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Research Article

MINIMIZING TOXICITY AND IMPROVING CELL UPTAKE OF DIMEGLUMINE GADOPENTETATE USING A NEW NON-TOXIC AND BIOCOMPATIBLE CHITOSAN-CARBON QUANTUM DOT HYBRID NANOGEL IN HEK293 AND MCF7 CELL LINES

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ABSTRACT

Magnetic resonance imaging (MRI) contrast agents have been used routinely for more than 20 years in order to increase sensitivity and specificity of lesion detection. Gadolinium contrast media are chemical substances used in MRI scans. dimeglumine gadopentetate is one of the gadolinium-based MRI contrast agents. Gadolinium based Contrast agents (CAs) show significantly toxicity profiles. Recently, a delayed serious adverse reaction known as nephrogenic systemic fibrosis (NSF) has been reported in patients, with a marked reduction in renal function after administration of gadolinium based CAs. For gadolinium based CAs, the emerging unexpected cytotoxicity has become a new concern. In this research, antitoxic biochemical and molecular potential basis of Chitosan coated on quantum carbon dot loaded on dimeglumine gadopentetate so as to reduce toxicity of dimeglumine gadopentetate was examined. To determine Chitosan loaded on quantum carbon dot AFM, FTIR and DLS were used. Also UV-Visible was used to prove Magnevist loaded on the new nanogel correctly. In order to evaluate the new nanogel- drug toxicity in coparson with the drug MTT was performed on, normal Kidney cell line, HEK-293 cell line. MTT, Flow cytometry and gene regulation assays were done to examine cytotoxic efficiency of new nanogel- drug compared to the drug alone when it exposed to, Brest cancer cell line, MCF7. As well as, cellular uptake of the new nanogel-drug was measured with ICP-Mass and compared with the free dug. Results showed, the new nanogel-drug was able to reduce toxicity of dimeglumine gadopentetate noticeably over HEK-293 cell line (P<0.05). Cytotoxic efficiency of new nanogel-drug showed it was a little more toxic than Magnevist when it exposed to MCF7 cell line. But this effect was negligible when it compared to anticancer drug Chlorambucil.Cellular uptake study was revealed the new nanogel-drug was able to enter cells five times more than Magnevist, however it was a little more in HEK-293 cell line. From our study, the new nanogel composed of quantum carbon dot and chitosan loaded on dimeglumine gadopentetate is a promising thranostic nanogel compared with the contrast agent and potentially suitable for MRI as a new contrast agent.

***KEYWORDS**: Renal failure, Dimeglumine gadopentetate, Quntum carbon dot, Chitosan.*

INTRODUCTION

Contrast Agent is a substance that used as a factor in increasing the amount of contrast in radiation in the field of medical imaging as well as in medical physics. The contrast media used in MRI causes a change in T1 rest time and T2 are different tissues. In addition, making these materials, most efforts are focused on the injection of paramagnetic and ferromagnetic materials. Magnevist is one of the gadolinium-containing compounds used in medical imaging in MRI. Gadopentetate dimeglumine is known as the Magnevist brand [1, 2]. The accumulation and toxicity of gadolinium-based contrast agents: Gadolinium-based contrast agents (GBCAs) after the US Food and Drug Administration authorized gadolinium gadodentinate in 1988 have found widespread use in MRI imaging studies. Different GBCAs are now available for clinical use in one or more regions of the world and it is estimated that more than 200 million doses of this substance are used worldwide [3, 4]. Gadolinium remains important in the body clinically important. Gadolinium is not found naturally in the body. When it enters the tissues of humans and animals, it remains for a long time. In addition, heavy metals are proven to be toxic. As a result, the risk of GBCAs injection into patients with severe renal impairment has been well documented and can lead to Nephrogenic Systemic Fibrosis (NSF) [5]. NSF is a rare, progressive, usually fatal disease characterized by skin thickening, painful joint contractions, and fibroids of multiple organs including the lungs, liver, muscles, and heart. Nearly all documented cases have occurred in patients with chronic severe renal insufficiency who has received gadolinium contrast. The association between gadolinium and NSF was first reported by Danish nephrologists in 2006. Between 2006 and 2010, several hundred cases were diagnosed worldwide .NSF usually develops clinically within days to months following gadolinium exposure, although rare cases have been reported years later. Nearly all patients have been in severe renal failure, and many were on dialysis [6]. Chitosan is a *polysaccharide* amino acid copolymer of 1, 4 D-glucosamine and N-acetyl-glucosamine [7]. Chitosan has a significant potential for tissue regeneration due to biocompatibility, biodegradability and re-absorption and reactivity, and has active amino and hydroxyl groups that can be chemically modified.

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Chitosan is also capable of modeling into porous scaffolds with controllable properties. In addition, chitosan levels support the attachment and expansion afterwards and the growth of various types of cells, which is attributed to high cationic chitosan [9]. Two important factors that determine the physical properties of chitosan specific applications include the antioxidant activity of chitosan (due to the high hydrogenation ability of chitosan in this nanoparticle) and the low molecular weight of chitosan in terms of antioxidant properties [10]. It is proved that the antioxidant properties of chitosan oligosaccharide in the balance of the antioxidant mechanism of biological systems [11]. Studies have shown that chitosan nanoparticles can reduce the oxidative stress caused by H2O2 in cells [12]. Quantum Carbon Dot is a new generation of carbon-containing oxygen compounds that have spherical geometry of less than 10 nm in size and have received considerable attention in recent years due to their superior physical and chemical properties [13, 14]. Compared to quantum dots of semiconducting and organic pigments, quantum carbon for high water solubility, biocompatibility and optimal bioavailability, chemical inactivity, ease of surface functionalization, high stability to fading and Low toxicity has a growing advantage [15, 16]. In this study, to increase the chitosan efficiency, it is loaded into a carbon-dot quantum, and then the Gadopentetate Dimeglumine *(Magnevist*) is made in a nanogel. Therefore, the toxicity of the magneto-drug loaded in the system (Nanogel Chitosan-Carbon quantum dots) compared with free magnetism has been studied.

MATERIAL AND METHODS

HCl (Sigma),EDTA (Sigma) , Glucose (Sigma) , Glutaraldehyde (Sigma) , Magnevist (Bayer) , HEK-293 and MCF-7 cells(Pasteur *Institute* of Iran (IPI) , Flow Cytometry (Merck) , MTT (Merck) , Powder DMEM medium.

Magnevist loading in the Nanogel (carbon-quantum dots):

The nanogel was first synthesized to load the drug. First, 2 g of glucose were dissolved in 10 ml of disintegrated water and after sonication, 30 ml of chloridric acid was added .Then the ultra-sonication solution was first and then autoclaved. Next, the solution was centrifuged and Carbon quantum dots separated .Then, 10 ml of chitosan was added to the Carbon quantum dots containing OH and COOH surface groups .Then the solution was placed on a stirrer. In order to make the nanogel, 6 mg of EDTA was first added and then the ethanol drop was added .Then, 90 µL of glutaraldehyde was added to form a complex between quantum carbon and chitosan .In the final step, the complex was first centrifuged and washed three times with distilled water in order to increase the purity of the complex for 72 hours in the purified dialysis bag to remove EDTA, ethanol and free carbon-quantum dots.

Determination of nanogel synthesis:

At this stage, AFM (Atomic force microscopy), DLS (Dynamic light scattering), and FTIR (Fourier-transform infrared spectroscopy) tests have been performed to prove the formation of quantum carbon-nanotubes in quantum dots.

FTIR:

In order to Solid sample preparation for spectroscopy ;at first, a small amount of nanoleg sample was selected. Then the resulting thin tablet was placed on a whole in an infrared spectrometer. The analyzes appear in many couriers on the machine's display screen, each courier representing the vibrational frequency of the atoms of a functional group in the structure of our desired molecule in the range of a particular wavelength .

DLS:

The DSL method is used to determine the particle size of this device. In this experiment, we transmitted the lyophilized nanogel content to the Pasteur Institute of Iran (IPI) of the Biotechnology Pilot Division, and after dispersing it in the aqueous phase using the Malvern U.S. Zeta Sizer device, the size and zeta potential of the sample It was measured at 25 ° C.

AFM:

At first dilute the sample to prevent particles from joining. Considering that the sample is organic. Here, with an organic solvent chloroform, the sample was diluted to about 0.001 mM or 5. 0 µM, and then placed 1 to 2 drops on the slurry. Then chloroform will dry. In the resulting images, we will have three-dimensional quality results of surface morphology, nanoparticle size, roughness and smoothness, and so forth.

Determination of Magnevist loaded on nanogel:

Although UV spectrometry could not use to approve synthesis and identification of unknown complex alone, UV spectrometry was carried out as auxiliary method to confirm Gd3+ loading on Glucosamine-dendrimer G2 conjugate.

UV spectrometry:

UV ultraviolet spectrometer test was used to confirm the loading of magnesium in the nanoparticle and the formation of the system. It is also determined by the intensity of absorption, the concentration of the material in a sample. One of the main ways to study and determine the specification of nanoparticles is to use spectroscopy methods.

In vitro cell culture:

MCF-7 and HEK-293 cell lines were delivered from the Pasteur Institute of Iran in a flask. After incubation, in an incubator containing CO2 for 2 days, cells were passaged in two 75 ml flasks. For this purpose, the previous cell culture media was discarded. Then, the cells were rinsed with PBS. With adding 5 ml Trypsin, the cells were separated with a 5-minute incubation in 370C. The residual cells were completely separated with pipetting as well, after adding 10 ml cell culture media containing FBS, cells were poured into a 15-ml falcon to neutralize Trypsin, 4 ml RPMI cell media containing FBS %10 and p/s %1 were added to the cells and it was completely mixed. Then, 2 ml of the cell suspension and 8 ml of the cell media was poured into two 75 ml flasks, and was left in the CO2 incubator.

In-vitro cellular uptake of nanogel-Magnevist Coupled Plasma-Mass Spectroscopy (ICP-MS):

Evaluating nanogel-Magnevist ICP-MS was done using HEK-293 and MCF-7 cell lines. In brief, after incubating nanogel-Magnevist and magnevist (200 µg/mL) with MCF-7 and HEK-293 cells (25,000 cells/well) into six-well plates for 60 min at 37 0C and 5% CO2, the cells were washed using 100 µL of phosphate-buffered saline and subsequently centrifuged at 1000 rpm (The step repeated for three times). After diluting samples in100 ml deionized water, they were ready to be analyzed with ICP-MS.

In vitro apoptosis necrosis assay:

An Annexin V-Propidium iodide staining kit was consumed to assess apoptosis according to the manufacturer's instruction. MCF-7 cell line (5000 cells/well) was used for the cell viability test. The cells were incubated with different amount of nanogel-drug and same amount of the drug for 48 hours with untreated cells as a positive control. Each concentration was tested in duplicate.

Real-Time PCR with SYBR Green. A SYBR Green Real time:

To investigate the expression of Bax and Bcl-2 gene in MCF-7 cell line quantitative PCR was done. RN easy Plus Mini Kit (Qiagen) was used in order to extract Total cellular RNA from the treated and untreated cells based on 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. BLAST program (https://blast.ncbi.nlm.nih.gov/blast) was performed to examine Primer specificity for real-time PCR. Total volume of 20 µl reaction mixture based to the protocol of DNA master SYBR Green mix (Roche Applied Sciences) was consumed for each real-time PCR reaction. The primer concentrations were selected 0.4 µM for genes. PCR cycling condition consists of 10min at 95◦C, 5mins at 95◦C for cycling parameters. Melting stage: at 95◦C for 20s, 60◦C for 60s, and 95◦C for 20s in an ABI 7300 real-time PCR system (Applied Biosystems, USA) were adjusted as an Amplification stage. Comparative threshold cycle (Ct) was used to evaluate the 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. Following Ct values were calculated as Values of each sample. The mRNA level obtained from each sample was adapted to human glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA level. Finally, the ratio formula (Ratio = 2−ΔΔCt) was considered to evaluate target/control gene expression ratio.

Statistical Analysis:

Statistical data 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 AND DISCUSSION

According to the obtained results from the FTIR test for chitosan and quantum dot and the comparison of these two combinations, the synergy of the spectra is observable .Meanwhile, the expansion of O-H group peak represents the formation of a nanogel (Fig. 1).In addition, due to the increased size of carbon quantum dot after loading chitosan (from 7.5 nm to 140nm), these changes represent the formation of a nanogel [Fig.2 (a and b].On the other hand, the chitosan zeta potential chart is due to the positive shift of NH2 (+31 mv) and the quantum dot Zeta potential due to the presence of a COOH (-16.3 mv) negative shift .However, when these two graphs were compared with the final plot of the nanogel formation, the nanogel plot was set to near zero inclined to negative point, which indicates the formation of a nanogel [Fig. 3 (a, b and c)]. The AFM image also confirmed the size of the dendrimer G2 conjugated to glucosamine and its morphology (Fig. 4). After confirming the formation of the nanogel by previous tests, ultraviolet spectroscopy was used to confirm the loading of Magnevist in the nanogel and determine the final structure. Also, it is observed in the ultraviolet peak field that the peak of the nanogel in the specified region has occurred, absorption that does not exist in the ultraviolet spectrum of chitosan. In other words, this peak represents the loading of Magnevist on the nanogel (Fig. 5). In Figures 6 and 7 the concentration of Magnevist in HEK-293 and MCF-7 cells are shown recpectively in comparison between Magnevist and Magnevist –Nanogel.The results clearly indicate that the new version of the nanogel-drug compared to its drug alone is close to 5 times more likely to enter the cell, which is slightly higher in healthy cells than cancer cells. According to the MTT Assay results derived from Magnevist –Nanogel and free Magnevist, the toxicity of Magnevist –Nanogel on HEK-293 cells is less than the drug alone. Especially in a dose of 400 µg / ml, which showed a significant difference between free drug and nanogel-drug (P <0.05) (Fig.8).As shown in the MTT Assay results over MCF-7 cell line with comparing these compounds ,it is observed that both compounds exhibit some degree of anticancer activity although this difference is not significant. When it comes to comparing *Chlorambucil* as standard anticancer drug control in this study with both compounds there is a significant difference between *Chlorambucil* with both compounds (P <0.05) (Fig. 9) . Results of the apoptosis- necrosis test also confirmed the data obtained from the MTT test. It is indicates a little improvement in the anticancer effect of nanogel-drug compared to Magnevist yeht nehw enil llec 7-FCM ot desopxe (Fig. 10). Results of the gene expression in MCF-7 cell line also confirmed the data obtained from the MTT and Flowcytometry tests. As a result, the increase of BCl2 expression followed by decrease in BAX expression when MCF-7 cell line exposed to drug-nanogel compared to free drug indicate more apoptosis originated from drug-nanogel compared to drug alone (Fig. 11). The results of loading Magnevist contrast agent in the new system of Quantum Carbon Dot -chitosan in reducing the magnitude of toxicity in the HEK-293 cell line with promising and impressive results in this study have been proven .As a result of the confirmation of results in reducing the toxicity of the cell line, it is suggested that animal studies of the pathology of this compound be tested in human clinical phases .If confirmed by the results of human clinical phases, this product can enter the pharmaceutical market.

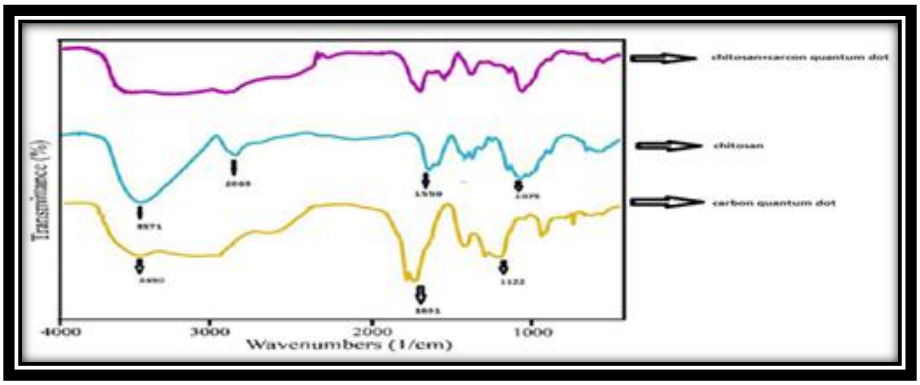


Fig. 1: Frequency vibration curve for functional groups of Chitosan-quantum carbon dots in the spectral range

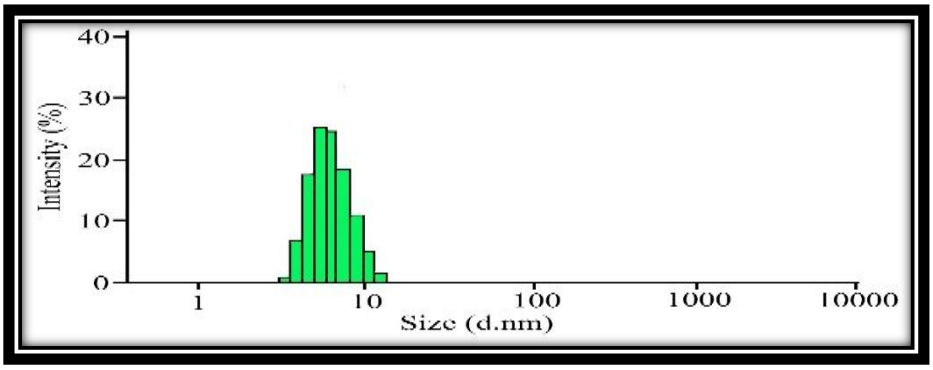


Fig. 2 (a): Size of quantum carbon dots

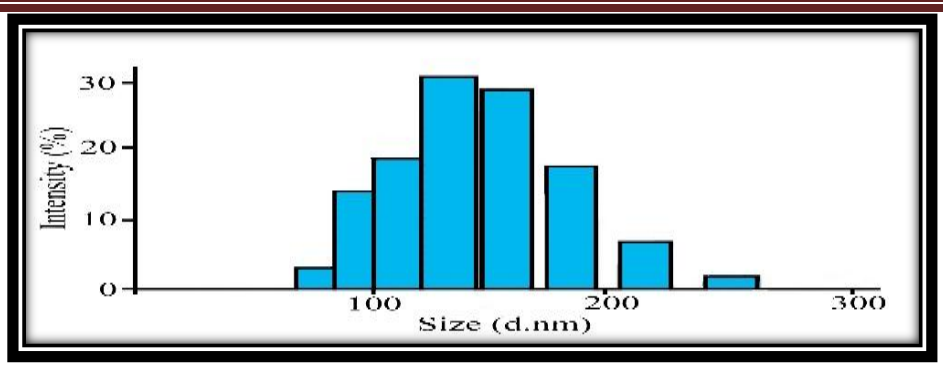


Fig. 2 (b): The size of the nanogel

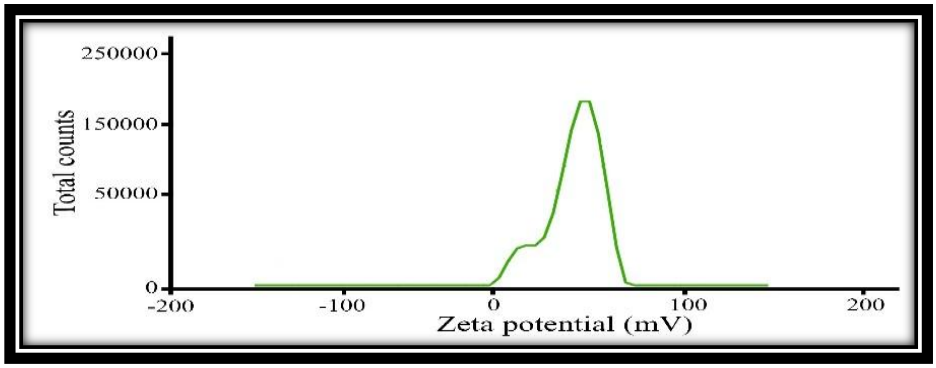


Fig. 3 (a): Chitosan zeta potential

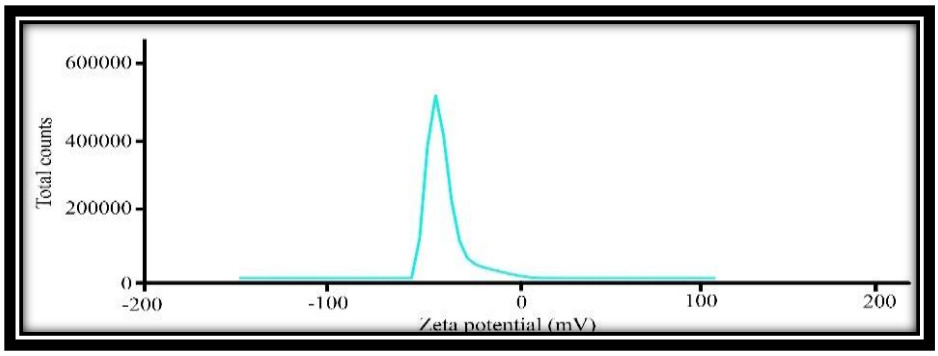


Fig. 3 (b): Carbon - Carbon Dot Zeta Potential

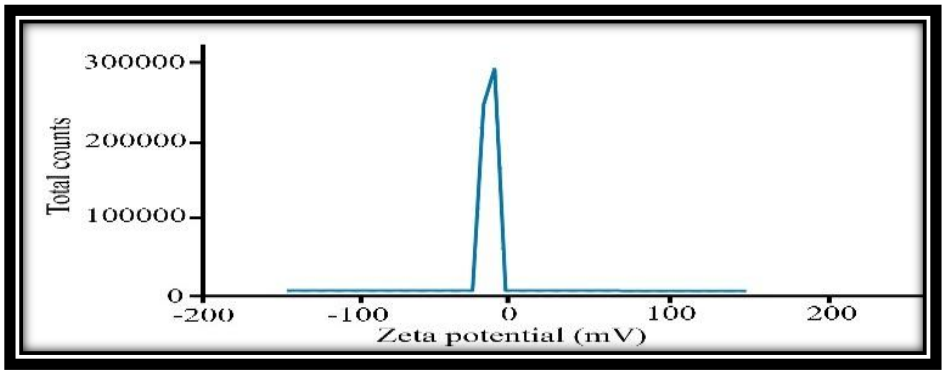
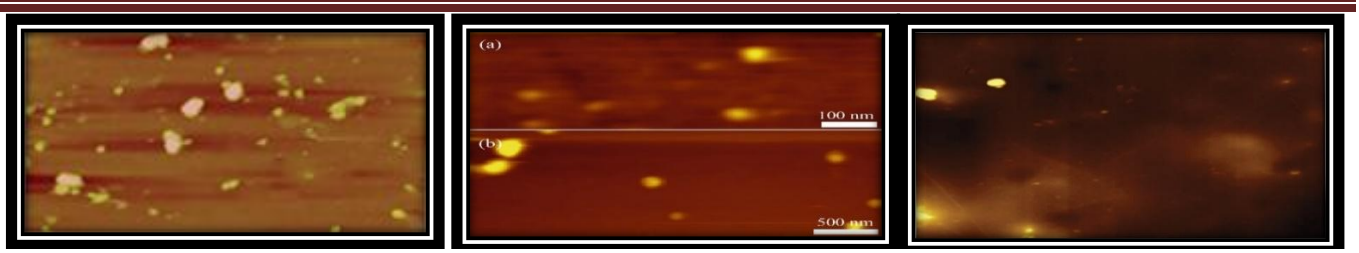


Fig. 3 (c): Nanogel Zeta Potential



A B C

Fig. 4: Atomic Force Microscopic images (A. Chitosan; B. Carbon quantom dot; C. Nanog

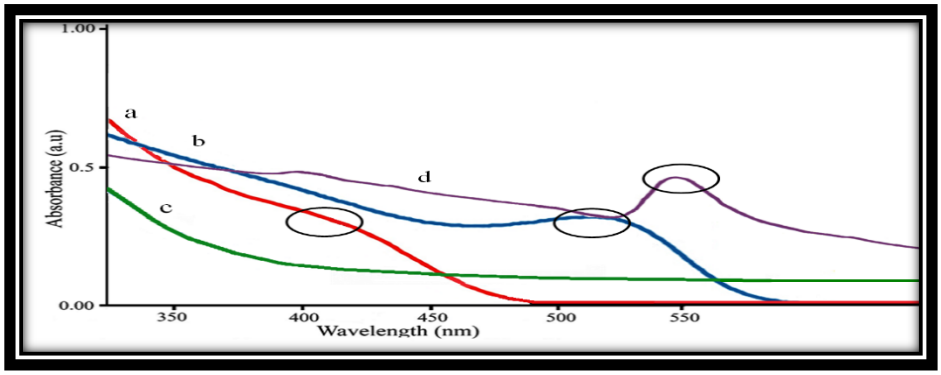


Fig. 5: The UV spectrum of the drug loaded on nanogel - a: Quantum dot + Chitosan, b: Quantum dot, c: Chitosan, d: Quantum dot + Chitosan + Magnevist

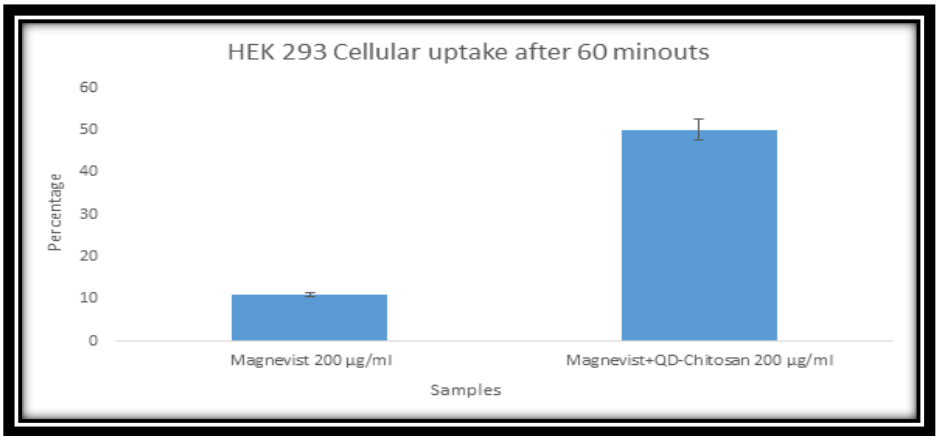


Fig. 6: In Vitro cellular upteke in HEK-293 cell line

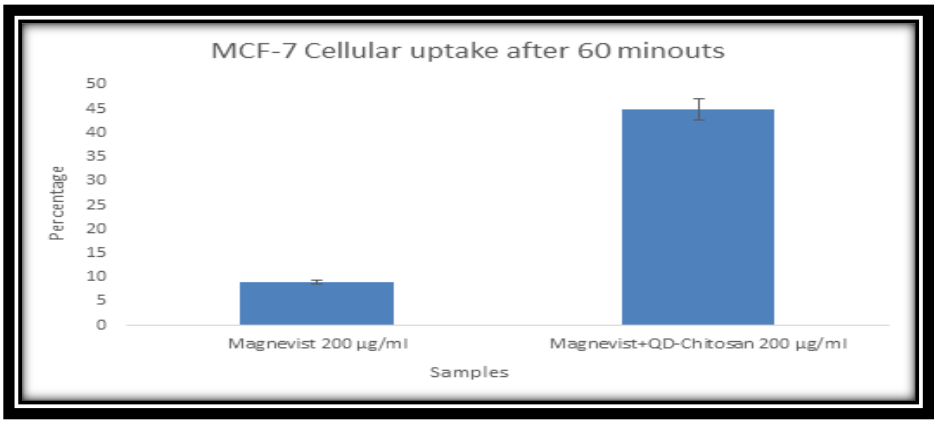


Fig. 7: In Vitro cellular upteke in MCF-7 cell line

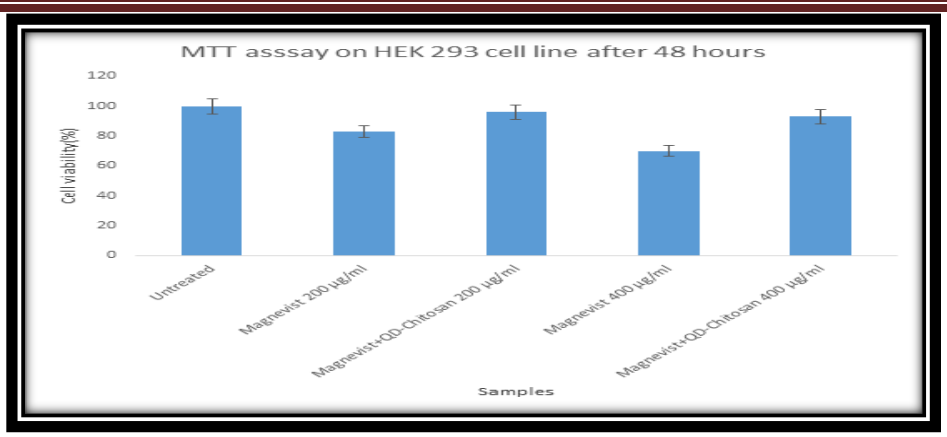


Fig. 8: MTT assay- HEK293 cells line were exposed to the samples for 48 hour

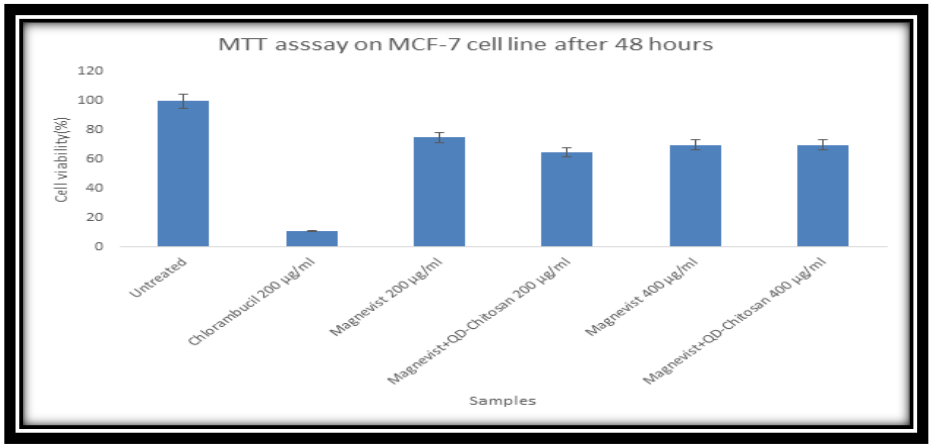


Fig. 9: MTT assay- MCF-7 cells line were exposed to the samples for 48 hour

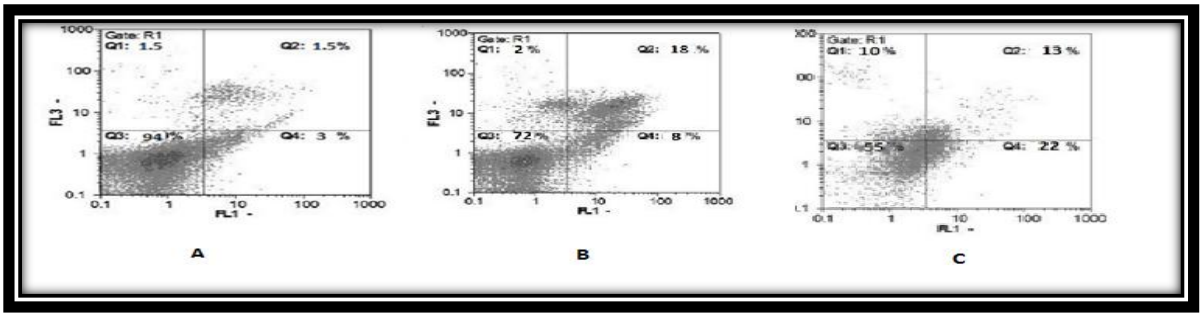


Fig. 10: Flowcytometry assay on MCF7 cell line incubated with A: Control B: Magnevist C: Nanogel-Magnevist

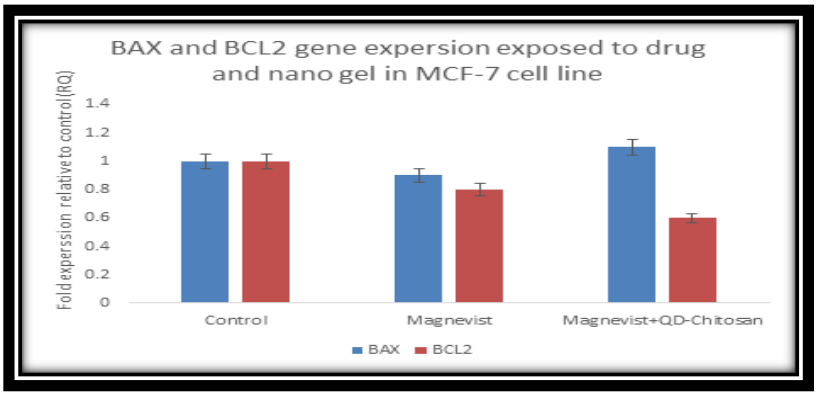


Fig. 11: The effect of samples on Bax and Bcl-2 gene expression

REFERENCE:

1. Thomson K, Varma D. Safe use of radiographic contrast media. Australian Prescriber, **2010**;33:19-22.
2. Irvine DJ. Drug delivery: One nanoparticle, one kill, Nature Materials **2011**;10(5):342-3.
3. Chen R et al. Parallel comparative studies on mouse toxicity of oxide nanoparticle- and gadolinium-based T1 MRI contrast agents. ACS Nano **2015**;9:12425–12435.
4. Stojanov DA, Aracki-Trenkic A, Vojinovic S, Benedeto-Stojanov D, Ljubisavljevic S. Increasing signal intensity within the dentate nucleus and globus pallidus on unenhanced T1 W magnetic resonance images in patients with relapsing-remitting multiple sclerosis: correlation with cumulative dose of a macrocyclic gadolinium-based contrast agent, gadobutrol. Eur Radiol **2016**; 26:807–815.
5. Wang YX et al .Total gadolinium tissue deposition and skin structural findings following the administration of structurally different gadolinium chelates in healthy and ovariectomized female rats. Quant Imaging Med Surg **2015**;5:534–545.
6. Laine M, Arjamaa O, Vuolteenaho O, Ruskoaho H, Weckstrom M .Block of stretch-activated atrial natriuretic peptide secretion by gadolinium in isolated rat atrium. J Physiol **1994**;480(Pt 3):553–561.
7. Chung TW. et al. Growth of human endothelial cells on different concentrations of Gly-Arg-Gly-Asp grafted chitosan surface. Artificial Organs **2003**;27(2):155-161.
8. Griffon DJ. et al. Chitosan scaffolds: Interconnective pore size and cartilage engineering. Acta Biomaterialia **2006**;2(3):313-320.
9. Lamarque G. et al. physicochemical behavior of homogeneous series of acetylated chitosans in aqueous solution: role of various structural parameters. Biomacromol **2005**;6(1):131-142.
10. V. Jarmila, E. Vavríková, Chitosan derivatives with antimicrobial, antitumour and antioxidant activities: a review. Cur Pharm Design **2011**;17:3596-3607.
11. C. Xue, G. Yu, T. Hirate, J. Terao, H. Lin. Antioxidative activities of several marine polysaccharides evaluated in a phospotidyl choline-liposomal suspension and organic solvents. Biosci Biotech and Biochem **1998**;62206-209.
12. ZS. Wen, LJ. Liu, YL. Qu, XK. Ou Yang, LY. Yang, ZR. Xu. Chitosan Nanoparticles Attenuate Hydrogen Peroxide-Induced Stress Injury in Mouse Macrophage RAW264.7 Cells, Marine Drugs. **2013**;11(10):3582-3600.
13. SC. Ray, A. Saha, NR. Jana, R. Sarkar. Fluorescent carbon nanoparticles: synthesis, characterization, and bioimaging application. J Phys Chem C **2009**;113:18546-18551.
14. HT. Li, XD. He, Y. Liu, H. Huang, SY. Lian, ST. Lee, ZH. Kang. One-step ultrasonic synthesis of water-soluble carbon nanoparticles with excellent photoluminescent properties. Carbon **2011**;49: 605-609.
15. SN. Baker, GA. Baker. Luminescent carbon nanodots: emergent nanolights, Angew. Chem., Int. Ed., **2010**;49:6726-6744.
16. CI. Weng, HT. Chang, CH. Lin, YW. Shen, B. Unnikrishnan, YJ. Li, CC. Huang. One-step synthesis of biofunctional carbon quantum dots for bacterial labeling. Biosens Bioelectron **2015**;68:1-6.

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