

Anti-inflammatory and antioxidant potential of hyaluronic acid mediated zinc nanoparticles

ABSTRACT

Introduction: Hyaluronic acid has a role as 'physiological selector' for spermatozoa prior to intracytoplasmic sperm injection (icisi). They are mostly seen in the synovial joints and act as a shock absorber. The objective of this study is to analyse the results achievable by the introduction of a routine HA. Hyaluronic acid is an immune neutral polysaccharide that is ubiquitous in the human body, and it is crucial for many cellular and tissue functions. Nanoparticles are the compounds which respond to the applied magnetic fields, magnetic separation labelled cells, therapeutic drug, gene and radionuclide delivery and other biological entities.

Materials and methods: Hyaluronic acid mediated with zinc nanoparticles were evaluated for its anti-inflammatory activity and antioxidant potential using photometry analysis.

Results and Discussion: Zinc nanoparticles show a higher efficiency of antibacterial activity, but sublethal concentration causes adverse effects and results in increased biofilm formation of *V.cholerae*. Hyaluronic acid mediated zinc oxide nanoparticles show good results in anti-inflammatory activity as well as in antioxidant activity. The biosynthesis of the nanoparticle was done by addition of hyaluronic acid with zinc nanoparticles in a scarce amount. This was kept in the orbital shaker, hence they take a long time for the synthesis. The colour change was observed, hence the synthesized nanoparticles proceeded with the anti-inflammatory and antioxidant tests.

Aim: The aim of the study is to interpret the hyaluronic acid mediated with zinc oxide nanoparticle and its anti-inflammatory and antioxidant activity.

Key Words: anti-inflammatory, hyaluronic acid, Anti-oxidant, Anti-microbial.

1.Introduction

Nanoparticles are the compounds which respond to the applied magnetic fields. They are used in magnetic separation labelled cells and other biological entities. They are used in therapeutic drugs, gene and radionuclide delivery¹. Zinc nanoparticles show a higher efficiency of antibacterial activity, but sublethal concentration causes adverse effects and results in increased biofilm formation of *V.cholerae*². Zinc oxide nanoparticles demonstrated moderate antioxidant activity by scavenging 45.47% DDPH at 1mg/mL and revealed excellent anti-inflammatory activity by dose dependent suppressing both mRNA and protein

expressions of INOS, COX-2, IL-1Beta, IL-6 and TNF-alpha³. A significant enhancement in DNA was observed starting from Zinc oxide nanoparticles concentrations of 10µg/mL⁴. Hyaluronic acid is a naturally occurring polysaccharide with distinct physicochemical properties which underlie its application as a viscoelastic tool in ophthalmological surgery. They have a major role in cataract surgery⁵. Hyaluronic acid is an immune neutral polysaccharide that is ubiquitous in the human body, and it is crucial for many cellular and tissue functions. They act as therapeutic agents for tissue repair and regenerations⁶. Hyaluronic acids are present in the synovial joint. Degradation of synovial fluid will cause osteoarthritis. They are artificially injected by an intra-articular injection for the relief of moderate to severe pain in people with OA⁷. Hyaluronic acid has a role as 'physiological selector' for spermatozoa prior to intracytoplasmic sperm injection (icsi)⁸. Hyaluronic acid is used to cleave the gel around the ovum, this is further useful in the fusion of gametes⁹. The objective of this study is to analyse the results achievable by the introduction of a routine HA. ICSI programme¹⁰. Immaturity based on hyaluronic acid binding and nuclear decondensation have exactly the same features¹¹. They can be used by vitamins of group B and zinc. High molecular weight hyaluronic acid displays anti-inflammatory and immunosuppressive properties. Whereas low molecular weight hyaluronic acid is a potent proinflammatory molecule¹². Low molecular weight hyaluronic acid was able to overcome CY-induced immune suppression and significantly raised the activity of superoxide mutase. Hence the results showed that the LMWHA, possessing pronounced free radical scavenging and antioxidant activity¹³. The encapsulated zinc nanoparticles cross linked by methacrylated hyaluronic acid provides active tumor accumulation ability by binding its order expressed receptors on the surface of cancer cells¹⁴.

2. Materials and Methods:

2.1 Biosynthesis of hyaluronic acid mediated zinc nanoparticles:

100mg of hyaluronic acid is mixed and dissolved with 10mL between 20mL, further 3mM of Zinc oxide was mixed along with prepared hyaluronic acid and then it was kept in the orbital shaker for 24- 72hrs until the color change was observed, which is the indication of nanoparticle synthesized¹⁵.

2.2 Anti-inflammatory activity:

BSA (Bovine serum albumin) was used as a reagent for the assay. it makes up almost 60% of all protein in animal serum. BSA undergoes denaturation upon heating and starts expressing antigen associated with type 3 hypersensitivity reaction which leads to inflammatory action. 2mL of 1% bovine albumin fraction was mixed with 400ul of adhatoda vasica extract containing copper nanoparticle was added in a different concentration ranging from 10-50ul. The P^H of the solution was adjusted to 6.8 by adding 1N hydrochloric acid to the solution. The reaction mixture was then incubated at room temperature for 20 minutes and the mixture was heated at 55⁰c for 20 minutes in a water bath. The mixture was allowed to cool at room temperature after incubation. The protein denaturation produced was assessed. The absorbance of the reaction mixture was measured using UV-spectrophotometer at 660nm. DMSO (dimethyl sulfoxide) was used as a control. The standard used for comparison of anti-inflammatory action was Diclofenac sodium.

% Inhibition = Absorbance of control - Absorbance of sample x 100 / Absorbance of control.

Colour change was recorded before and after incubation.

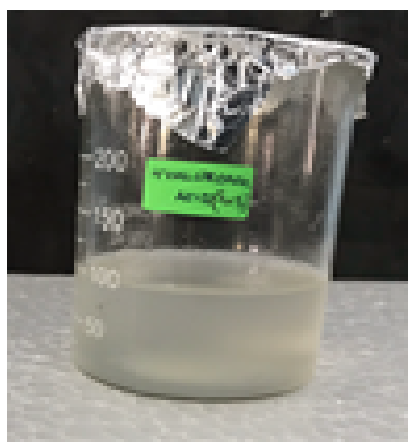
2.3 Antioxidant activity:

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical which reacts with compounds that can donate a hydrogen atom. It contains stable lipophilic free radical, nitrogen centered with purple color. DPPH acts as free radical which induces oxidation. The anti-oxidant can donate an electron to DPPH radical and change in absorbance at 517nm will follow. There was a color change to pale yellow gradually. 2mL of extract was added to five test tubes RANGING FROM 10 -50ul. 50% of the methanol solution (buffer), 0.1mm of DPPH solution was added to five test tubes. The mixture was then incubated for 30 minutes in a dark place at room temperature. The absorbance value was spectrophotometrically analyzed at 517nm. The blank used was methanol solution. Methanol solution mixed with 0.1mM of DPPH solution was used as a control. Ascorbic acid was used as a standard. The IC₅₀ value (minimum inhibitory concentration) was calculated. Percentage of inhibition was estimated using the equation,

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Colour change was recorded before and after incubation.

3.Results and Discussion:



(a)



(b)

Figure - 1 Showing the synthesis of Hyaluronic acid mediated zinc nanoparticle

3.1 Anti-inflammatory activity:

The test for anti inflammatory properties was assessed using photometry¹⁶. The hyaluronic acid mediated zns are added with Bovine serum albumin and they are checked for the anti-inflammatory activity with nanoparticle concentrations 10 μL , 20 μL , 30 μL , 40 μL and 50 μL . Diclofenac sodium in different concentrations was used as standard and then incubated for 55[^]c for 20 min and then the result was analysed spectrometrically. There is an increased activity with the increase in the constituents. Hence they show a good anti inflammatory property^{17,18}.(FIG-2)

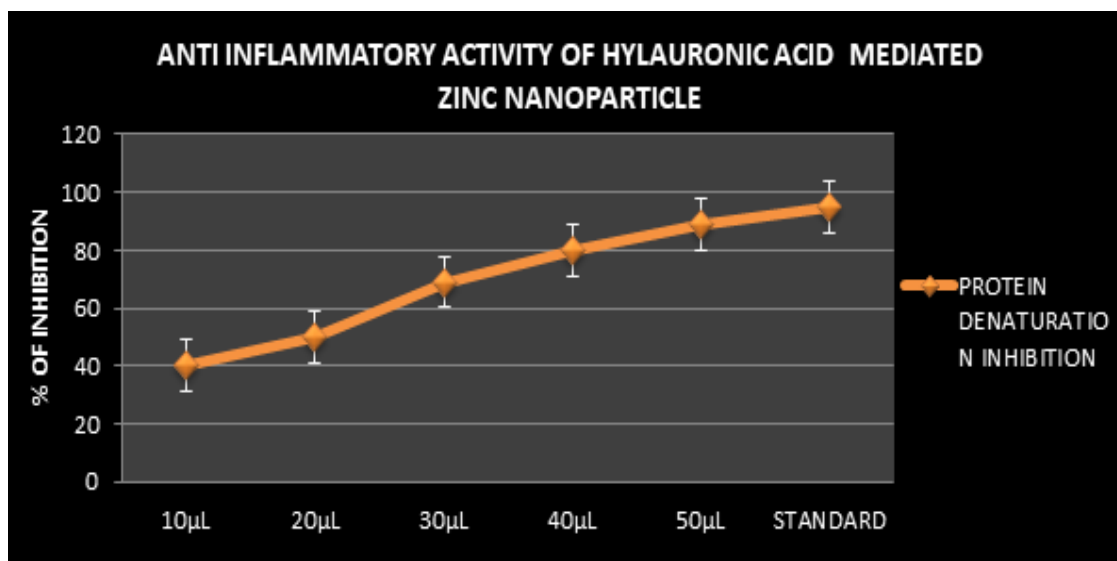


Fig 2: Graph showing the antiinflammatory activity of hyaluronic acid mediated zinc nanoparticles

3.2 Antioxidant activity:

The test for anti-inflammatory properties was assessed using photometry. The hyaluronic acid mediated zns are added with 50% methanol, DPPH solution and they are checked for the anti-inflammatory activity with nanoparticle concentrations 10 µL, 20 µL, 30 µL, 40 µL and 50 µL. They are kept in a dark place for 10 minutes for incubation and the reading was noted and then the result was analysed spectrometrically There is an increased activity with the increase in the constituents (FIG-3). Hence they show a good anti-inflammatory property¹⁹. Both the activities show an increased activity with an increased constituent; hence this compound got a good anti-inflammatory activity and good antioxidant potential. They can be used for the production of anti-inflammatory and antioxidant drugs²⁰.

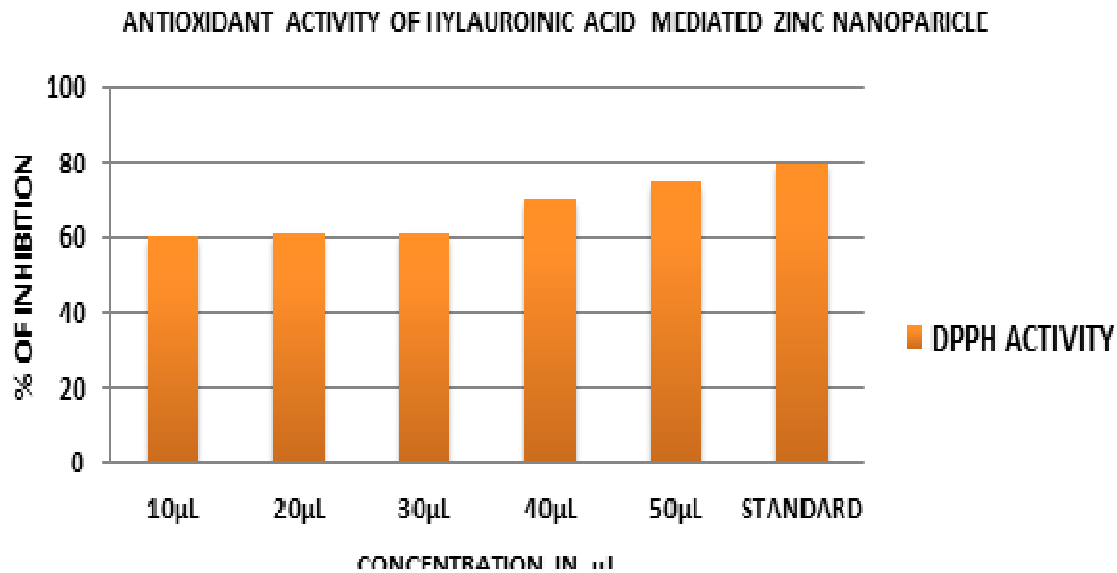


Fig 3: Graph showing the antioxidant activity of hyaluronic acid mediated zinc nanoparticles

4. Conclusion:

The study concludes that hyaluronic acid mediated zinc nanoparticles show very good anti-inflammatory activity and antioxidant activity due to its capability to reduced to nano size so that it can perform its activity in a specific pathway, hyaluronic acid as a individual compound has its own properties and when it is mediated through Zinc it get enhanced in may ways to be a beneficial compound in many ways. Since it shows a good anti-inflammatory and antioxidant property at 50 μ L when compared to standard used and also paves way for further evaluation at higher doses and they can be used for further pharmacological investigations also. These compounds are natural, hence there is a very less chance to obtain side effects. Hence this can be a cost efficient compound too.

5. Reference:

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