cancer cells and HBlEpC normal cells

Flow Cytometry

Apoptosis analysis was carried out by Flowjo 7.6.1 software. As indicated in Fig. 6, the significant enhancement was showed in apoptotic cancer cells compared to normal type. As seen in fig. 6 the FA-CS-5-FU-SPION has indicated significant apoptosis in cancer cells whereas has no remarkable effect on normal cells. The FA-CS-SPION didn't reveal any apoptotic effect in the various concentrations as shown from fig. 6 on bladder cancer cell line (T24) as well as normal cell line (HBlEpC) which proved this fact that the FA-CS-SPION does not stimulate any apoptosis on both T24 and HBlEpC cell lines. The achieved data have also exhibited that free 5-FU, does not reveal any apoptotic effect in both T24 and HBlEpC cell lines.

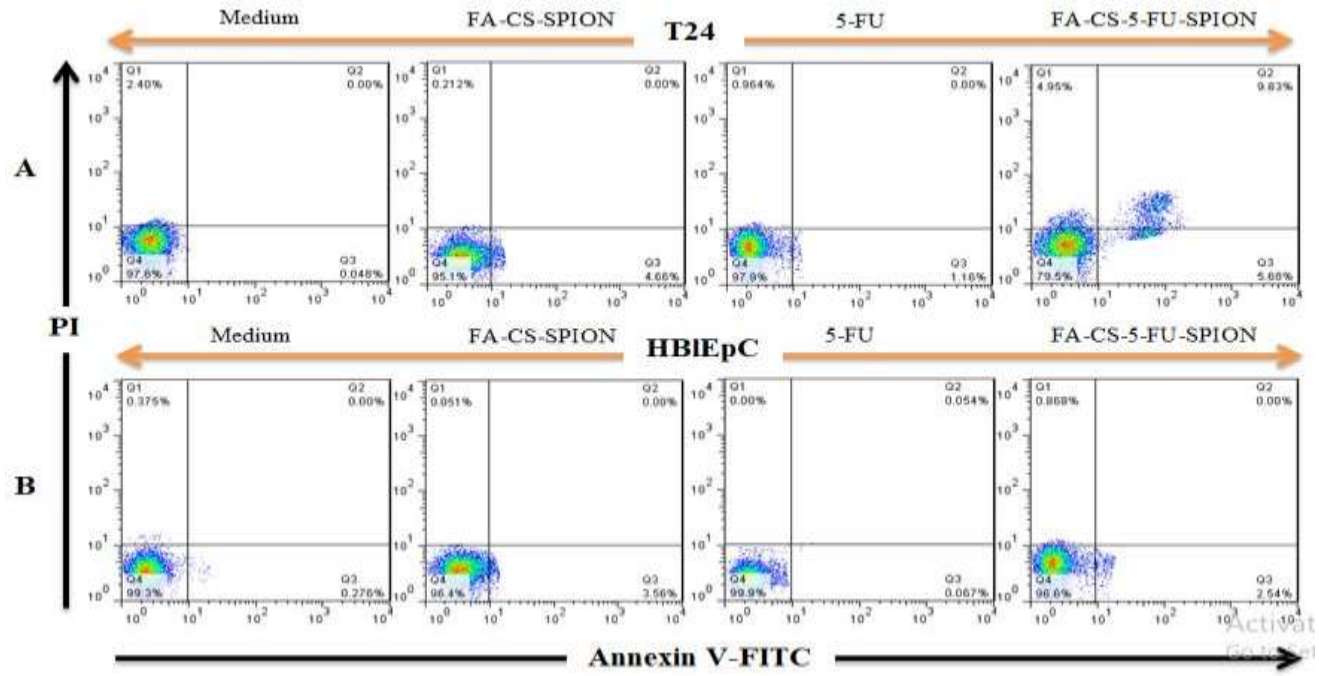

Fig 6: Apoptosis assay using FA-CS-5-FU-SPION on T24 and HBlEpC cell lines

Discussion

The anti metabolite 5-Fluorouracil (5-FU) drug work by preventing vital biosynthetic processes, or inhibiting the normal function of main macromolecules of cells such as DNA and RNA duo to incorporating with them. Despite the strong cancer therapeutic potency of 5-FU, its low water solubility and negligible bioavailability, conduct the researches to apply new strategies to pass this problems and enhance this drug efficiency in the treatment of different diseases specially cancers. Several studies reports that 5-FU solubility was enhanced when it encapsulated or loaded in suitable drug carriers [35]. In this paper, we suggested that folated chitosan covered SPION (FA-CS-SPION) can improve several essential factors such as water solubility, biocompatibility, blood circulation time (durability or stability) and consequence the bioavailability of 5-FU so facilitate applying this therapeutic agent for the antitumoral purpose.

So in this study, we prepared a nanosystem by encapsulation of 5-FU into the FA-CS-SPION nanoparticles and then evaluate its therapeutic effect on the treatment of bladder cancer and normal cell lines. Several studies proved the efficient potency of FA-CS-SPION as a drug nanocarrier for different anticancer agent and demonstrate successful result in treating numerous cancers such as breast and leukemia [36, 37]. Consequently, the FA-CS-5-FU-SPION nanoformulation was prepared with appropriate size (79 ± 13 nm), stable morphology, and good polydispersity for drug delivery purpose (fig 1, 2). After combination the 5-FU in FA-CS-SPION system, we measured the nanosystem characteristics. The encapsulation drug efficiency of 5-FU in FA-CS-SPION nanoparticle was ~73% that display consequence the supreme colloidal stability and good drug feeding. These results indicate almost same encapsulation drug efficiency compare with similar work with this nanoparticle [38,39]. Furthermore, the FA-CS-5-FU-SPION show controlled behavior in drug release process so that in acidic pH (pH of cancer cells microenvironment) presents more drug release compare with normal PH (fig 3).

So the cytotoxicity effect of the FA-CS-5-FU-SPION system, stimulated by internalization of 5-FU into the treated cells and its combination potency to DNA and RNA within the cell (fig 4). In this paper, the cytotoxicity studies exhibit reducing in vitality rate of T24 cancer cell lines due to enhancing the dose of FA-CS-5-FU-SPION treatment after 24 and 48 hours respectively.

The IC50 for both 24 and 48 hours has occurred in 38.13 and 27.96 µM respectively. The FA-CS-5-FU-SPION toxicity in the HBlEpC cells was negligible without any noteworthy IC50 for treated dose (Fig 5). Measurement of the cytotoxicity effect of FA-CS-5-FU-SPION and void 5-FU separately with the all different applied concentration presented negligible cytotoxicity on both T24 cancer and HBlEpC normal cell lines. These data were in accordance with the results of

the study the similar treatment effects of nanoformulated 5-FU in chitosan coated SPION [40]. The apoptosis study demonstrated different result in relation to the T24 cancer and HBlEpC normal cell lines (Fig. 6). The apoptosis induction study proved this fact that the cell death process is influenced remarkably by 5-FU in its nanoformulation (FA-CS-5-FU-SPION) and there is different sensitivity to the two cancerous (chronic myeloid bladder T24) and normal (human bladder epithelial cells HBlEpC) cell lines (Fig. 6).

The treated normal human bladder epithelial cells (HBlEpC) with FA-CS-5-FU-SPION nanoformulation presented similar result in apoptosis induction compare with cytotoxicity assay so the HBlEpC didn't showed any apoptosis induction. Consequently, the effect of FA-CS-5-FU-SPION is specifically in chronic myeloid bladder T24 cancer cells and has not any impact on human bladder epithelial cells HBlEpC normal cells that is due to the specific effect of nanoformulated 5-FU in the efficient targeting of cancer cells than normal cell. In return, the bare FA-CS-SPION has not shown any cytotoxicity effect and is completely biocompatible. Also the void 5-FU drug has not presented any cytotoxic effect on cell proliferation because of drug efficiency losing due to the low water solubility, negligible half-life and poor bioavailability of 5-FU (Fig 5).

Conclusion

The cytotoxicity effect and apoptosis induction studies on cancer (T24) and normal (HBlEpC) cells demonstrated the specific effect of 5-FU formulated nanoparticle (FA-CS-5FU-SPION on T24 cells whereas has not significant cytotoxic effect on HBlEpC cells. The selective behavior of 5-FU drug in impressive killing the cancer cells compare with normal cells is the basic cause for this finding. Additionally, the bare nanoparticle (FA-CS-SPION) didn't show any noticeable cytotoxicity and has remarkable biocompatibility that consequently leads to negligible effect on cell proliferation decreasing.

References

1. Urruticoechea A et al (2010) Recent advances in cancer therapy: an overview. Curr. Pharm. Des., 16 (1): 3-10.
2. Al-Musawi S et al (2018) Folated-nanocarrier for paclitaxel drug delivery in leukemia cancer therapy. J Pharm Sc. J. Pharm Sci. & Res, 10 (4):749-754.
3. Farooqi SI et al (2018) Synthesis, theoretical, spectroscopic and electrochemical DNA binding investigations of 1, 3, 4-thiadiazole derivatives of ibuprofen and ciprofloxacin: Cancer cell line studies. J Photochem Photobiol B., 189: 104-118.
4. Khanjani F et al (2018) Rapid screening of drug candidates against EGFR/HER2 signaling pathway using fluorescence assay. Anal. Bio. Anal. Chem., 410(30): 7827-7835.
5. Sara JD et al (2018) 5-fluorouracil and cardiotoxicity: a review. Ther. Adv. Med. Oncol., 18 (10): 1758835918780140.
6. Longley DB et al (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer, 3 (5): 330-8.
7. Carrillo E et al (2015) 5-Fluorouracil derivatives: a patent review. Expert Opin. Ther. Pat., 25 (10):1131-44.
8. Kumari S, Kondapi AK (2017) Lactoferrin nanoparticle mediated targeted delivery of 5-fluorouracil for enhanced therapeutic efficacy. Int. J. Biol. Macromol., 95: 232-237.
9. Feng Y et al (2018) Autophagy Inhibitor (LY294002) and 5-fluorouracil (5-FU) Combination-Based Nanoliposome for Enhanced Efficacy against Esophageal Squamous Cell Carcinoma. Nanoscale Res Lett. 17:13(1); 2018: 325.
10. Silva JSF et al (2018) Multifunctional System Polyaniline-Decorated ZIF-8 Nanoparticles as a New Chemo-Photo thermal Platform for Cancer Therapy, 3(9): 12147-12157.
11. Wang Z et al (2018) Colorectal cancer combination therapy using drug and gene co-delivered, targeted poly (ethylene glycol)-ε-poly (caprolactone) nanocarriers. Drug Des Devel. Ther., 12: 3171-3180.
12. Syed AA et al (2018) Applications of nanoparticle systems in drug delivery technology. Saudi Pharm J., 26(1): 64-70.
13. Naskar S et al (2018) J Drug Target. Chitosan-based nanoparticles as drug delivery systems: a review on two decades of research, 5: 1-15.

14. Divya L et al (2014) Chitosan-Based Micro and Nanoparticles: A Promising System for Drug Delivery. Research Journal of Pharmacy and Technology, 7 (12): 1463-71.
15. Patel MP et al (2010) Chitosan mediated targeted drug delivery system: a review. J. Pharm. Pharm. Sci., 13(4): 536-57.
16. Singh N et al (2010) Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). Nano rev., 1: 234-39.
17. Schlachter EK et al (2011) Metabolic pathway and distribution of superparamagnetic iron oxide nanoparticles: in vivo study. Int. J. Nanomedicine, 6: 1793-1800.
18. Friedrich RP et al (2015) Flow cytometry for intracellular SPION quantification: specificity and sensitivity in comparison with spectroscopic methods. Int. J. Nanomedicine, 10: 4185-4201.
19. Jain H (2013) Magnetic Nanoparticles: As a Drug Targeting Carrier. Expert Opin. Drug Deliv., 10(1): 73-87.
20. Kievit FM et al (2011) Magnetite Nanoparticles for Medical MR Imaging. Research Journal of Pharmacy and Technology, 4 (7): 1040-45.
21. Li L et al (2013) Super-paramagnetic Iron Oxide Nanoparticles as MRI contrast agents for Non-invasive Stem Cell Labeling and Tracking. Theranostics, 3(8): 595-615.
22. Kumar P et al (2018) Preparation and characterization of superparamagnetic iron oxide nanoparticles for magnetically guided drug delivery. Int. J. Nanomedicine, 13: 43-46.
23. Samani S et al (2013) Preparation and assessment of chitosan-coated superparamagnetic Fe3O4 nanoparticles for controlled delivery of methotrexate. Res Pharm. Sci., 8(1): 25-33.
24. Assa F et al (2017) Chitosan magnetic nanoparticles for drug delivery systems. Crit. Rev. Biotechnol., 37(4): 492-509.
25. Abdul-Husin IF et al (2018) Aqueous lemon extracts as antimicrobial agent against some pathogenic bacteria. Plant Archives, 18(1): 431- 434.
26. Senapati S et al (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther., 16 (3): 7.
27. Fathi M et al (2017) Stimuli-responsive chitosan-based nanocarriers for cancer therapy. Bio impacts, 7(4): 269-277.
28. Revia RA, Zhang M (2016) Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances. Mater Today (Kidlington), 19(3): 157-168.
29. Khedri B et al (2018) Preparation of chitosan-coated Fe3O4 nanoparticles and assessment of their effects on enzymatic antioxidant system as well as high-density lipoprotein/low-density lipoprotein lipoproteins on wistar rat. Biomedical and Biotechnology Research Journal, 2 (1): 68-73.
30. Wang W et al (2015) Poly (lactic acid)/chitosan hybrid nanoparticles for controlled release of anticancer drug. Mater. Sci. Eng. C Mater. Biol. Appl., 46: 514-20.
31. Kadhum SA et al (2017) Evaluation of Antimicrobial Activity of the Aquatic Extract against Bacterial isolates from URTI in Babylon Province, Iraq. J. Global Pharma. Tech., 12(9): 296-301.
32. Sağir T et al (2016) Preparation and in vitro evaluation of 5-flourouracil loaded magnetite-zeolite nanocomposite (5-FU-MZNC) for cancer drug delivery applications. Biomed Pharmacother, 77:182-90.
33. Asadi L et al (2018) Anticancer Agents Med Chem. Cytotoxic effect of 5-Fluorouracil-loaded polymer-coated magnetite nanographene oxide combined with radiofrequency. Anticancer agents Med Chem., 18(8): 1148-58.
34. Hajikarimi Z et al (2014) Evaluation of the cytotoxic effects of PLGA coated iron oxide nanoparticles as a carrier of 5- fluorouracil and mega-voltage X-ray radiation in DU145 prostate cancer cell line. IEEE Trans Nanobioscience, 13(4): 403-8.
35. Metterle L et al (2016) Intralesional 5-fluorouracil (FU) as a treatment for nonmelanoma skin cancer (NMSC): A review. J. Am Acad. Dermatol., 74(3): 552-7.
36. Kumari S, Kondapi AK (2017) Lactoferrin nanoparticle mediated targeted delivery of 5-fluorouracil for enhanced therapeutic efficacy. Int. J. Biol. Macromol., 95: 232-237.

37. Ni W et al (2018) Dual-targeting nanoparticles: co-delivery of curcumin and 5-fluorouracil for synergistic treatment of hepatocarcinoma. J. Pharm Sci., 18: (18) 30674-9.

38. Handali S et al (2018) A novel 5-Fluorouracil targeted delivery to colon cancer using folic acid conjugated liposomes. Biomed Pharmacother, 108: 1259-1273.

39. Mohiyuddin S et al (2018) Enhanced antineoplastic/therapeutic efficacy using 5-fluorouracil-loaded calcium phosphate nanoparticles. Beilstein J. Nanotechnol., 20 (9): 2499-2515.

40. Morán M et al (2018) Dual responsive gelatin-based nanoparticles for enhanced 5-fluorouracil efficiency. Colloids Surf B Bio interfaces, 13 (172): 646-654.

