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Composite Nanoparticles and Coated With Hydrogel to Slow Drug Delivery of Cyclophosphamide Test it Against Breast Cancer Cell Lines (GJE, MCF7)

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Abstract

Introduction & Objective: Magnetic nanoparticles have found many applications because of their characteristics such as large surface area, small volume and simple separation with external magnetic field. These characteristics are very critical for drug delivery. In the current study, magnetic nanoparticles of iron oxide have been used as the central core of PAMAM dendrimer. The aim of current study was to evaluate the cytotoxic effects of this dandrimer on cancerous cell lines.

Materials and methods: magnetic nanoparticles were synthesized through the co-precipitation method and its size was measured by an electron microscope. following, NH2 @ methyl acrylate + ethylintiamine groups were added as branches and hydroxyl group with the negative charge was placed on the end terminus of the dendrimer branchs. The size of the synthesized dendrimer was measured by SEM. In order to investigate the accuracy of the steric bond reaction between the ethylene diamine and methyl acrylate functional groups, a FTIR infrared absorption spectrometer was used to investigate the absorption peak in a range of 1720-1720 nm. In order to release the drug continuously, after loading the methyl Prednisolone drug onto a synthesized nano dendrimer, the entire collection was coated with chitosan quaternary derivative. The absorption spectrum of Prednisolone was measured at 284 nm wavelength (which is the maximum absorption peak) by the UV-visible light absorption device, and the amount of drug release was detected within 24 hours. Finally, the fatal effect of this nano-particle complex on the cancerous types GEJ-MCF 7 was investigated by inverse electron microscopy and the amount of nano-particle toxicity was measured using lactate dehydrogenase Kit and ELISA.

Results: Based on the results of the electron microscope, the Fe3O4 size was in the range of 4.79 nm-6.37 nm range, which is smaller than nano-dendrimers with a range of 6.30 nm-43.67 nm. The FT-IR chart for the Methylacrylate @ Ethylendiamin esteric bond was obtained in the range of 1720 and 1730 cm -1. In this chart, the values of 600 cm-1 and 1000 cm-1 were related to Fe3O4 and Fe3O4 @ NH2, respectively. Triethyl Chitosan coated with Nanoparticle @ Drug coated with Nanoparticle @ Drug, along with trimethyl chitosan and glutaraldehyde creates a connection between TMC monomers. In this condition, glutaraldehyde helps to limit the delivery time of the drug. Also, cytotoxicity results indicated that the cell death rate in the incubated media with a complete nano-particle complex was dramatically greater than cell death rate with drug alone as well as nano-particle alone, which expresses the efficacy of the dendrimer-drug + chitosan drug complex.

Conclusion: The results indicated that the synthesized nanoparticle complex by the proposed method increased the death rates in the studied cells. In addition, a low dose application of a drug in any nanoparticle compound reduces the side effects of drug, and this method is safe for cancer treatment.

Introduction

The development of molecular nanoscale structures in particle size dimensions with proper shape is considered for biomedical applications such as the delivery of active pharmaceutical ingredients, imaging materials, or gene transfer. For example, the structures used as vector in the drug delivery should be in the range of nm and of a uniform size in order to increase their ability to cross the membrane and reduce the risk of their cleansing from the body through the liver and spleen (1). Dendrimer polymers or dendrimers are kind of the structures for carrying the drugs. The ability to adjust the properties of dendrimers for therapeutic needs has made them ideal vectors for small molecule drugs and biomolecules. Dendrimeric vectors can deliver drugs through the various types of links to the skin, eyes, mouth and lungs. In fact, dendrimers are nanostructures that are nowadays considered in many biomedical fields. Dendrimers are composed of a central core that branched out many branches, step by step like tree. Each group of these particles is very similar in terms of size, shape, branch length, particle density, and functional groups on the particle surface as well as the overall structure of the particle

(whether internal or external). These particles have the potential to accommodate different molecules within their branches and to enclose them, thus protecting them against external factors, or releasing them in controlled environments. They also increase the solubility of drugs by enclosing drugs.

Dendrimers can attach and transport a variety of molecules to their surface due to the presence of numerous functional groups at their surface, therefore they are used for active targeting for a specific tissue. With the advancement of nano-drug delivery, we observe the dendrimers applications as good vectors for drugs. The structure, characteristics and central core of the dendrimer and its synthesis methods guide the use of these compounds in targeted drugs to the body.

The aim of current study was to synthesis and investigate the effects of *polyamidoamine* (PAMAM) nano-dendrimer in in-vitro in Sanandaj Islamic Azad University (2). Nano- dendrimes have numerous biological properties such as antimicrobial and cytotoxic properties. In current study, the anti-tumor effects of synthesized nano-dendrimer compounds on breast cancer cell line compared with the drug alone were studied (46). In current study, a new method for making nano-dendrimers PAMAM was designed. Dendrimer is fixed on magnetic iron oxide nanoparticles with covalent bonds. acetic acid is used to load carboxyl group with a negative charge on the nano-composite end terminus. Nanocomposite properties are measured by SEM and FTIR. The PAMAM Poly amido amin dendrimer with a negative charge at its carboxyl end has the required conditions for better binding to chitosan with a positive cationic charge.

Materials and methods

Materials

Nanoparticle and quaternary chitosan synthesis material: FeCl3.6H2O (Sigma), FeCl2.4H2O (Sigma), ammonia solution (25%), methyl acrylate (Sigma), chitosan, methyl iodide, ethylenediamine, triethoxysiliylpropilamine, N-methyl pyrolidone, methyl prednisolone , sodium iodide , acetic acid, glutaraldehyde all were purchased from Sigma Aldrich.

Cell culture material: RPMI1640 (Gibco, USA), FBS (Gibco, USA), NEAA (Gibco, USA), L-Glutamine (Sigma, USA), Penicillin (Sigms, USA), Streptomycin (Sigma, USA), LDH Cytotoxicity kit (Roche, Germany), Hoechst33342 (Sigma, USA), Propodium Iodide (Sigma, USA).

Characterization: SEM images were achieved by MIRAS TESCAN – SEM HV: 30.0 KV –WD: 4/25 mm. FTIR analysis were obtained by utilization KBR discs in 400 - 4000 cm-1 area with ABB Boomed MB – 100 FT- IR spectrophotometer. Release of loaded drug was measured with UV spectrometer at 284 nm in 40 min interval times for 24 hours. MCF -7 and Jeg- 3 apoptosis and necrosis acquired by cell attribute an invert- florescence microscopic (Olympus IX-71, Japan). Cytotoxicity was dealt by LDH cytotoxicity kit. Eliza reader (EL800: USA) used to LDH activity colorimetric. all cell culture analysis was done via SPSS soft war.

Synthesis of magnetic NanoFe3O4

Magnetic nanoparticles were synthesized through the co-precipitation method. Briefly, 7.6 g FeCl3.6H2O, 3.1736 g FeCl2.4H2O were dissolved in 320 ml deionized water under N2 atmosphere at 80 oC using magnetic string for one hour. Then, pH of the reaction mixture was adjusted on 12 by addition 40 mL NH3. Dark black nanoparticles instantly formed after addition of ammonia. Stirring was continuing for another one hour and room temperature under nitrogen atmosphere. Finally, magnetic Fe3O4 nanoparticles were separated using neodymium magnet. The nanoparticles were washed two more times with deoxygenated distilled water, two time with ethanol and one time with diethyl ether. Finally, nanoparticles were separated by neodymium magnet and dried in under vacuum at room temperature.

Surface modification of Fe3O4 by the amine group (Fe3O4@NH2)

6 ml triethoxylsilyl propylamine was mixed with to 3 gFe3O4 in 40 ml toluene and stirred using magnetic stirrer for 3 days under N2 atmosphere at 60 oC. The produced Fe3O4@NH2 nanoparticles were separated by neodymium magnet and washed two times with ethanol and then dried in desiccator under vacuum.

Incorporation of PAMAM dendrimer on the surface of Fe3O4@NH2 nanoparticles

Sequential reaction of Fe3O4@NH2 with methyl acrylate (MA) (Michael reaction) and then with ethylene diamine (EA) (amidation) was performed to created PAMAM on the surface of magnetic nanoparticles. Briefly, 10 mL methyl acrylate was mixed with 3 g Fe3O4@NH2 in 20 mL of methanol and the mixture was stirred for 3 days at room temperature to complete the Michael reaction between primary amino groups of the Fe3O4@NH2 with methyl acrylate. The obtained Fe3O4@MA1 product was separated by neodymium magnet and washed two times with methanol and separated again with neodymium magnet. Afterward, Fe3O4@MA1 was mixed with 7.5 mL EA and 20 mL methanol and the mixture was stirred was stirred for 3 days at room temperature to complete the amidation reaction between ester groups of the Fe3O4@MA1 with EA and afford Fe3O4@EA1. The sequential reaction with MA and EA was repeated two more time to produce Fe3O4@EA3.

Incorporation of negative charges on the surface of Fe3O4@PAMAM nanoparticles

Fe3O4@PAMAM nanoparticles (2 g) were dispersed in 30 mL ethanol. Afterward, 12 g chloroacetic acid and 5 g sodium hydroxide were added to the mixture and the mixture was stirred for a week at room temperature. Finally, the product was separated using neodymium magnet and washed several times with ethanol. Finally, the Fe3O4@PAMAM@COONa product was separated and dried at room temperature under vacuum.

Synthesis of trimethyl chitosan (TMC)

2 chitosan and 4.8 g sodium iodide were mixed in 80 mL N-methyl pyrrolidone. Then, 11 mL of NaOH aqueous solution (0.15 M) and 11 mL methyl iodide were added to the mixture. The mixture was stirred at 50 oC for 24 h to obtain a brown solution. The solution was poured into ethanol to afford the quaternary ammonium salt of chitosan as a yellow precipitate. Product was separated and washed several time with ethanol and finally with diethyl ether and then dried at room temperature.

Loading complex of methylprednisolone (MP) in the magnetic nanoparticles and subsequent coating with TMC.4 g of Fe3O4@PAMAM@COONa nanoparticles were dispersed in 2 mL of distilled water, then, 0.3 g MP was added to the mixture. The mixture was stirred for 24 hours, then 0.4 g TMC was added to the mixture to coat the nanoparticles. After 2 h, 320 µL glutaraldehyde was added to the mixture to create some covalent crosslinking on the TMC structure to fix it as a shell on the surface of nanoparticles. Finally, Fe3O4@PAMAM@COONa@TMC nanoparticles loaded with MP were separated neodymium magnet and washed one time with distilled water and then dried at room temperature and used of further experiments.

Release of MP from magnetic nanoparticles

0.4 g of MP-loaded nanoparticles were dispersed in 10 mL bicarbonate buffer (pH ~ 7.3, C ~ 0.05 M) and in different interval times 500 µL of the clear solution was taken and replaced by 500 µL of fresh buffer to keep the total volume of the release medium constant. The UV adsorption of each taken sample was recorded via UV spectrometer at 284 nm.

Cell Culture

JEG-3 and MCF-7 cells were grown in RPMI1640 culture media (Gibco,USA), supplemented with 10% fetal bovine serum (FBS, Gibco,USA), 1% amino acid (NEAA, Sigma), 2mM L-glutamine (Sigma), 100IU/ml penicillin (Sigma), and 100µg/ml streptomycin (Sigma) in T-25 cm2 tissue culture flasks. The cultures were incubated in 5% CO2 at 37ºC.

Cell Treatment

Overnight after plating the cells, cells were washed with PBS (Phosphate-buffered saline), pH 7.4. There were three groups: Group I: incubated with drug, Group II: incubated with nano-particles, and Group III: incubated with nanopartical complex. There were eight treatments in each group including; control: 0.0µg, treatment 1: 2.5 µg, treatment 2: 5 µg, treatment 3: 10 µg, treatment 4: 20 µg, treatment 5: 40 µg, treatment 6: 80 µg, and treatment 7: 160 µg. Then, the cells were placed in the incubator with 5% CO2at 37◦C. The cells were cultured in RPMI1640 culture medium.

Cell viability measurement

Cell viability was quantified by measuring the release of lactate dehydrogenase (LDH) from damaged or destroyed cells in the medium. Cytotoxicity was measured with LDH cytoxicity detection Kit (Roche, Germany) which detected LDH release from dead cells. The increase of LDH activity in each treatment showed that the treatment solution had cytotoxicity effects and caused further dead cells. Cells were plated in 24 well culture plates with 104 cells/mL densities for overnight. Then, cells were cultured by the different treatment media for 24 h. The percentage of cytotoxicity was measured by the protocol from company (Roche, Germany); the colorimetric of LDH activity measured by the calculated absorbance of samples at 490 or 492nm using an ELISA Reader (EL800; USA). The reference wavelength should be more than 600nm. All experiments were replicated independently for at least 3 times. Within each experiment, we replicated each condition 4 times.

Quantification of cell death incidence

Hoechst/PI nuclear staining was carried out as previously described by others (Yuan, Guo et al. 2009). Briefly, cells were plated in 24 well culture plates with 104 cells/mL density for overnight. then, cell s were cultured in different treatment media for 24 hours. Then, cells were incubated for 15 min at 37°C with Hoechst 33342 dye (10 µg/ml in PBS), washed twice in PBS PI (50 µg/ml in PBS) before characterization of cells using an inverted-florescence microscope (Olympus IX-71, Japan). The apoptotic index was calculated by the ratio of apoptotic cells over the total cell count (at least 500 cells) in (). All experiments were independently replicated for at least 3 times. Within each experiment, we replicated each condition 4 times. Overall process for modification of Fe3O4 nanoparticles and subsequent loading with MP has depicted in Figure 1.

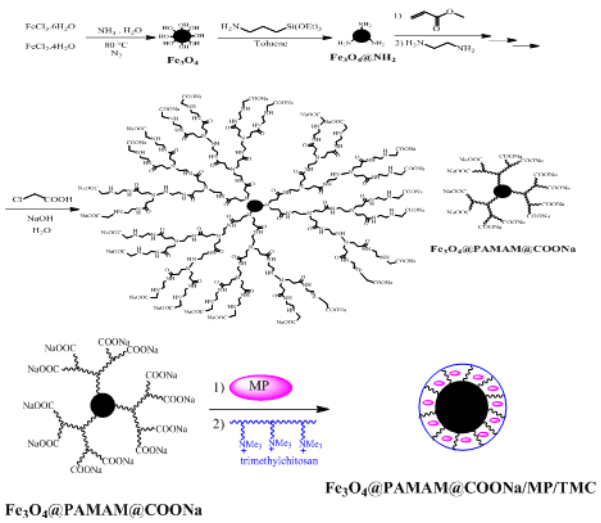

Figure 1: Schematic process for modification of Fe3O4 nanoparticles and its loading with MP.

Results

The results of SEM related to nanoparticles of iron oxide and nano-dendrimer using electron microscopy indicated the values in the range of 5.61-17.46 and 43.7-6.3 nm, respectively (Figs. 2 , 3).

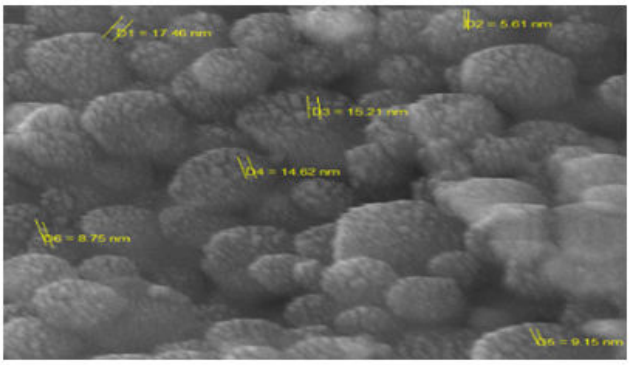

Figure 2: Electronic image of iron oxide size.

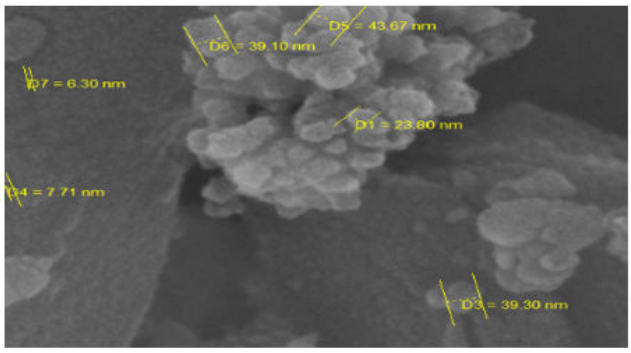

Figure 3: Electronic image of nano- dendrimer size.

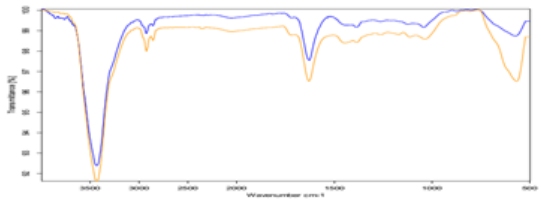

Chart1. Comparison of ethylenediamine III and II absorption spectrum

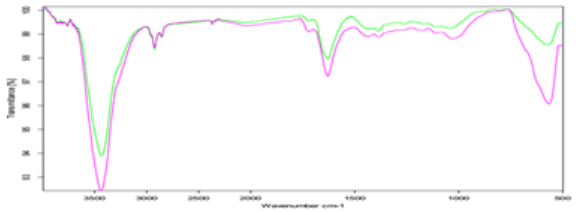

Chart 2. Comparison of Methyl acrylate III and II absorption spectrum

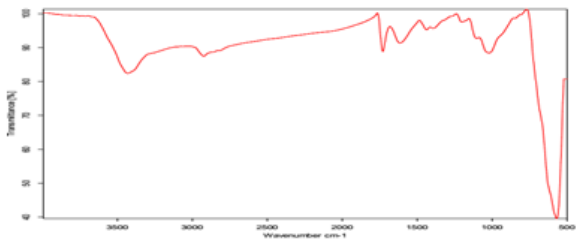

Chart 3. Methyl acrylate absorption spectrum at 1730 cm^{-1}

Results of drug release from the nano-particle complex

Drug release was measured by a UV-Visible spectrophotometer. Since methyl Prednisolone has the highest absorbance of visible light at 248 nm, ten samples were taken over a period of 24 hours.

Each sample was taken at 40 to 360 minutes apart from each other, and finally the last sample was taken after 24 hours. The volume of each sample was 500 λ and per 500 λ of the sample, the equal volume of the buffer was added to the reactor (table 1). Chart of release changes has an increasing trend (chart 4).

Table 1: Absorption spectrum of drug release Cell.

Uv absorbtion	Each 40 mint untell 6 hour last sample after 24 hour
4.370	1
4.552	2
4.424	3
4.462	4
4.396	5
.4.429	6
4.692	7
4.510	8
4.470	9
4.470	10

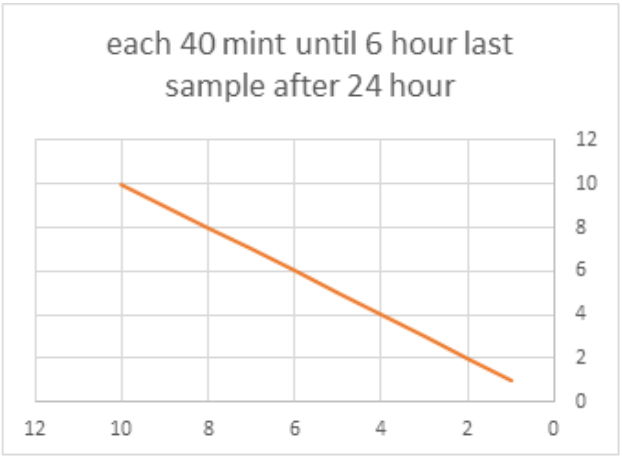

Chart 4: Increasing trend of drug release.

The lactate dehydrogenase assay is used in cell death estimation. In cell death, the amount of lactate dehydrogenase enzyme is released from the cell, and the results are as follows in the eight treated groups.

Group 1, which was incubated with drug, had a lower cell survival than the control group in the treatment group 7-4.

Group 2, which was incubated with drugs and was exposed in different concentrations of nanoparticles, had a lower cell survival in group 4-7 in comparison to the control group and cell survival percentage in treatment 7 decreased compared to treatment 1 and 4.

In group 3, the cell was exposed to various concentrations of the nanoparticle complex, which resulting in a decrease in the cell survival in the treatment group 7-2 compared to the control group. cell survival percentage in group 7 were less than group 6 and group 6 less than group 5, group 5 was less than group 4

and group 4 was less than group 3, group 3 was less than group 2 and group 2 was less than group 1 (chart 5)

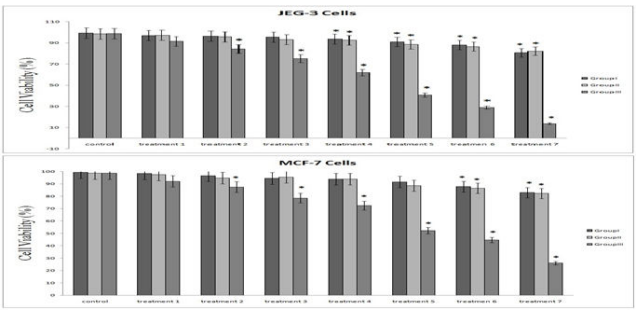

Chart 5: JEG-3 and MCF-7 cell survival index.

Results of cell death indices

In first group, the percentage of death in treatment of 7- 5 was increased in comparison with the control group. In the second group, the cells were exposed to various concentrations of PAMAM nanoparticles, which resulted in an increase in cell death in the treatment group 5-7 compared to the control group.

In the third group, the cells were exposed to different groups of nanoparticle complexes, which resulted in an increase in cell death in the treatment group 2-7 compared to the control group. According to the explanation given for different amounts of treatment and type of treatment in first, second and third groups in the case of cell death, it can be easily understood that the cell survival rate in the control group is higher, in which no inhibitor has been used.

In all groups, Cell survival rate was reduced from treatments one to seven, respectively. Also, the cell survival rate in third group in the third group was lower than the first and the second group was less than the first group in all cell lines, and the nanoparticle complex produced the highest cell death.

In the control groups, no cell inhibitor has been used. In Group 1 from treatment 1 to 7, only Methyl Prednisolone was used for treatment In The second group from treatment 1 to 7, only nanoparticle was used for treatment. In The third group from treatment 1 to 7, only nanoparticles complexes were used for treatment In all treatment groups, which have a nanoparticle complex, had the highest rate of cell death.

Discussion

In this study, a novel PAMAM nano-dendrimer synthesis method was designed. The method was fixed on the magnetic nanoparticles of dendrimer iron PAMAM with covalent bond. The applied method was to fix the PAMAM dendrimer on iron oxide magnetic nanoparticles with a covalent bond. Acetic acid was used to load carboxyl group with negative charge on the terminal end of the nano-composite. Nanocomposite properties are measured by SEM and FTIR.

The size of these particles, using a SEM electron microscope, was between 5.61 -17.46 nm (Fig. 1).Also, all of the bonds were covalent and the size of the nanoscale dendrimer using electron microscopes ranged from 6.3 to 43.7 (Fig. 2).

The PAMAM Poly amido amin dendrimer with the negative charge on its terminal carboxylic has the necessary conditions for better binding to chitosan with a positive cationic charge.

The rate of drug release from the complete nano-particle has been measured by UV absorption within 24 hours. The release rate in the absorption spectrum of 284 nm, which is the maximum absorption wavelength is measured (charts 1, 2, 3). The absorption rate of the visible light by the released drug over a period of 24 hours are plotted, during this time period, drug release has increased. (chart 4) (Table 1). In the following, the effects of apoptotic and necrotizing effects of nanocomposite prepared on breast cancer cell lines, JEG-3, which are belong to series of embryonic trophoblastic cells, were investigated on MCF-7 cancer cells.

Lactate dehydrogenase kit is used to estimate cell death. In group 2: The group exposed to various concentrations of nanoparticles which resulted in a decrease in the cell survival in the group 7-4 compared to the control group. cell survival percentage in treatment 7 decreased compared to treatment 1 and 4.

Group 3: Cells were exposed to different concentrations of nano-particle complexes, which resulted in decreased cell survival in the group 2-7 compared to the control group.

Cell survival percentage in group 7 were less than group 6 and group 6 less than group 5, group 5 was less than group 4 and group 4 less than group 3, group 3 was less than group 2 and group 2 was less than group 1 (chart 5) (Table 2).

Also, regarding the different levels of treatment and the type of treatment in groups 1, 2 and 3, about for cell death chart, it can be easily understood that the amount of cells survival rate in the control group, which did not use any inhibitory substances, had the highest amount in all groups.

The rate of cell survival in the treatment 1 to 7 is reduced. Also, cell survival in the third group, in all treatment levels, were less than the second group, and the second group was less than the first group and had the highest cell death in all treatment groups that had the nanoparticle complex.

Drug delivery through nano-vectors due to drug pharmacokinetic changes, prolonged drug availability in the bloodstream, reduced toxicity, increased drug half-life, decreased systemic drug distribution, decreased drug use and more precise targeting are considered as one of the most promising solutions for cure the cancer and **difficult to cure** illness.

These particles are superior to other nano-vectors because of properties related to their inherent magnetism. In general, the structure of these particles has facilitated the use of these particles in various sciences and techniques, especially in medicine, due to low toxicity of these particle in various branches, in addition to drug delivery, has been applied in the field of magnetic resonance imaging and diathermy.

The best advantage of these particles is the ability to control their movement through the application of an external magnetic field to the particles, which facilitated the goal of drug delivery,

that is, the targeted transfer of drug to the target tissue. However, application of nanoparticles and microparticles based on targeted transmission in animals and clinical trials has been relatively successful and more research is underway (62). Theses particle has been discovered since ancient Greece and so far has been used in the diagnosis and treatment of diseases such as cancer, heart disease and neurological diseases as well as targeted delivery in drug delivery. These particles have unique properties that the collection of these properties with superficial coatings gives the nanoscale magnetic particles a favorable biomedical property. According to this, the applications of magnetic nanoparticles in the medicine were the preferred priorities outlined in this paper. current research has focused on the various types of magnetic particles, the effects of the field on them, and their characteristics, benefits of using nanoparticles in medicine and so on (63). PAMAM nanoparticles is designed in many different drug delivery systems for various body components. However, such studies have been performed on mice or in culture media or nanoparticle systems have been used in a fibroblast culture medium that focuses on the size, surface, surface development, nano-particle compression ratio, percentage of cell absorption and the amount of cell harvesting, that is involved in estimating the amount of toxicity. The iron oxide magnetic core coated with polyethylene glycol indicated that in absorption and development of the surface of the nanoparticle, hydrophobic and hydrophilic protein absorption were involved in cell harvesting and intracellular effects as well as the result of these cellular interactions, such that less than 0.68 of the membrane anomalies are created by the uncoated magnetic core, which calls for the synthesis of a suitable coating on the nanoparticle. Toxicity in the range of 0-1000 µg / ml in 40-50 nm is very low. The toxicity is appeared in higher doses. Nanoparticles show a moderate ability to enter the cell even in the submicron scale, but they do not have this ability in micron scale (64, 65). In many cases absorption occurs through endocytosis. Endocytosis absorption in human cancer cells is occurs in different amounts. In various studies, the effects of concentration in endocytosis have been measured with Green-Fluorescent flow cytometry analysis. nanoparticles with a negative charge are released from the endosomes after six hours. While the nanoparticles with positive charge in the endosome are released after a longer time. With endothetic harvesting, depending on the surface activity of different nanoparticles, the binding power to the target cell which plays an important role in the release of intracellular drugs.

Nanoparticles are allowed to enter the sub-mocuse environment, while micro particles do not have permission to do so, and are concentrated on the epithelial layer. The cationic nanoparticles that are used for Plasmid transfer, DNA, are so called Nanoplex. The optimal size of these nanopolets varies between 10-10 nm and the zeta potential is 7 to 31 milv. The nano-particle with a size of 8 nm has been very successful in treating cancer by gene therapy (66, 67, 68).

In research that has been conducted on cancer, the most reliance has been on Target Therapy, therefor the new generation of research has advanced beyond nanoparticle synthesis to release its drug at the target, and the drug itself has been used as a dendrimer branches in nano-particle synthesis. The drug is then loaded on a nano-particle and is bonded with nano-particles through covalent bonds, non- covalent bonds or through physical interaction, and is released in the target tissue within a very long time. For example, by creating a dendrimer based on folic acid and loading the methotrexate, the highest therapeutic effect has been achieved, which has the lowest cytotoxic effect on healthy tissue compared with the use of medication alone. Studies which has been conducted on dendrimer solubility and activity indicated that dendrimers do not have any saturated critical concentration of or Critical mecilar concentrations, to unstable the dendrimer. This issue is a Milestone point in the stability of the dendrimer. Therefore, drug release occurs even at low concentrations. Even covalent binding of ligands to the nanoparticles for Target therapy as long as weeks or months will be beneficial (69).

By studying the size, which is the most important factor for nanoparticle to enter the cell, the size of 100 nm is two and a half times better than the 1µm and six times better than 10µm for entrance into the cell. The type of bond involved in dendrimer synthesis determines the time required for destruction and the stability of the dendrimer by the cell (70). Nanoparticles have the ability to cross the blood-brain barrier and enter the brain's cancerous tumors. Nanoparticles have been very effective in treating central nervous system diseases, Alzheimer and malignant tumors of the brain because of non-availability of these areas. The cerebro-vascular barrier allow the materials with molecular weight less than 400-600 Dalton to pass. Increasing the half-life of stability, solubility, low toxicity, and the power of passing through the blood-brain barrier has transformed this nano-particle characters into a good case for solving brain problems (71).

Endocytosis into the cytoplasm is dependent on clatterin, caveolin and receptor. Even endocytosis without clatterine and caveolin has also been observed. Nanoparticles are entered into the human cell and are concentrated in mitochondria and golgi apparatus. The surface scheme and nano-particle size is effective in its the cellular assimilation. Also, the cellular assimilation is saturable. Also, cell harvesting is saturable (72). The nano-particle covalent band with the ligand leads to unrelease the nano-particle or late release from the drug, which should be Synthesized according to the type of use in different parts of the body and the type of transplant. Even occasionally, due to the lack of drug release, it may not have a healing effect (73).

Different oral doses of nano-parethicle lead to various complications that are more common in colitis and also sometimes precipitated by the Helicobacter pylori bacteria in the stomach. The effects of a nano-particle drug depend on the strength of the nanoparticle bond, not on the amount of drug loaded on the nano-particle. Nanoparticles of nm 200 exhibit toxic effects on neuronal endothelial cells. This toxic effect depends on the charge of the nano-particle. Anionic nanoparticles at low concentrations have no toxic effects on the cell as well as on the vascular-blood-brain barrier. The cationic type is anti-coagulant, and its toxicity is much greater than its anionic type that leads to an increase in urine nitrogen levels and alanine transaminase enzyme in the kidney and liver

toxicity. Anionic use does not show any effect on the release of cytokines (74). Anionic type application does not show any effect on the release of cytokines (74).

The results of the present study are achieved good results according to the mentioned principles. The use of magnetic nanoparticles due to the controllability of size, surface, surface area, electrical charge, hydrophobicity, hydrophilicity, critical concentrations of stream, high absorption through the endocytosis, endocytosis with and without clathrin, biocompatibility and biodegradability, proper ability of drug release in desired target, determining how the ligand interacts with nanoparticles, magnetic conduction to the target, in some cases long drug release, low side effects and no toxic effects on healthy cells, ability to detect based on using green fluorescence, the ability to cross the blood-brain barrier and access to inaccessible parts of the body, are the best choice for advanced research in human society in future years.

Conclusion

The results indicated that the synthesized nanoparticle complex by the mentioned method increased the death rates in the studied cells. In addition, a low dose application of a drug in any nanoparticle compound reduces the side effects of drug, and this method is safe for cancer treatment.

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