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Synthesis and evaluation of a novel nanosized anionic linear globular dendrimer G2-ciprofloxacin conjugate against prostate cancer

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Abstract: Prostate cancer is the second most common cancer in the world and the fifth cause of cancer deaths in men. Ciprofloxacin enables the inhabitation of the development of prostate cancer. In this regard, we plan to improve the anticancer effect of ciprofloxacin using the anionic G2 dendrimer in conjunction with ciprofloxacin. In the current study, we measured the size and the zeta potential as well as LC Mass to prove the fact that the conjugation was synthesized correctly. The anticancer activity among three groups including Ciprofloxacin, Ciprofloxacin –G2 dendrimer, and control was measured *in vitro. In vitro* studies showed that G2 anionic linear-globular polyethylene-glycol-based dendrimer, which conjugated to ciprofloxacin, was able to significantly improve the treatment efficacy over clinical ciprofloxacin alone with respect to proliferation assay. Maximal inhibitory concentration (IC_{50}) was calculated as 200 µg/mL for ciprofloxacin alone and 30µg/mL for ciprofloxacin–G2 dendrimer. In addition, the growth of DU-145 cancerous cells was inhibited by ciprofloxacin–G2 dendrimer conjugate and the number of apoptotic and necrotic cells was increased significantly as evaluated by an annexin V-fluorescein isothiocyanate assay. Ciprofloxacin –G2 dendrimer conjugate was able to increase Bcl-2/Bax ratio in a large scale as compared with the control group and CBL alone. According to the above results, this compound could be considered as a good candidate for functional cancer treatment with low side effects.

Keywords: Prostate cancer, ciprofloxacin, dendrimer, nanosize conjugate, anticancer drug.

INTRODUCTION

Currently, cancer is considered a new and not completely understood disease. Despite fast and deep developments in research through the last decades, cancer issue is still remaining a worldwide killer (Jamal *et al.,* 2011). Prostate cancer has a major role in men mortality which progresses compare to other cancers (Torre *et al.,* 2012). Statistics show the prostate cancer is ranked second in men death among other cancers in the USA and Europe (Jadvar, 2011). The primary treatment for prostate cancer is surgical excision of the prostate (radical prostatectomy) (Virgolini *et al.,* 2018). Drug treatment of prostate cancer with finasteride (5a-reductase inhibitor) has shown to be able to decline dihydrotestosterone levels leading to reduce the size of the prostate (Marberger, 2006). However, the use of finasteride has been controversial since it has been showed that it might develop high-grade prostate cancer during clinical trial studies (Patel, 2014). Ciprofloxacin is an antibacterial drug that is commonly used to treat a wide range of bacterial strains such as E. coli Puoci *et al.,* 2012), especially in urinary tract infections. This drug is mostly concentrated in the lung tissue, the prostate gland, and the urinary tract. The concentration of ciprofloxacin in the above tissues is incredibly more than it in the serum and other tissues (Haraguchi *et al.,* 2007). Ciprofloxacin has been shown to be able to halt topoisomerase II in eukaryotic, including mammalian cells (Idowu and Schweizer, 2017). The inhibition activity of this drug is through breaking the double-strand of the nucleic acids and arresting the cell cycle which promotes apoptosis in cancerous cells (Lim *et al.,* 2018). There are several studies indicating ciprofloxacin role in adjuvant therapy of certain cancers (Kloskowski *et al.,* 2010, Kloskowski *et al.,* 2011). It has also been shown that ciprofloxacin is able to decline prostate cancer cell proliferation (Maj *et al.,* 2017). Dendrimers, on the other hand, are highly branched macromolecules. Their structure consists of three moieties: a central core, repetitive branching units and terminal groups which enable modifiable surface groups. The generation of dendrimers is determined by the number of repetitive branching units (Svenson and Tomalia, 2012, Lee *at el,* 2005). Dendrimers draw attention to drug delivery due to their specific properties and their adjustable size and shapes (Kalomiraki *et al.,* 2016). Drugs and chemicals can be loaded in the dendrimers' cavities or bound to their external group of the surface through hydrophobic or electrostatic interactions. The terminal functional groups of dendrimers are also able to covalently attaché to various drugs (Buhleier *et al.,* 1978). The broad spectrum effect of anticancer drugs faces two major difficulties. First is their inefficiency in the aquatic medium and the second is their unspecific effects and their toxic effects on normal tissues. Dendrimers are assumed to be able to overcome

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these difficulties significantly when they are used in a drug delivery system (Van Dongen *et al.,* 2014, Zhong *et al.,* 2016). In this study, a dendrimer-drug delivery system (Anionic Linear Globular Dendrimer G2- Ciprofloxacin) was introduced and applied to improve the anticancer effect of ciprofloxacin against prostate cancer.

2- Experimental details

Determination of ciprofloxacin–G2 dendrimer conjugate

To determine the structure and the size distribution of the conjugation between G2 dendrimer and Ciprofloxacin, a thermo microscope (model AP-2001, Waltham, MA, USA) and a dynamic light scattering zetasizer (Malvern Instruments, Malvern, UK) were used. Liquid chromatography/mass spectrometry (LC/MS) analysis was performed by Agilent 6410 Triple Quadrupole LC/MS in order to confirm the molecular structure of the CBL–G2 dendrimer conjugate.

Chemical synthesis of the CBL–G2 dendrimer conjugates

Anionic linear-globular dendrimer G2 was synthesized according to the method previously reported by Assadi *et al* with some modifications. Briefly, 1 mL (3.7 mmol) polyethylene glycol (PEG) 600 (Merck, Darmstadt, Germany) was dissolved in 10 mL Dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany). Then, 0.75 g (2*3.7 mmol) N, N′-Dicyclohexylcarbodiimide (DCC) (Merck, Darmstadt, Germany) was added to the solution. The reaction was incubated for 30 min at room temperature while stirring. Next, 0.71 g (2*3.7 mmol) citric acid (Merck, Darmstadt, Germany) was added to the solution and the reaction remained stirred at room temperature for 1 h. Afterward, 2.25 g (6*3.7 mmol) DCC and 5 mL DMSO were added and the reaction continued stirring under the above-mentioned conditions for approximately 15 min. Finally, 2.1 g (222 mmol) citric acid was added and the reaction was continued stirring for 1 week at room temperature. The solution containing G2 dendrimer was then filtered and the purification was performed using a Sephadex G-50 fine column (GE Healthcare Life Sciences, UK). Finally, the purified product was lyophilized and the greenish doughy product was collected.

The product was then mixed with adipic acid dihydrizide (ADH) (Merck, Darmstadt, Germany), which acted as a linker spacer to facilitate the conjugation to ciprofloxacin (in a ratio of 1mmole dendrimer to 10 mmol ADH) in the presence of 10 mmol water soluble ethyl dimethyl propylamine carbodiimide (EDC) (Merck, Darmstadt, Germany). Subsequently, 500 mg ciprofloxacin (Merck, Darmstadt, Germany) in 1 ml water was added dropwise to the solution containing functionalized dendrimer in the presence of 10 mmole EDC and the reaction mixture was stirred at room temperature for at least 24 hours.

In vitro cell culture

DU-145 cell line was obtained from the Pasteur Institute of Iran. The cells were incubated at 37°C in an incubator containing CO2 for 48 hours. Cells were removed from the flask using 5 ml Trypsin/EDTA, centrifuged, washed, resuspended in RPMI containing 10% FBS and split into two 75 ml flasks and incubated at 37°C until use.

In vitro apoptosis necrosis assay

An Annexin V-propidium iodide staining kit was consumed to assess apoptosis according to the manufacturer's instruction. Du-145 cell line (5000 cells/well) was used for the cell viability test. The cells were incubated within the presence of different rate of conjugation and the same amount of ciprofloxacin for 48 hours. Each concentration was tested in duplicate .

Real-Time PCR with SYBR Green

To investigate the expression of Bax and Bcl-2 gene in DU-145 cell line a quantitative PCR was performed. RNeasy Plus Mini Kit (Qiagen) was used in order to extract the total cellular RNA from the treated and untreated cells according to the manufacturer's protocol. Quanti-Tect Reverse Transcription Kit (Qiagen) was applied to isolate High quality of RNA for cDNA synthesis based on the manufacturer's instructions. To examine the primer specificity for real-time PCR the BLAST program (https://blast.ncbi.nlm.nih.gov/blast) was performed. A total volume of 20l reaction mixture was consumed for each real-time PCR reaction based on the DNA master SYBR Green kit protocol (Roche Applied Sciences). The PCR cycling condition consisted of one cycle at 95°C for 15min and 30 cycles at 95°C for the 20s, 60°C for 60s using an ABI 7300 real-time PCR system (Applied Biosystems, USA). The comparative threshold cycle (Ct) was used to evaluate gene expression. To provide ΔCt and ΔΔCt mean, threshold cycle (mCt) value of internal housekeeping gene (GAPDH) was taken off from mCt value of the target genes and the Ct values were calculated as the value of each sample. The mRNA level obtained from each sample was compared with the human glyceraldehydes-3- phosphate dehydrogenase (GAPDH) mRNA level as the control. Finally, the ratio (Ratio=2−ΔΔCt) was considered to evaluate the target/control gene expression ratio.

STATISTICAL ANALYSIS

Statistical analysis was performed using Prism5 and excels software (Microsoft Office 2013). For quantitative data analysis, One Way ANOVA in case of cluster comparison was applied. $P<0.05$ was considered statistically significant.

RESULTS

Determination of ciprofloxacin–G2 dendrimer conjugate

LC/MS results confirmed the accuracy of the ciprofloxacin–G2 dendrimer conjugation as illustrated in

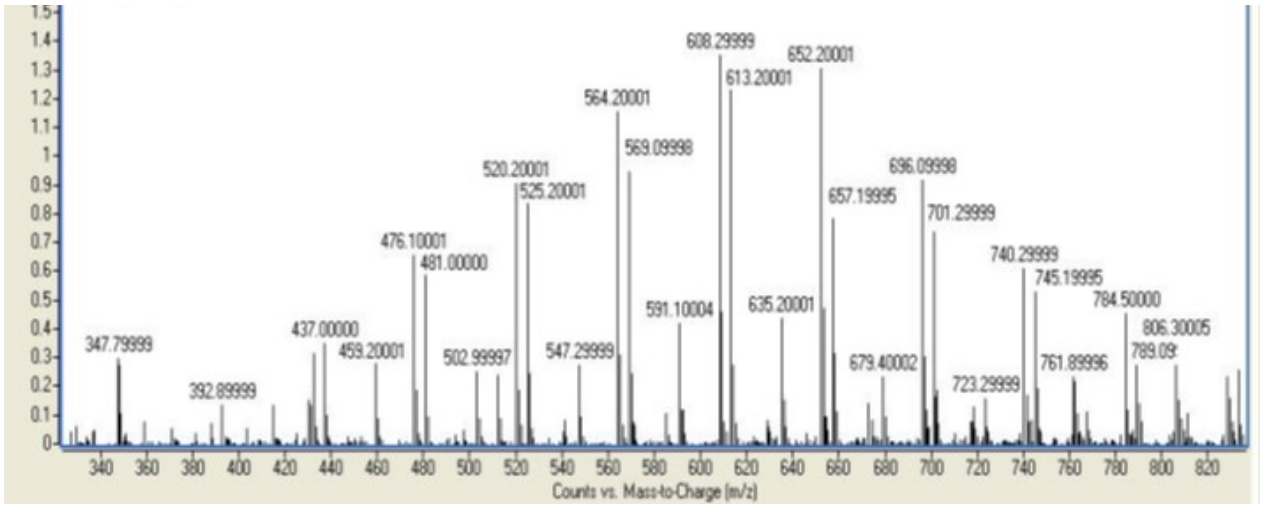

Fig. 1: Ciprofloxacin conjugation to dendrimer, obtained by LC/MS

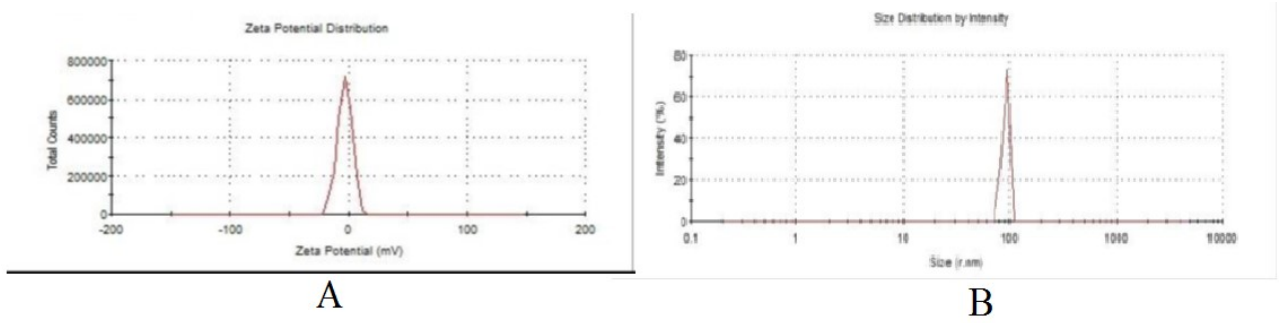

Fig. 2: Size distribution and zeta potential of G2. (A), Zeta potential distribution; (B), size distribution of G2.

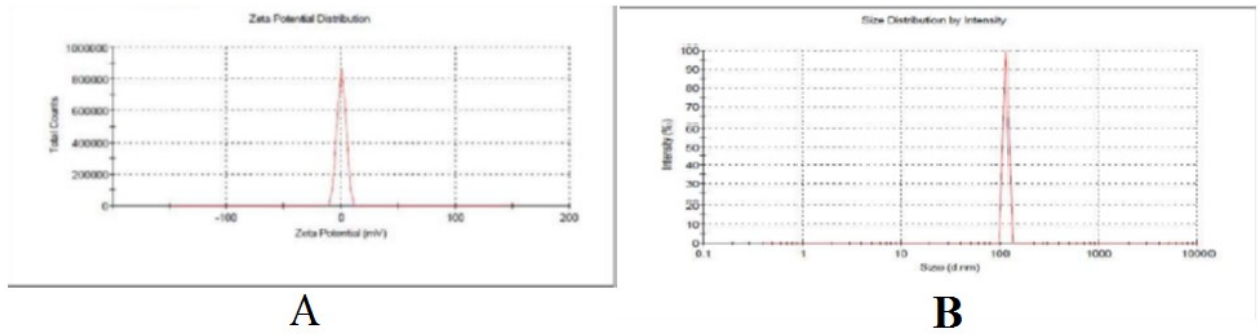

Fig. 3: Size distribution and zeta potential of Ciprofloxacin –G2 dendrimer. (A), Zeta potential distribution; (B), size distribution of G2.

Fig. 1. The most important peak of m/z was 547, which represent the final conjugated Ciprofloxacin–G2 dendrimer. Another important m/z peak was identified on 437 spot that belongs to PEG. Other important peaks are shown in a fig. 3. By comparing the molecular weights of dendrimer-G2, Ciprofloxacin and the conjugate, the average efficacy of conjugation process is predictable to be 2:1 (Ciprofloxacin: dendrimer-G2) molar ratio per particle.

The average size and zeta potential of the conjugate were 105 nm and 0.559 mV respectively (fig. 2). Size and zeta potential of the intact dendrimer were evaluated as 91 nm and -3.38 mV respectively (fig. 3). The conjugation was confirmed as the complex became larger in size and getting a slightly positive charge as compared to the intact dendrimer (Hajmohammadi *et al.,* 2015).

In vitro apoptosis necrosis assay

The results of MTT assay for both ciprofloxacin and ciprofloxacin–G2 dendrimer in concentrations of 10, 50, 75 and 100µM are shown in fig. 4.

It is crystal clear from the fig. 4 that by increasing the concentration of both the drug and conjugation the cell viability was decreased significantly. The significant statistical differences can be seen in the control with 10, 50, 75 and 100µM of ciprofloxacin and ciprofloxacin–G2 dendrimer ($p<0.001$). A significant difference ($p<0.05$) was observed between the 75 and 100 µM ciprofloxacin and the same dose inconjugate. Nano drug conjugation compared to the drug alone in 75 and 100 µM illustrated a significant high toxicity to the cancer cells. The same conforming evidence was observed using a flowcytometry assay (fig. 5). In order to measure the IC_{50} (half maximal inhibitory concentration) of the samples, a linear model chart was created. The IC_{50} of ciprofloxacin was 200 µM and IC50 of Ciprofloxacin–G2 dendrimer was 30µM (Calculated by equation: y=2.172x-121.3) (fig. 6). Comparing the results, it was determined that the lower concentration of ciprofloxacin in the new conjugation was able to kill 50% of cancerous cells as compared the higher concentration of intact Ciprofloxacin.

DISCUSSION

The ratio of Bax/Bcl-2 has been determines to be the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis (Farhoudi and Ghorbani, 2018). Analysis of apoptosis-related gene expression data revealed that the mRNA level of Bax gene was upregulated significantly whilst the expression of antiapoptotic Bcl-2 was relatively down-regulated in cells treated with the conjugated nano druge in comparison with untreated control. It appears that these changes may refer to apoptotic potential of new nano drug conjugation. Consequently, the Bax/Bcl-2 ratio increased significantly as shown in fig. 7.

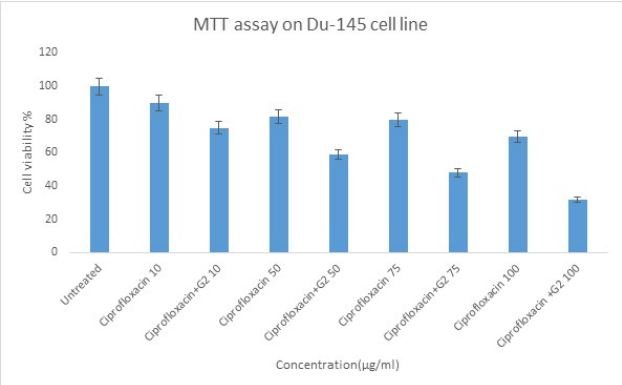

Fig. 4: Results of MTT assay on DU-145 cells, as percentage cell viability at different concentrations, compared using Ciprofloxacin and Ciprofloxacin –G2 dendrimer.

Some new drugs in markets have been faced with a series of inefficiency like low aqueous solubility and short half-life (Hajmohammadi *et al.,* 2015). Nanoparticles have been used to overcome these difficulties because of their ability to modify the basic properties of drug molecules, solubility, half-life, biocompatibility and its release characteristics (Hu *et al.,* 2010). Dendrimers are polymeric architectures that are diagnosed by their differentiation and versatile structure used in drug delivery (Hu *et al.,* 2010). These biodegradable nanostructure macromolecules have shown their potential abilities in entrapping and/or conjugating high molecular weight hydrophilic/ hydrophobic entities by host-guest interactions and covalent bonding (pro-drug approach) respectively (Alavidjeh *et al.,* 2010, Martinez *et al.,* 2017). Recently polymers have been used largely in drug delivery since the pharmacokinetics, bio-distribution and controlled release of the drug has been improved (Bello *et al.,* 2017). Thanks to the above mentioned properties, dendrimers have drawn attention in biological application especially in drug delivery compared to traditional polymers (Soto-Castro *et al.,* 2012, Hossen *et al.,* 2018, Tomalia, 2005). Therefore, this study was focused on conjugation of Anionic linear globular dendrimer G2 with Ciprofloxacin by considering dendrimer properties such as biocompatibility and biodegradability to improve anticancer efficacy of drug.

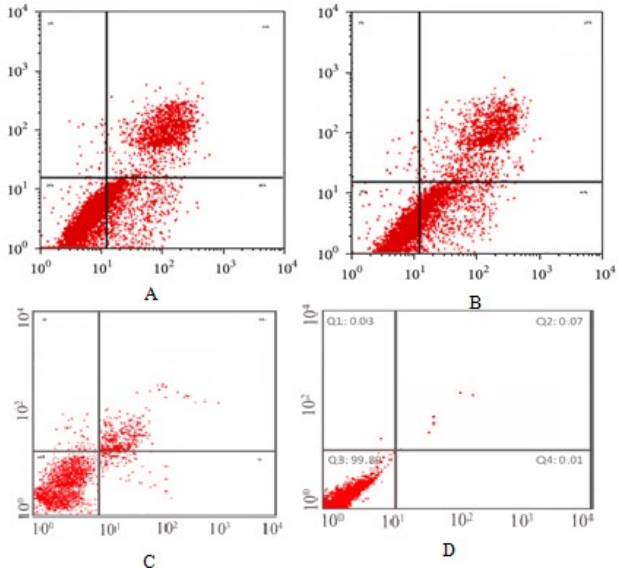

Fig. 5: Dot plots of Annexin V-propidium iodide staining are shown in (A) DU-145 cells treated with 100 µg/mLG2 dendrimer- Ciprofloxacin after 24 hours; (B) DU-145 cells treated with 100 µg/mLG2 dendrimer- Ciprofloxacin after 48 hours (C) MCF-7 cells treated with 100 µg/mL Ciprofloxacin; and (D) untreated DU-145 cells.

Ciprofloxacin is able to halt mitochondrial topoisomerase II resulted in effects on cellular energy metabolism. A study was done recently showed the concentration beyond 80µg/ml of Ciprofloxacin has apoptotic effects whilst at

25µg/ml it stops the proliferation of Jurkat cells without cell death ability (Koziel *et al.,* 2010).

Anticancer dendrimer-based drugs have shown efficient results against cancer cells both in vitro and in vivo because of their especial features. In a research was performed on anticancer efficiency of Anticancer dendrimer-based drugs, it was concluded that Anionic linear globular dendrimer G2 conjugated with cisplatin [cis-diaminedichloroplatinum; (CDDP)] has extremely anticancer potency rather than drug alone when it exposed to several cancer cell lines (Haririan *et al.,* 2010).

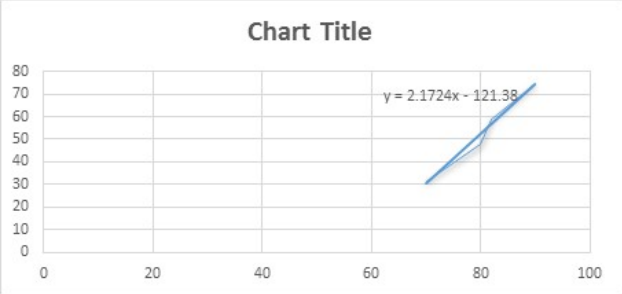

Fig. 6: Results of linear model of G2 dendrimer-Ciprofloxacin MTT assay for DU-145 cells determined by percentage cell viability at different concentrations

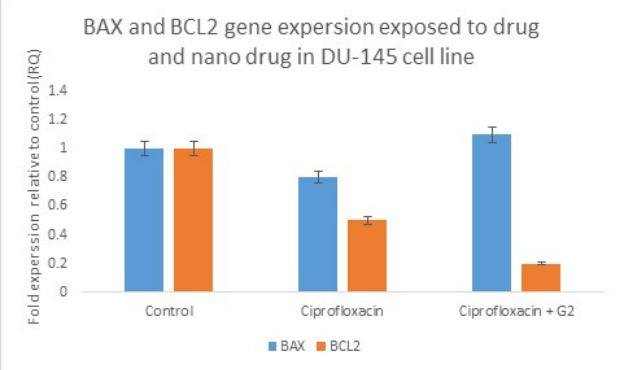

Fig. 7: The effect of Ciprofloxacin and G2 dendrimer-ciprofloxacin on Bax and Bcl-2 gene expression.

The *in vitro* cytotoxicity assay disclosed that Ciprofloxacin G2 dendrimer was more toxic against cancer than drug alone following interaction of Ciprofloxacin G2 dendrimer 48 h with DU-145 cell line. The effect of ciprofloxacin G2 dendrimer on the apoptotic cells was much stronger than the mode of cell death that occurs under normal physiological conditions and in which the cell is an active participant in its own death.

CONCLUSION

Conjugated ciprofloxacin in the ciprofloxacin-G2 dendrimer complex is more effective on tumor cells by increasing the expression of Bax gene as compared with the ciprofloxacin alone. Moreover, further analyses on patients with similar therapeutic status and also tumor characteristics such as tumor stage and differentiation may be very useful from different aspects, including prognosis of the disease, predicting patient's survival, tumor relapse, and also response to chemotherapeutic agents. It is obvious that more studies are also needed to understand the characteristics of the nano-conjugates. The advantages of nano conjugates such as Ciprofloxacin-G2 dendrimer include biocompatibility and biodegradability and the anticancer potency as compared to the intact drugs.

REFERENCES

Alavidjeh MS, Haririan I, Khorramizadeh MR, Ghane ZZ, Ardestani MS and Namazi H (2010). Anionic linear-globular dendrimers: Biocompatible hybrid materials with potential uses in nanomedicine. *J. Mater. Sci-Mater. M.,* **21**(4): 1121-1133.

Assadi A, Najafabadi VS, Shandiz SAS, Boroujeni AS, Ashrafi S, Vaziri AZ, Ghoreishi SM, Aghasadeghi MR, Sadat Ebrahimi SE, Pirali-Hamedani M, Shafiee Ardestani M (2016). Novel chlorambucil-conjugated anionic linear-globular PEG-based second-generation dendrimer: *in vitro/in vivo* improved anticancer activity. *Onco. Targets Ther.,* **9**: 5531-5543.

Bello M, Fragoso-Vázquez J and Correa-Basurto J (1978). Theoretical Studies for Dendrimer-Based Drug Delivery. *Curr. Pharm. Des.,* **23**(21): 3048-3061.

Buhleier E, Wehner W and VoGtle F (1978). Cascade"- and "Nonskid-Chain-like" Syntheses of Molecular Cavity Topologies. Synthesis, **2**:155-158.

Farhoudi R and M Ghorbani (2018). Construction of Gold nanorods carrying IFNγ and methionine to target MCF-7 cells. *Dig. J. Nanomater. Biostruct.,* **13**(1): 225-234.

Hajmohammadi M, Siadat SD, Ghorbani M, Shafiee Ardestani M, Teimourian S, Asgari V, Ahangari Cohan R, Hajmohammadi M, Hajmohammadi A, Behzadi R, Nezhad SR and Namvar Asl N (2015). Synthesis and characterization of a novel chemically designed (Globo) 3-DTPA-KLHantigen. *Drug Des., Devel. and Ther.,* **9**: 217-239.

Haraguchi S, Hioki M and Yamashita K, Orii K, Yamashita Y, Kawamura J, Takushima M, Endo N, Koizumi K and Shimizu K (2007). Ciprofloxacin penetration into the pulmonary parenchyma in Japanese Patients. *Surg. Today,* **37**: 282-284.

Haririan I, Alavidjeh MS, Khorramizadeh MR, Ardestani MS, Ghane ZZ and Namazi H (2010). Anionic linear-globular dendrimer-cis-platinum (II) conjugates promote cytotoxicity in vitro against different cancer cell lines. *Int. J. Nanomed..* **5**: 63-75.

Hossen S, Hossain MK, Basher MK, Mia MNH, Rahman MT, Uddin MJ.Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. *J. Adv. Res.,* **15**: 1-18.

Hu CM, Aryal S and Zhang L (2010). Nanoparticle-assisted combination therapies for effective cancer treatment. *Ther. Deliv.,* **1**: 323-334.

Idowu T and Schweizer F (2017). Ubiquitous nature of fluoroquinolones: The oscillation between antibacterial and anticancer activities. *Antibiotics (Basel),* **6**(4): 1-24.

Jadvar H (2011). Prostate Cancer. *Methods Mol. Biol.,* **727**: 265-290.

Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D (2011). Global cancer statistics. *CA Cancer J. Clin.,* **61**(2): 69-90.

Kalomiraki M, Thermos K and Chaniotakis NA (2016). Dendrimers as tunable vectors of drug delivery systems and biomedical and ocular applications. *Int. J. Nanomed.,* **11**: 1-12.

Kloskowski T, Gurtowska N and Drewa T (2010). Does ciprofloxacin have an obverse or reverse? *Pulm. Pharmacol. Ther.,* **23**: 373-375.

Kloskowski T, Gurtowska N, Nowak M, Joachimiak R, Bajek A and Olkowska J and Drewa T (2011). The influence of ciprofloxacin on viability of A549, HepG2, A375.S2, B16 and C6 cell lines *in vitro*. *Acta. Pol. Pharm.,* **68**: 859-865.

Koziel R, Szczepanowska J, Magalska A, Piwocka K, Duszynski J and Zablocki K (2010). Ciprofloxacin inhibits proliferation and promotes generation of aneuploidy in jurkat cells. *J. Physiol. Pharmcol.,* **61**(2): 233-239.

Lee CC, MacKay JA, Frechet JMJ and Szoka FC (2005). Designing dendrimers for biological applications. *Nat. Biotechnol.,* **23**: 1517-1526.

Lim EJ, Yoon YJ, Heo J, Lee TH and Kim YH (2018). Ciprofloxacin enhances trail-induced apoptosis in lung cancer cells by upregulating the expression and protein stability of death receptors through chop expression. *Int. J. Mol. Sci.,* **19**(10): 1-12.

Maj M, Bajek A, Nalejska E, Porowinska D, Kloskowski T, Gackowska L, Drewa T (2016). Influence of mesenchymal stem cells conditioned media on proliferation of urinary tract cancer cell lines and their sensitivity to ciprofloxacin. *J. Cell Biochem.,* **118**(6): 1361-1368.

Marberger M (2006). The MTOPS study: New findings, new insights and clinical implications for the management of BPH. *Eur. Urol. Suppl.,* **5**: 628-633.

Martínez-Muñoz A, Bello M, Romero-Castro A, Rodríguez-Fonseca RA, Rodrigues J, Sánchez-Espinosa VA and Correa-Basurto J (2017). Binding free energy calculations using MMPB/GBSA approaches for PAMAM-G4-drug complexes at neutral, basic and acid pH conditions. *J. Mol. Graph Model.,* **76**: 330-341.

Patel VH (2014). Nutrition and prostate cancer: an overview. *Expert Rev. Anticancer Ther.,* **14**(11): 1295-304.

Puoci F, Piangiolino C, Givigliano F, Parisi OI, Cassano R, Trombino S, Curcio M, Iemma F, Cirillo G, Spizzirri UG, Restuccia D, Muzzalupo R and Picci N (2012). Ciprofloxacin-collagen conjugate in the wound healing treatment. *J. Funct. Biomater.,* **3**(2): 361-371.

Soto-Castro D, Cruz-Morales JA, Ramírez Apan MT, Guadarrama P (2012). Solubilization and anticancer-activity enhancement of Methotrexate by novel dendrimeric nanodevices synthesized in one-step reaction. *Bioorg. Chem.,* **41**(2): 13-21.

Svenson S and Tomalia DA (2012). Dendrimers in biomedical applications Reflections on the field. *Adv. Drug Deliv. Rev.,* **64**: 102-115.

Tomalia DA (2005). Birth of a new macromolecular architecture: Dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. *Prog. Polym. Sci.,* **30**: 294-324.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A (2015). Global cancer statistics, 2012. *CA Cancer J. Clin.,* **65**: 87-108.

Van Dongen MA, Rattan R, Silpe J, Dougherty C, Michmerhuizen NL, Van Winkle M, Huang B, Choi SK, Sinniah K, Orr BG and Holl MMB (2014). Poly (amidoamine) dendrimer-methotrexate conjugates: The mechanism of interaction with folate binding protein. *Mol. Pharm.,* **11**: 4049-4058.

Virgolini I, Decristoforo C, Haug A, Fanti S, Uprimny C (2018). Current status of theranostics in prostate cancer. *Eur. J. Nucl. Med. Mol. Imaging.,* **45**(3): 471-495.

Zhong Q, Bielski ER, Rodrigues LS, Brown MR, Reineke JJ and da Rocha SR (2016). Conjugation to poly (amidoamine) dendrimers and pulmonary delivery reduce cardiac accumulation and enhance antitumor activity of doxorubicin in lung metastasis. *Mol. Pharm.,* **13**: 2363-2375.