



Hyaluronic Acid Mediated Zinc Nanoparticles against Oral Pathogens and Its Cytotoxic Potential

V. Karthik¹, Lakshminarayanan Arivarasu^{2*} and S. Rajeshkumar²

¹Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

²Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-77, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Idea and study was conceptualized by authors LA and SR collection of the literature and drafting the manuscript was by authors VK and LA. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1930716

Editor(s):

(1) Dr. Paola Angelini, University of Perugia, Italy.

Reviewers:

(1) Mary Ucheagha Ememe, Okpara University of Agriculture, Nigeria.

(2) Ana Henriques Mota, Universidade de Lisboa, Portugal.

(3) Luca Gazzabin, Villa Donatello Private Clinic, Italy.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/59849>

Original Research Article

Received 01 June 2020
Accepted 06 August 2020
Published 26 August 2020

ABSTRACT

Aim: To determine the Hyaluronic acid mediated zinc nanoparticles against oral pathogens and cytotoxic potential.

Introduction: Hyaluronic acid is a non sulfated glycosaminoglycan. Bacterial invasion can also be repressed by an inhibitor Interfering with receptor interaction for bacterial invasion, Hyaluronic acid is an example of inhibitor. Nanoparticles is considered as one of the most promising studies in science and technology study and Maintaining the shape, size and distribution of nanoparticles helps in its function and interaction with other molecules.

Materials and Methods: 0.1 g of Hyaluronic acid was added to a flask containing 100 ml of distilled water and heated for an hour. After observing the solubility, 0.574 g of Zn was added to the mixture and then kept on a magnetic stirrer for 1 hour at 100 degree Celsius.

Anti microbial activity: Agar well diffusion and Disc diffusion method is used. Then incubated for 37 degree Celsius for 48 hour. The zone of inhibition is recorded.

Cytotoxic potential: Different concentrations of Hyaluronic acid mediated zinc nanoparticles are incorporated to the wells. After 24 hrs the results were analysed.

*Corresponding author: E-mail: lakshminarayanan512@gmail.com;

Results and Discussion: Hyaluronic mediated zinc nanoparticles is proved to be effective against a wide range of foodborne and clinically relevant Gram-positive and Gram-negative bacteria using several assays such as disk diffusion, agar or broth dilution. Hyaluronic acid mediated Zinc nanoparticles has high potent cytotoxic potential it had been proved with the help of brine shrimps. **Conclusion:** From the observed results, it has been concluded that Hyaluronic acid has a lot of medicinal values and it has antimicrobial activity and it has good cytotoxic potential.

Keywords: Hyaluronic acid; antimicrobial; cytotoxicity; zinc oxide; nanoparticle; brine shrimp.

1. INTRODUCTION

Hyaluronic acid is a non sulfated glycosaminoglycan . Bacterial invasion can also be Inhibited by an inhibitor Interfering with receptor interaction for bacterial invasion . Based on these inhibitors, a main example is a hyaluronic acid [1]. Nanoparticles is considered as one of the most promising studies in science and technology study [2]. By maintaining the shape, size and distribution of nanoparticles, it can be used to successfully achieve their properties and the nature and also the intensity of their interaction with the subsequent molecules .A main method to edit or modify the final result of a nanomaterial is to use polymeric support [3,4].

The antimicrobial activity of the metal nanoparticles have been inscribed rapidly in the last year, as an auto treatment for infected wounds, mainly due to antimicrobial resistant bacteria, and hence nanoparticles of different metals had been studied [5,6]. It has been demonstrated that gold mediated nanoparticles are inert or they have a nontoxic effect on the human cells [7]. The results have been predicted that Gold NanoParticles are not cytotoxic or immunogenic but are bio compatible, collaborating their potential in the area of nanomedicine [8]. On the other hand , good anti microbial activity has been explained against various pathogenic bacteria [9,10]. In contrast to the gold nanoparticles, silver mediated nanoparticles shows high toxicity associated with the oxidative and inflammatory nature [11]. It is insisted that Silver NanoParticles can inhibit the main mechanism of antioxidant defence through decrease in glutathione and the promotion of the lipid peroxidation [12]. Mitochondria is the cellular compartment with high sensitivity to Silver NanoParticle toxicity [13]. Hyaluronic acid is a basic component of the extracellular matrix of the skin, mucosal tissue, joints, eyes, and many other organs and tissues. It takes part in tissue repair processes and is a required component in the resurfacing of the skin and the prevention of scar formation. Its osmotic capability to bring

back tissue hydration during the inflammatory process, and its viscosity helps to prevent the passage of bacteria and viruses into the pericellular area . It is a known stimulator of the inflammatory process because it acts as a barrier to tissue degradation and has antioxidant properties, including the ability to eliminate free radicals [14,15]. 1. The study was aimed to determine the effect of Hyaluronic acid mediated zinc nanoparticles on oral pathogens and their cytotoxic potential.

2. MATERIALS AND METHODS

2.1 Biosynthesis of Nanoparticles

In a flask 0.1 g of Hyaluronic acid is added to the distilled water and heated for an hour. Meanwhile the solubility is checked . After checking the solubility 0.574 g of Zns is added to 0.1 g/100 ml of Hyaluronic acid. Mixed solution is kept Ina magnetisms stirrer for 1 hour at 90 degree Celsius (Fig. 1).

2.2 Anti Microbial Activity

The agar well diffusion method was used to determine the antibacterial activity of Hyaluronic acid medicated Zinc nanoparticles. Different concentrations of compounds were tested against *streptococcus mutans*, *Lactobacillus*. and *Candida albicans*. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates and Rose bengal agar for Antifungal activity respectively. Different concentrations of nanoparticles (50, 100 & 150 μ L) were incorporated into the wells and the plates were incubated at 37°C for 24 h. The antibiotics were used as positive control. Zone of inhibition was recorded in each plate. (Figs. 2 and 3).

2.3 Cytotoxicity Activity

Brine shrimp eggs were added to saline water in a hatching chamber. After 24 hours, exactly 10 hatched larvae (nauplii) were suspended in 6 wells containing 10 ml of saline water, each.

Different concentrations being 5 μL , 10 μL , 15 μL , 20 μL and 25 μL of the nanoparticles synthesised was dispersed in each well with the last well as a control (without any nanoparticles). Post 24 hours, the number of surviving nauplii were counted manually under dissection microscope and recorded. (Fig.- 4)

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

The antimicrobial activity was carried out using the Agar well diffusion method. Three agar plates for identifying the inhibitory effect over *Lactobacillus*, *S. mutans* and *C. albicans* respectively, were used (Figs. 2 & 3). Each plate had four wells each with different nanoparticle concentrations being 50 μL , 100 μL and 150 μL , while the fourth was a standard. Against

Lactobacillus, the diameter of zone of inhibition is observed to be 8 mm, 11 mm and 15 mm respectively, with *S. mutans*, the diameter of zone of inhibition was obtained as 9 mm, 14 mm and 16 mm respectively and against *C. albicans*, the diameter of zone of inhibition was observed as 11 mm, 15 mm and 16 mm respectively. Thus, maximum activity for all the three was observed at 150 μL against standard.

3.2 Cytotoxic Activity

The test for cytotoxic properties was assessed using brine shrimps. Ten nauplii were placed in each of six wells with one standard and the remaining with nanoparticle concentrations 5 μL , 10 μL , 15 μL , 20 μL and 25 μL . LD50 concentration was obtained to be 25 μL , with half the population of nauplii in the respective well surviving post incubation.



Fig. 1. Showing process of preparation of hyaluronic acid mediated zinc nanoparticles



Fig. 2. Culture plates showing antimicrobial activity in MIC (minimal inhibitory concentration) of hyaluronic acid mediated zinc nano particle

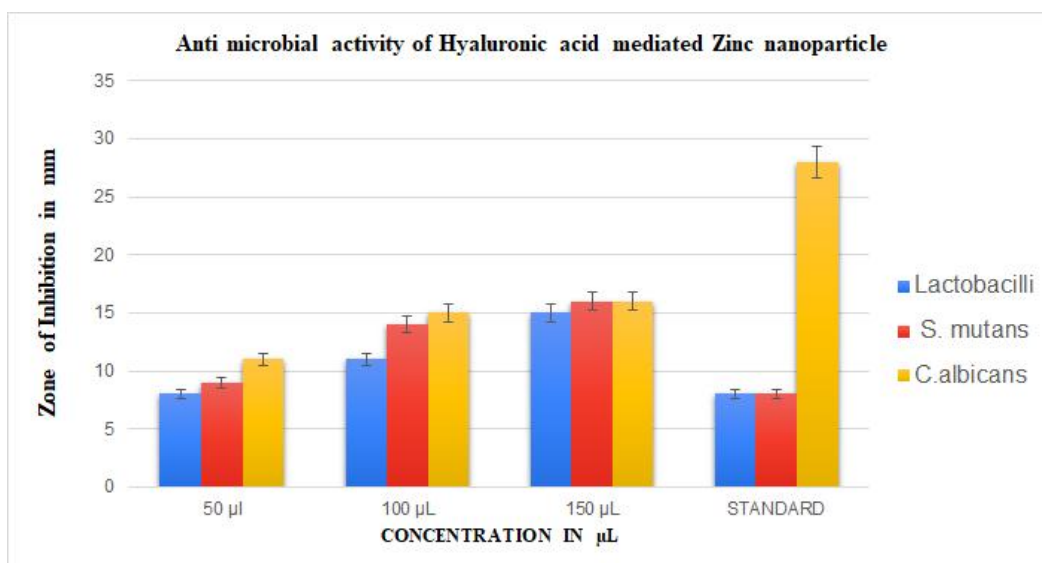


Fig. 3. Graph showing antimicrobial activity of hyaluronic acid mediated zinc nano particle

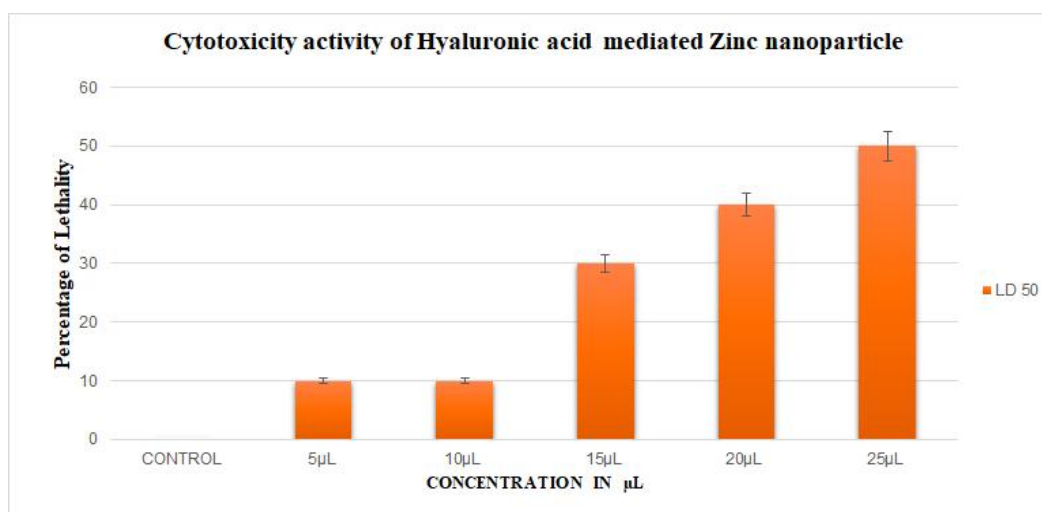


Fig. 4. Graph showing cytotoxic activity of hyaluronic acid mediated zinc nanoparticles

The biologically synthesized zinc nanoparticles using Hyaluronic acid were found to be highly toxic against different pathogenic bacteria and fungi of selected species. The zinc nanoparticles synthesized are highly toxic towards fungal species when compared to bacterial species. Hyaluronic mediated zinc nanoparticles is proved to be effective against a wide range of foodborne and clinically relevant Gram-positive and Gram-negative bacteria using several assays such as disk diffusion, agar or broth dilution. Hyaluronic acid mediated Zinc nanoparticles have high potent cytotoxic potential; it has been proved with the help of brine shrimps.

4. CONCLUSION

From the observed result, it has been concluded that Hyaluronic acid has a lot of medicinal values and it has antimicrobial activity and it has good cytotoxic potential.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors are thankful to Saveetha Dental College for providing a platform to express our knowledge and for the support to conduct the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Leach JB, Schmidt CE. Hyaluronan. Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. 2015;3733–3742.
2. Scott N, Chen H. Nanoscale science and engineering for agriculture and food systems. Industrial Biotechnology. 2013;9: 17–18.
3. Mirkin CA. The beginning of a small revolution. Small. 2004;1:14–16.
4. Campelo JM, Luna D, Luque R, et al. Sustainable preparation of supported metal nanoparticles and their applications in catalysis. Chem Sus Chem. 2009;2:18–45.
5. Maaz K. Silver nanoparticles: Fabrication, characterization and applications; 2018.
6. Dhanalakshmi V, Nimal TR, Sabitha M, et al. Skin and muscle permeating antibacterial nanoparticles for treating Staphylococcus aureus infected wounds. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2016;104: 797–807.
7. Connor EE, Mwamuka J, Gole A, et al. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small. 2005;1:325–327.
8. Shukla R, Bansal V, Chaudhary M, et al. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. Langmuir. 2005;21:10644–10654.
9. Prema P, Iniya PA, Immanuel G. Microbial mediated synthesis, characterization, antibacterial and synergistic effect of gold nanoparticles using *Klebsiella pneumoniae* (MTCC-4030). RSC Advances. 2016;6: 4601–4607.
10. Penders J, Stolzoff M, Hickey DJ, et al. Shape-dependent antibacterial effects of non-cytotoxic gold nanoparticles. International Journal of Nanomedicine 2017;12:2457–2468.
11. Bastos V. Silver nanoparticles: Potential hazards of silver nanoparticles to the environment and human health. CRC Concise Encyclopedia of Nanotechnology. 2015;1008–1019.
12. Piao MJ, Kang KA, Lee IK, et al. Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. Toxicology Letters. 2011;201:92–100.
13. Carlson C, Hussain SM, Schrand AM, et al. Unique cellular interaction of silver nanoparticles: Size-Dependent generation of reactive oxygen species. The Journal of Physical Chemistry B. 2008;112:13608–13619.
14. Chen WYJ, John Chen WY, Abatangelo G. Functions of hyaluronan in wound repair. Wound Repair and Regeneration. 1999;7: 79–89.
15. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 2009;27:76–83.

© 2020 Karthik et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/59849>