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Anti-inflammatory activity of chitosan coated iron nanoparticles

Hebzi Emalda Rani M
Reg: 19113012032008, Research scholar, Department of Chemistry, Annai Velankanni College, Tholayavattam, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli)
Email: hebsiemalda@gmail.com

Dr. S. Mary Helen
Associate professor and Head, Department of Chemistry, Annai Velankanni College, Tholayavattam
Email: chemhelen1976@gmail.com

***Abstract*---**Chitosan coated iron nanoparticles were synthesized by co-precipitation method and also Chitosan coated Nanoparticles evaluate the effect of surface coating on the stability and toxicity of nanoparticles. FTIR amino groups were acted as a capping site for the Iron nanoparticles stabilization. Invitro cytotoxicity of the nanoparticles is dependent dose-based method and their reduction was observed in SKMEL cancer cells administered with different concentration of the samples. I_{50} value obtained for the Iron nanoparticles is 217.75µg/Ml. Cyclooxygenase and Lipoxygenase which is nonsteroidal anti-inflammatory agent.

***Keywords*---**Chitosan, MTT assay, FTIR, Cyclooxygenase and Lipoxygenase.

1. Introduction

Chitosan is a characteristic decidedly charged biopolymer got from the hydrolysis of the polysaccharide's chitin. Chitin is an amino polysaccharide bounteously accessible regular biopolymer found in the exoskeletons of scavengers like shrimp, crab, lobster and other shellfish. Chitosan is a biopolymer with a wide scope of biomedical applications, which is including of wound recuperating tissue fix, and neighborhood conveyance of cells, drugs, proteins, qualities and different therapeutics. Tetrazolium salts have widely been used to study the mitochondrial respiratory chain since the 1960s [Mosman et al., 1983: Atterwill C. K et al., 1993]. COX-2 is an inducible enzyme that presents in low amounts in the human kidney, brain, ovaries (Pairet et al., 2010). 5-LOX pathways end product is

leukotriene B4, a mediator of several inflammatory and allergic diseases, including atherosclerosis, cancer, and cardiovascular diseases (Kaur et al., 2017; Dwyer et al., 2017; Hyde et al., 2009; Koukoulitsa et al., 2007). Several studies have recently shown that reduction of tetrazolium salts is related not only with mitochondria reductase, but also with many intracellular reductases (Berridge et al., 2005: Cookson et al., 1995). The main concern in this study how can benefit more from these natural resources to get rid of a sickness which is life killing.

2. Materials and Methods

2.1 Preparation of Chitosan

Crab shells were collected from the seashore of Rameswaram. The shells were first washed in the sea water and again washed with fresh water and dried. The well dried crab shell wastes are made powdered by crushing it in a pestle and mortar and sieved. Then subjected into demineralisation, deproteinization deacetylation and finally the chitosan powder was obtained.

2.2 Preparation of Iron Oxide Nanoparticles

0.1g of Iron(lll)Chloride Hexahydrate $FeCl_3.6H_2o$ and 0.50g of Iron(ll)Sulfate Hexahydrate $FeSo_4.7H_2o$ were taken in a 250ml beaker. To this added 20ml of Millipore double distilled water. The mixture was stirred in a magnetic stirrer for 10 minutes. Then 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 precipitates of iron oxide nanoparticles were obtained. It was collected and washed several times with water.

2.3 Synthesis of Chitosan Coated Iron Oxide Nanoparticles

The iron oxide nanoparticles were dried and made into a fine powder. 200mg of chitosan was made into a gel using 2-5ml of formaldehyde under magnetic stirrer for one hour. Finally, a homogenous mixture of chitosan coated iron oxide particles obtained which were filtered, dried and stored for further analysis.

3. Result and Discussion

Ftir Spectra Analysis

FTIR was performed to confirm the functional groups on the surface of the synthetic materials. Spectra had been recorded in the range of 500-4000cm-1

FTIR Spectral Studies of Chitosan Coated Iron Nanoparticles

The spectra of chitosan coated with Iron nanoparticles indicated that the manifested absorption peaks at about 3419.56cm-1, 3049.25cm-1, 2360.71cm-1, 1592.13cm-1,1418.55cm-1,1381cm-1,1352.97cm-1, 1103.21cm-1, 1012.56cm-1 and 873.69cm-1. The peaks near 3419.56cm-1 correspond to free OH, NH intermolecular conversion. The band at 3049 cm-1 corresponded to OH vibrations. The peak at 2360.71cm-1 corresponded to N-H bending vibrations. The peak at 873cm-1 belonged to the C-BR stretch of alkyl halides. The peak at 603.68cm-1 corresponded to C-Fe bond formation of iron nanoparticles (Xuan Nui Pham et al., 2016). Primary amino groups in the interaction with the metal

surface brings the result of the amino groups as acted as a capping site for the Fe NPs (Fig.1)

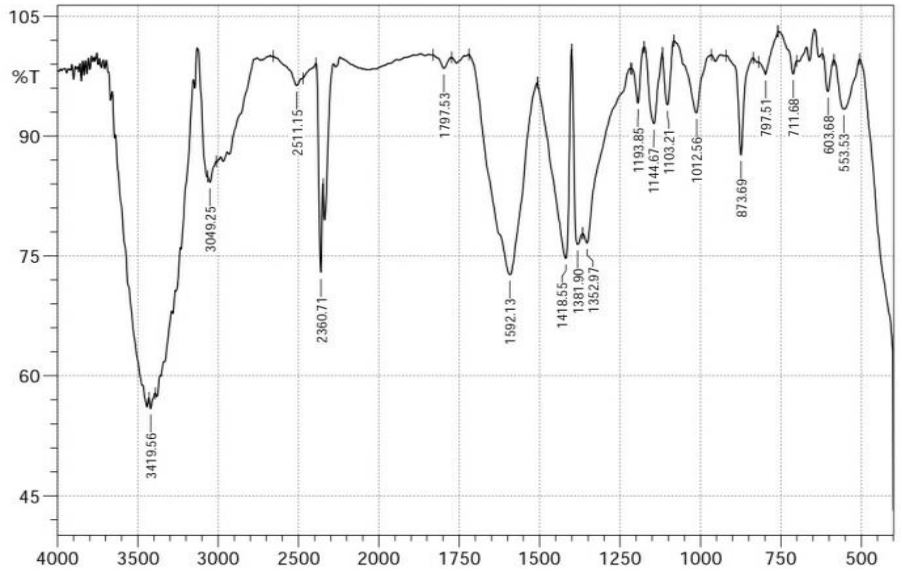

Figure 1: FTIR spectra of Chitosan using Iron nanoparticles

MTT Assay

MTT Assay used to measure cellular metabolic activity as an indicator of cell viability proliferation and cytotoxicity. This colorimetric assay is primarily based on the reduction of a yellow tetrazolium salt (three-(four, five-dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide or MTT) to red formazan crystals by using metabolically actives of cells. The viable cells comprise NAD(P)H dependent oxido reductase enzymes which reduce the MTT to formazan (Mosmann et al.1983). Below Table 1 shows that chitosan coated iron nanoparticles solution are in different concentration of 6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml, and 100µg/ml which gives the percentage viability reports are99.39%,96.66%, 94.01%, 90.02% and 76.81 %.

Table 1: MTT assay-Viability of Chitosan coated Iron nanoparticles

S. No	Concentration (µg/ml)	Percentage of viability
1.	6.25	99.39
2.	`12.5	96.66
3.	25	94.01
4.	50	90.02
5.	100	76.81
IC_{50}	217.75µg/ml	

For 6.25µg/ml of the chitosan coated Iron nanoparticles showed the 99.39% of cell viability similarly 100(µg/ml) of the chitosan solution has given the

percentage of viability is 76.81%. It was concluded that cell viability decreases as per increase in the concentration of chitosan.

This experiment was carried out in triplicates and the mean values. IC_{50} value calculated using the equation for slope (t = mx + c) obtained by plotting the average absorbance of the different concentrations of the test sample (6.25-100 µg/ml). This toxicity of nanoparticles becomes determined to be dose based. The results truly suggested that the cellular viability decreased with increased of dose. Dose dependent reduction in cells viability was observed in SKMEL cancer cell administered with different concentrations of the chitosan coated Iron nanoparticles. The IC_{50} value obtained for a chitosan coated iron nanoparticles is 217.75µg/ml.

Cyclooxygenase (COX) and Lipoxygenase (LOX) Assay

Inflammation is a protective response of the body by itself. COX and LOX enzymes have significant roles to play in the regulation of inflammatory responses (M. Leach et al., 1998). Anti-inflammatory drugs and agents decrease these responses by suppressing the production pathway of the inflammatory mediators, which in turn block the initiation and progression of inflammation-associated diseases (Rådmark & Samuelsson 2007). Dual inhibitors are tablets able to block both the COX and the 5-LOX metabolic pathways.2-COX and 5-LOX inhibitors are used in this method. This analysis was done using chitosan coated Iron nanoparticles.

COX and LOX Enzyme Activity of Chitosan Coated Iron Nanoparticles

When the percentage of inhibition COX enzyme are at various concentration of standard Diclofenac sodium of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml were the outcome for chitosan coated iron nanoparticles as follows 6.27%, 13.38%, 28.43%, 53.42% and 63.04% (Melita Loncaric et al., 2020). In LOX inhibition assay the percentage inhibition was found to be 2.62%, 6.37%, 16.71%, 26.67% and 32.77% (Fig.2) for the concentration of chitosan coated iron nanoparticles 6.25 µg/ml, 12.5µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml respectively. It revealed that when 100µl/ml bacterial melanin exhibited is used the outcome is also found to be 32.77 radical which show that increase of the concentration also increases the percentage of inhibition. The low concentration of 6.25µg/ml is used; the outcome has come 2.62% of inhibition. This shows that decrease of the concentration of the solution decreases the percentage of inhibition

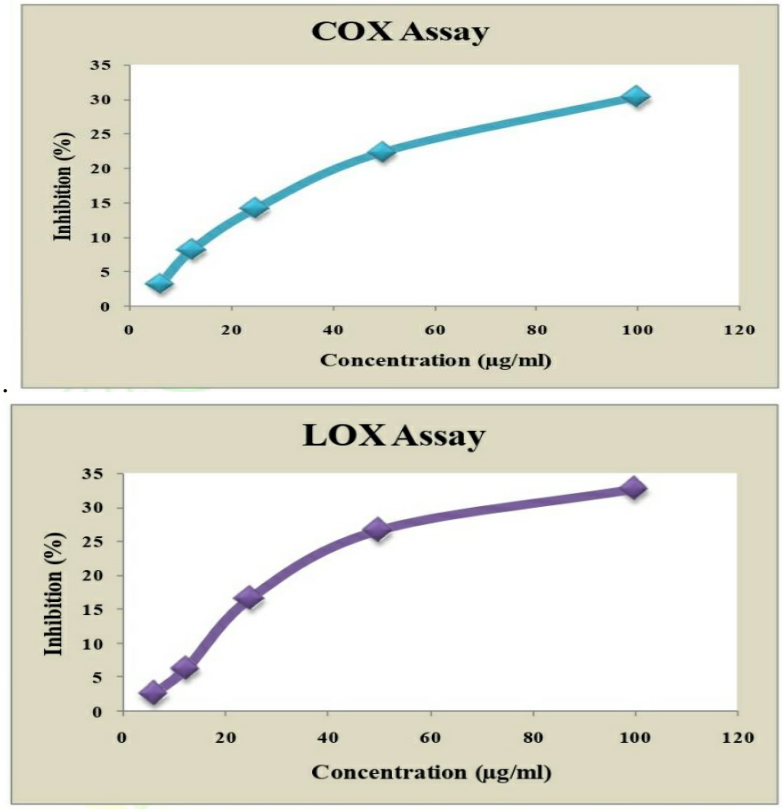

Fig 2: The percentage inhibition of COX and LOX enzyme activity by chitosan coated with iron nanoparticles

4. Conclusion

Chitosan is a biodegradable and inexpensive and affordable polymer. They have in numerable applications in biomedical as well as pharmaceutical diligence. Sundry research work has been done on chitosan and its derivations for the purpose of medicine delivery, water treatment, cancer treatment, crack mending, antitumor and antimicrobial activity. Chitosan and its origin have been extensively studied for couched towel engineering biomaterials as they will be besmirched at reasonable rate without causing any seditious or generate any ill-intentioned products when the new tissues are formed. Iron nanoparticles explore through co-precipitation method. FTIR was performed to confirm the functional groups on the surface of the synthetic material. MTT Assay used to measure cellular metabolic activity as a gauge of cell viability proliferation and cytotoxicity. The I_{50} value obtained for Iron nanoparticles is 217.75µg/ml. COX and LOX enzymes have momentous roles to play in the regulation of inflammatory responses. Chitosan coated iron oxide samples, exhibited cyclooxygenase (COX) inhibitory and Lipoxygenase activity. It publicized that when 100µl/ml bacterial melanin is used the outcome is also found to be 32.77 radical which shows that increase of the concentration also increases the percentage of inhibition. They have anti-inflammatory properties and also the potential to be developed as non-steroidal anti-inflammatory drugs (NSAIDs).

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