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Study of the Effectiveness of Chitosan Encapsulated Iron Oxide Nanoparticles

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The magnetic iron oxide nanoparticles have been synthesized by using co-precipitation approach. Chitosan coated nanoparticles compare the effect of surface coating on the stability and toxicity of nanoparticles. Invitro cytotoxicity of the nanoparticles are evaluated by three-(four-5) dimethyl thiazole-2-yl and -two,5, diphenyl thiazoniam bromide (MTT assay) along with glide cytometry examine for cell viability. Does dependent reduction was observed in SKMEL cancer cells administered with different concentration of the sample. I_{50} value obtained for the Iron nanoparticle is 217.75µg/Ml. The fluorescence primarily based real time opposite transcription PCR(RT-PCR) is widely used for the quantification of constant state mRNA ranges and is a crucial device for primary research molecular medicine and biotechnology. FTIR amino companies were acted as capping sites for the iron nanoparticles stabilization. In antibacterial studies Gentamycin 80mcg showed the higher activity of Iron nanoparticles.

Keywords: Chitosan; MTT assay; mRNA; FTIR and antimicrobial activity.

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1. INTRODUCTION

Chitosan has been widely used for different biological and biomedical applications due to its unique properties. For instance, it can be used in water treatment [1], wound healing materials [2] pharmaceutical excipient or drug carrier [3] obesity treatment [4] and as a scaffold for tissue engineering [5]. It is commonly used as an excipient in tablet formulation for oral medication. High molecular weight chitosan is more viscous and delays the release of active ingredient prolongs the duration of drug activity improves therapeutic efficiency as well as reducing the side effects of oral tablets [6]. Gene therapy has drawn vast interest over the past many years for the treatment of inherited and acquired diseases. Major studies efforts have focussed on designing suitable provider vectors that compact and defend oligonucleotides for gene remedy. DNA or RNA is large, hydrophilic macromolecule with negative charge very unstable in physiological environment and do not cross biological membranes effectively since both the gens and cell surfaces are negatively charged spontaneously entry of unprotected genes inside cells is difficult [7]. The MTT assay is the most widely used cell viability assay and several modifications of the original method have been described [8]. In the present study, an attempt has been made to prepare the Iron oxide nanoparticles through co-precipitation method that has been explain the gene expression study, effective antimicrobial activity and MTT assay.

2. MATERIALS AND METHODS

2.1 Preparation of Chitosan

Crab shells had been accumulated from Rameswaram seashore. The shells were washed with sea water and again with sparkling water and dried. The nicely dried crab shell wastes are powdered by crushing it in a pestle and mortar and sieved. Then subjected to demineralisation, deproteinization deacetylation and eventually the chitosan powder become obtained.

2.2 Preparation of Iron Oxide Nanoparticles

0.1g of Iron (III) chloride hexahydrate $Fecl_3.6H_2o$ and 0.50g of Iron (II) sulphate hexahydrate $Feso_4.7H_2o$ have been taken in a 250ml beaker. To this delivered 20ml of Millipore water (double distilled). The mixture changed into stirred in a magnetic stirrer for 10 minutes. To this 7% freshly prepared ammonia solution was added to make the mixture more basic. The addition is continued till the PH of reaction mixture reached 11. This mixture was kept for drying in oven at 110^0.Finally black precipitate of iron oxide nanoparticles was obtained. It became collected and washed numerous times with water.

2.3 Synthesis of Chitosan Coated Iron Oxide Nanoparticles

The iron oxide nanoparticles were dried and made proper right into a fine powder. 200mg of chitosan changed into made proper into a gel the use of 2-5 ml of formaldehyde beneath magnetic stirrer for one hour. Finally, a homogenous combination of chitosan coated iron oxide particles received which have been filtered, dried and saved for addition assessment.

3. RESULTS AND DISCUSSION

3.1 FTIR Spectra Analysis

FTIR study was finished to verify the functional group of materials. The spectra of natural Fe nanoparticles indicated that the manifested absorption peaks at approximately 3419, 3049.25, 2360.71, 1592.13, 873 and 553.53cm-1 (Fig. 1). The surprisingly severe height at 3419 cm-1 corresponded to the stretching vibrations of free OH and CH groups [9]. O H vibrations obtained for the peak of 3049 cm-1. The peak at 2360.71 cm-1 corresponded to N-H bending vibrations. The peak at 873cm-1 belonged to the C-BR stretch of alkyl halides given in Table 1. The end result sincerely indicated the involvement of primary amino companies inside the interaction with metal surface and the amino groups have been acted as capping web sites for the Iron nanoparticles stabilization.

3.2 Antibacterial Activity

The chitosan nanoparticles pretence to have properly bactericidal activity. The synthesized chitosan with nanoparticles has been tested in opposition to two extraordinary pathogenic micro-organisms which include Escherichia coli, *Staphylococcus aureus*. From this examine, the chitosan act as an antibacterial agent against *Escherichia coli* and *Staphylococcus aureus*. When the concentration of widespread Gentamycin at 80mcg zone of inhibition for each S.aureus and E.coli were 25mm. In addition

Gentamycin at 400mcg zone of inhibition for *S. aureus* and *E. coli* were identical of 12mm while when gentamycin at 800 mcg zone of inhibition for *S. aureus* is 14 mm and for *E. coli* thirteen (Fig. 2). Comparing this region of inhibition for standard Gentamycin 80 mcg showed the higher activity than the others.

3.3 MTT Assay

MTT Assay used to measure cell metabolic activity as an indicator of cell viability proliferation and cytotoxicity. This colorimetric assay is primarily based the reduction of a yello tetrazonium salt (three-(four, five-dimethylthiazol-2-yl)-2, five-diphenyl tetrazolium bromide or MTT) to pink formazan crystals by using metabolically active cells. The cytotoxic effect of the chitosan, mobile viability become observed in SKMEL most cancer cells at a wavelength of 570nm administrated with distinctive concentration of 6.25, 12.5, 25, 50, and 100µg/ml. Percentage of viability obtain for chitosan coated iron nanoparticle is 99.39, 96.66, 94.01, 90.02 and 76.81%. The cell viability decreases as per increase in the concentration of chitosan. This showed that the percentage of cancer cells decreased by increases the volume of test solution [10]. This experiment was carried out in triplicates and the mean values are depicted in Table 1. The IC_{50} value was obtained for chitosan coated iron nanoparticle 217.75 µg/ml.

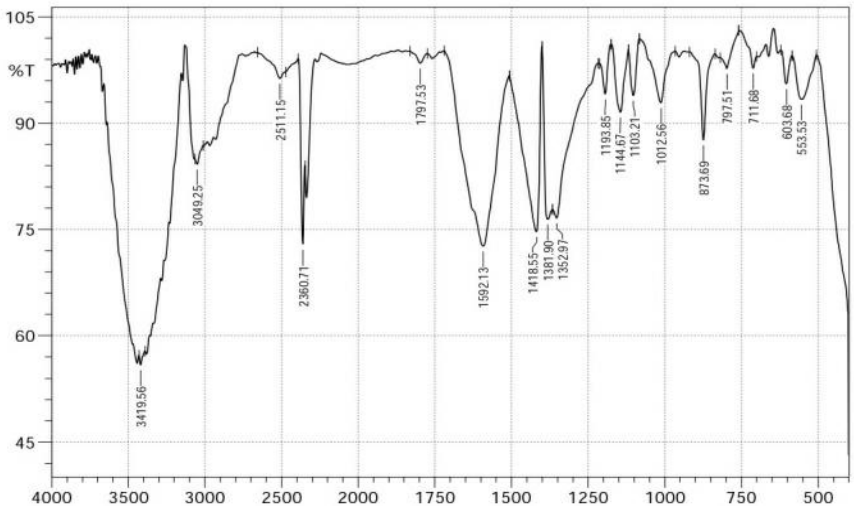

Fig. 1. FTIR spectra of Chitosan encapsulated Iron nanoparticles

Fig. 2. Antimicrobial activity of chitosan coated Iron oxide nanoparticles

Table 1. Viability of chitosan coated Iron nanoparticle

Concentration(µg/mL)	6.25	12.5	25	50	100
Percentage of viability	99.39	96.67	94.01	90.02	76.81
IC50	217.75 µg/mL				

The IC50 values were calculated using the equation for slope (y=mx+ C) obtained by plotting the absorbance of the different concentrations of the sample (6.25 µg/mL) (Fig. 3).

3.4 mRNA-agarose Electrophoresis

It is mainly used for the purification of DNA fragments. The RNA quality was checked by 1% agarose gel electrophoresis by loading 5µl of the four differ samples. They are HER 1 109, HER2 192, HER1 218 and HER2 96.RNA was obtained was quantitated at 260nm and purity was judged with 260/280 nm. The image of the picture was taken using Medicare, Gelstan geldoc system and is represented in Fig. 4.

Result of this component is RNA sample received as a good integrity and purity. So, before cDNA synthesis attempt to quantify the samples in nanodrop and test the integrity via electrophoresis on a denaturing gel. A right RNA sample will have a 260/230 ratio ranging 2. Zero - 2.2 and 260/280 ratio ranging 1.8 - 2. Zero. All the four samples mRNA purified exact great. They are non-poisonous, in the main used for DNA and RNA separation.

3.5 Real Time Analysis

Analysis of expression levels of BCL2 and beta-actin in the treated samples with respect to control. The primers used for the real time PCR wereBCl2 F-GAACTGGGGGAGGATTGTGG and BCl2 R-ACTTCACTTGTGGCCAGAT. β-Actin selected as a control gene which was detected as the reference gene using the senser primer. They are Beta actin F-ACTATTGGCAACGAGCGGTT and Beta actin R- AGGGTAAAACGCAGCTCA. All gene will amplify around 300 bp.

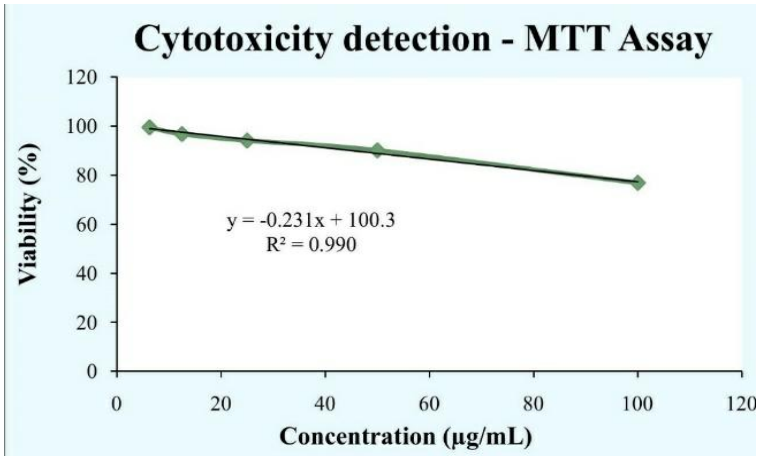

Fig. 3. Cytotoxicity detection-MTT assay

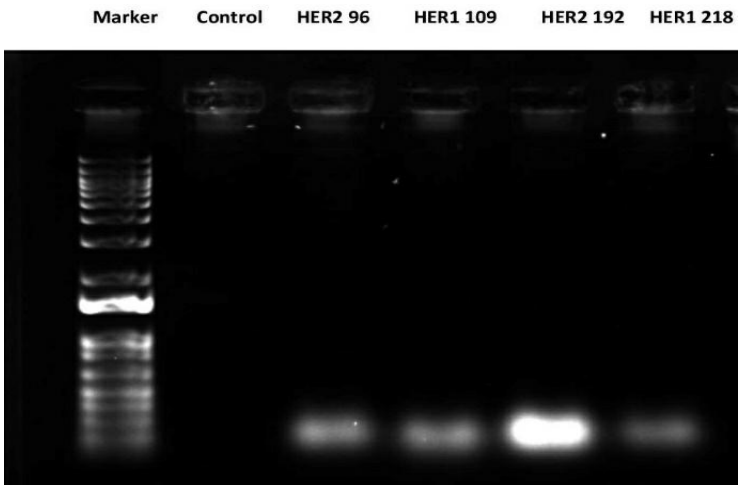

Fig. 4. Lane 1: DNA Ladder, lane 2: lane 3: HER2 96, Lane 4: HER1 109, Lane 5: HER2 192, Lane 6: HER1 218

Table 2. mRNA quantification

Samples	OD at 260	OD at 280	260/ 280
Control	0.2659	0.1466	1.81
HER296	3.113	1.1935	1.60
HER1 109	2.815	1.52	1.85
HER2 192	2.908	2.525	1.15
HER1 218	3.089	1.625	1.90

3.6 Average CT Values

Quantitative analysis was performed by the measurement of threshold cycle CT value during the exponential phase of amplification. In these studies, HER2 96, HER1 109, HER2 192, and HER1 218 are used as a control. Average CT values for BCl2 are 36.64, 37.61, 38.02, and 35.09. When the CT value is lowering higher in viral loaded. Lowest CT vales are 35.09 and highest value is 38.02. The obtained CT values for β-actin are 28.16, 32.08, 23.72, and 31.13. Lower the CT value is 23.72 which are lightly affected by viral gene. Higher the CT value is 32.08 which is moderately affected by viral gene. These are given in Table 3. Comparing this Bcl2 showed the good positive results.

Table 3. Relative expression of BCl2 with respect to Actin

Samples	BCl2	Actin
CNTL	38.89	30.19
96	36.64	28.16
109	37.61	32.08
192	38.2	23.72
218	35.09	31.13

4. CONCLUSION

This study shows that chitosan is a promising polymer for biomacromolecules delivery especially for gene therapy purposes. Invitro cytotoxicity assays, MTT assay used to measure cell viability. The cell viability decreases as per increase in the chitosan coated iron nanoparticles. A precise RNA pattern will have a 260/230 ratio ranging 2.0 - 2.2 and 260/280 ratio ranging 1. Eight - 2.0. All the four samples mRNA properly first-rate. They are non-toxic, mostly used for DNA and RNA separation. When the CT value is lowering higher in viral loaded. Average CT values for BCl2 are 36.64, 37.61, 38.02, and 35.09. These are all higher the value so it is good quality.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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