EVALUATION OF CYTOTOXICITY OF A NOVEL MOUTHWASH CONTAINING SELENIUM NANOPARTICLES AND CHITOSAN COLLOID

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Abstract

Background: Nanoparticles are now widely gaining popularity in the field of dentistry They are being incorporated into major ingredients in mouthwashes possessing antimicrobial properties and in the treatment of white spot lesions in the field of orthodontics.There is an increased need for alcohol free herbal mouth rinses which are non cytotoxic and biocompatible such that they can be used as an adjunct to mechanical plaque removal techniques in orthodontic patients.

Aim: The aim of this study was to evaluate if a novel non alcohol based herbal mouthwash containing selenium nanoparticles(SeNP) and chitosan colloid is safe and biocompatible for use in patients undergoing orthodontic treatment to reduce plaque accumulation.

Materials and Methods: 15 gms of brine shrimps were hatched in salt solution after incubating them for 24 hrs. Oral rinse prepared from selenium nanoparticles was added at concentrations 5µL, 10µL, 20µL, 40µL, 80µL in a microtiter plate. 10 Hatched nauplii (brine shrimps) were added in each of these microtiter plates and incubated for 24 - hours.

Results: SeNP's based mouthwash showed excellent compatibility at lower concentrations. Mild toxic effects were elicited at higher concentrations.

Conclusion: Oral rinse with 5-10µL of SeNP's was found to be biocompatible and can be used as a potential adjunct along with mechanical plaque removal techniques in orthodontic patients.

Keywords: Nanoparticles,brine shrimps,herbal mouthwash,orthodontics.

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1. Introduction

Nanoparticles are essential building blocks of nanotechnology. The most distinct property of nanoparticles is that they exhibit greater surface to volume ratio.(1).They possess unique features like small size, high surface area, surface charge, surface chemistry, solubility and multi-functionality .Nanoparticles are also very strong carriers for the delivery of therapeutic molecules. They increase the therapeutic efficiency of ionised drugs; improve the penetration of water soluble compounds, proteins, peptides, vaccines, siRNA, miRNA, DNA and other biological therapeutics. Surface modification of nanoparticles with targeting ligands makes the drug delivery system much more versatile and can selectively deliver at target site.(2).

Nanoparticles are now widely gaining popularity in the field of dentistry as well.They are being incorporated into major ingredients in mouthwashes possessing antimicrobial properties and in the treatment of white spot lesions in the field of orthodontics.(3).

Selenium nanoparticles have superior properties as they show better bioavailability , biological activity, better antioxidant properties and reduced toxicity as compared to organic selenium compounds(4).However, all these properties are rendered when selenium is present in its zero oxidation state-which is highly unstable .(5).It is for this purpose that chitosan colloid can be used along with selenium nanoparticles to stabilize them. Chitosan possesses antibacterial, biodegradable and biocompatible properties which has led to increased application in the fields of drug delivery and biomedicine (6).

There is an increased need for alcohol free herbal mouth rinses having effective antimicrobial properties and biocompatibility owning to the fact that certain commercially available mouthwashes could cause less desirable effects like extrinsic staining,antimicrobial resistance against oral pathogens, rare but fatal allergic reactions.(7).

In this study, Selenium nanoparticles were green synthesised and incorporated along with chitosan colloid to form a herbal mouthrinse.

The objective of this study is to assess the cytotoxicity of the mouthrinse and thus assess its biocompatibility as an alternative to commercially available mouthwashes.

2. Materials and Methods

Synthesis And Antimicrobial Evaluation Of Herbal Mouthrinse :

Synthesis of nanoparticles :

Owing to the good antioxidant and natural sweetening properties possessed by Stevia plant,dried stevia leaves were used to extract selenium nanoparticles.Stevia extract was mixed with Sodium Selenite solution and the mixture was heated at 70 degrees .The mixture was homogeneously mixed on an orbital shaker. The colour was pale yellow and it turned purple indicating the formation of SeNP's. This formation of SeNp's was confirmed using spectroscopic analysis which showed a peak of 420 nm corresponding

to the surface plasmon resonance of SeNPs. Characterization using Transmission Electron

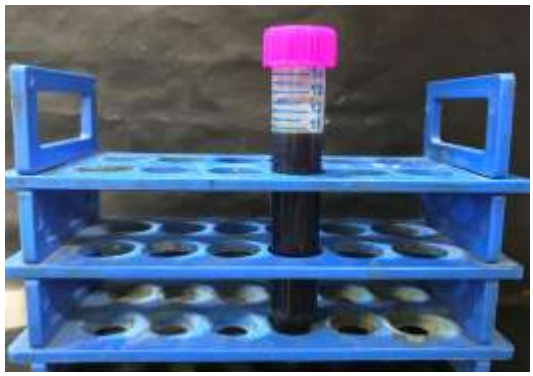
Microscopy revealed spherical SeNP's with a size in the range of 4-45 nm. (figure 1)

FIGURE 1:

Synthesis of Selenium nanoparticles.

Synthesis of Herbal Mouthwash :
Using the SeNP's synthesised from Stevia leaves a herbal alcohol based mouthwash was prepared.Along with Selenium Nanoparticles ,Chitosan Colloid was incorporated as a major ingredient owing to its good antimicrobial properties.(table 1,figure

FIGURE 2 :



Herbal Mouthwash containing Selenium nanoparticles and Chitosan colloid.

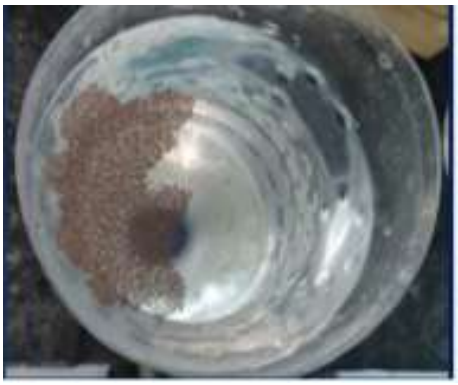
Table 1:Components Of The Mouthwash

COMPONENT	ACTION
selenium nanoparticles -1ml	antimicrobial activity
chitosan colloid -1ml	antimicrobial activity
sucrose -0.3 mg	sweetening agent
sodium benzoate -0.001 g	preservative
sodium lauryl sulfate -0.01g	foaming agent
peppermint oil-100 mules	flavouring agent.

Test for Cytotoxicity:
In this in-vitro study, brine shrimp assay was used to evaluate the toxicity of prepared mouthwash containing SeNP's and Chitosan Colloid.
The larvae of Brine shrimp is referred to as nauplii. (8)

Hatching the brine shrimp:
27g of table salt was weighed and added to 3 litres of distilled water in a cylindrical jar and thoroughly stirred. For good aeration, an air pump was placed into the bottom of the jar. 15 g of brine shrimp eggs were added at the top-level water of the jar and mixed. A light bulb (60–100-Watt bulb) was placed a few inches from the jar. After 20-24 - h of incubation period, nauplii hatched. Hatched nauplii were separated from the empty egg by turning off air and switching off the lamp. This was done to make sure that the empty eggs were floating on top and the brine shrimp were concentrated at the bottom of the water column.(figure 3)

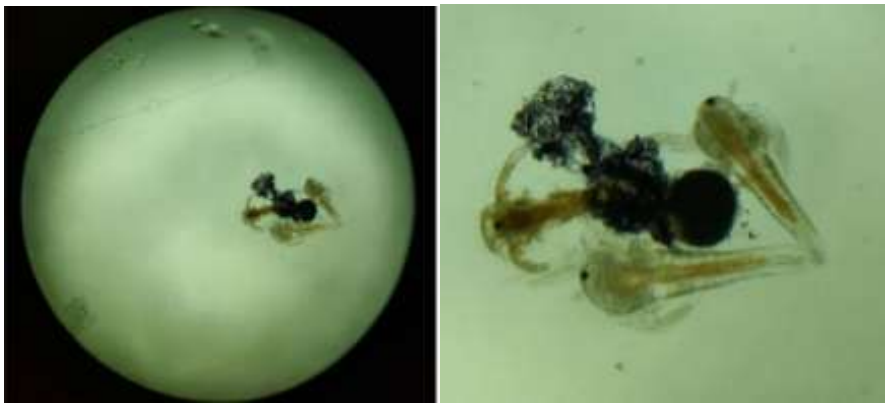
FIGURE 3 :



Hatched nauplii eggs.

Microscopic evaluation of the brine shrimps:
Two brine shrimp eggs were inoculated on a clean slide with 2 drops of distilled water. They were observed under a light microscope at 40X and 10X magnification. Shrimps had an unsegmented body and a single eye, about 22 mm long.(figure 4)

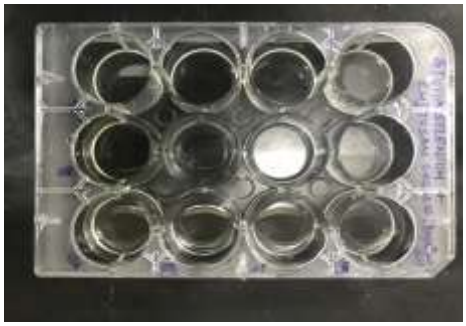
FIGURE 4:



Light microscope - A.nauplii : 40X (left) , 10X (right); represents the microscopic image of the brine shrimp attached to a Selenium nanoparticle.

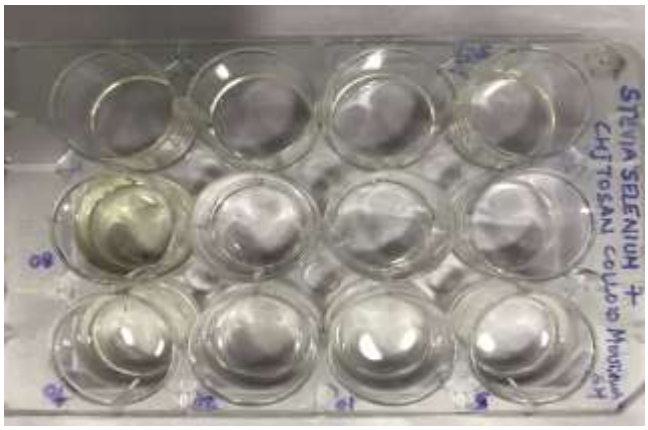
Toxicity testing on theArtemia nauplii:
At the end of 24 - h, wells of microtiter plates were inoculated with SeNP based mouthwash concentrations of 5µL, 10µL, 20µL, 40µL and 80µL. In one of the wells,a sterile salt solution was used as control. A. nauplii were collected in a petri dish from the cylindrical jar. 10
hatched nauplii were collected using a dropper and added in each well. They were
counted using a magnifying glass in duplicate. This was cross checked by another researcher in duplicate to avoid any error. At the end of 24 - h, nauplii in each well in the microtiter plate were counted.(figure 5 and 6)

FIGURE 5:



Elisa plates with mouthwash before 24 hours.

FIGURE 6 :



Elisa plates with mouthwash after 24 hours.

3. Results

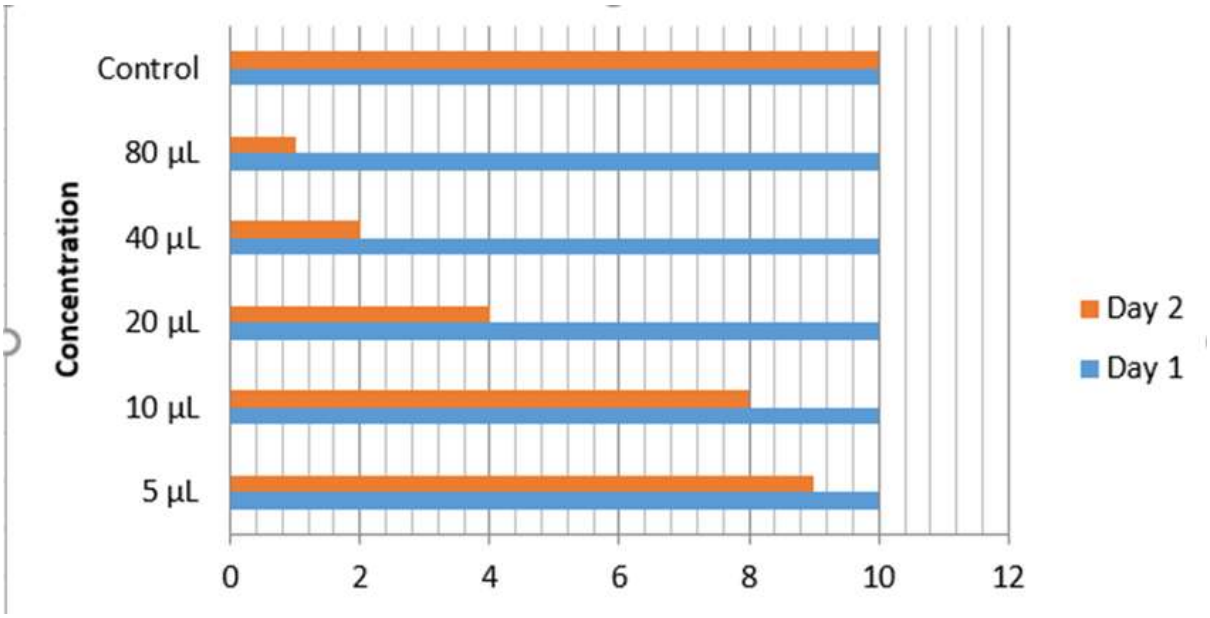
Mortality Rate:

The mortality rate was calculated as follows :

Mortality (%) = (Number of dead nauplii / Total number of A. nauplii ×100)

At the end of 24 hours ,9 nauplii were alive at low concentrations of the mouthwash(10µL)and 1 nauplii to be alive at high concentrations(80µL). At 5µL concentration the mortality rate of the nauplii was 10 %,20% at 10µL ,60 % at 20µL ,80 % at 40µL and 90% at 80µL(figure 7).The cytotoxic effect of SeNP based indigenous mouthwash on A. Nauplii revealed that all brine shrimps are alive at the end of 24 - h at low concentrations which indicate no cytotoxic effect of SeNP based mouthwash on the brine shrimps. At higher concentrations, the brine shrimps are not alive indicating the toxic effect of the mouthwash on brine shrimps at higher concentrations.These results indicated that as the concentration of the mouthwash increased its cytotoxicity also increased.

FIGURE 7 :



The graph indicates the number of nauplii alive at different concentrations of herbal mouthwash after 24 hours in comparison to control.

4. Discussion

In this study,Selenium nanoparticle based mouthwash containing chitosan colloid was prepared and its biocompatibility was assessed. In an attempt to use it as an adjunct to mechanical cleansing in orthodontic patients for reduction of plaque load and white spot lesions, its cytotoxic evaluation was carried out on brine shrimps.(9–12)

In this in-vitro study, brine shrimp assay was used to evaluate the toxicity of prepared SeNP based mouthwash. Brine shrimp assay was first proposed by Michael et al. in 1956, then developed by Vanhaecke et al. in 1981. It has had its applications in detecting fungal toxins,, plant extract toxicity,heavy metals and cytotoxicity testing of dental materials(13,14).Artemia(A.Nauplii) is a genus of aquatic crustaceans, a zooplankton, known as brine shrimps. These species have small size, short life span, large offspring production, and high adaptability to hypersaline environments at various temperatures. Various nanotoxicology conducted on animals proved to be time consuming due to the tedious procedure of obtaining ethical clearance.To overcome this, preference is given to in-vitro studies for evaluating toxicity. Hence, A.nauplii have been used as invertebrate models in nanotoxicology.

Initially, in-vivo animal studies were largely used for assessing toxicity. However animal rights activists from IACUC were not in favour of this idea. Due to increased costs and time in in-vivo studies, certain in – vitro methods were employed. These techniques were XTT assay, MTT assay, cell culture, the WST-1 assay, BrdU assay, fluorescence Microscopy and LDH(13). However,in spite of being in-vitro these techniques were also time consuming and expensive. In an attempt to obtain a rapid and cost-effective screening technique brine shrimp assay test was introduced and is being currently used in assessing the toxicity of nanoparticles.

Many studies available in literature have used brine shrimps for evaluating biocompatibility(15–21).A similar study by (22) assessed the cytotoxicity of selenium nanoparticles extracted from the fruit of Capparis decidua using brine shrimp assay and found that the selenium nanoparticles had no significant cytotoxicity.
(23) in their study of phytofabrication of selenium nanoparticles from emblica officinalis fruit extract also found that cytotoxicity of selenium nanoparticles which are herbally synthesised is lesser than that of chemically synthesized nanoparticles which validates the results of our study. Another study by (24) also concluded that the cytotoxic effects of green synthesized SeNPs from *Allium Sativum o*n Vero cells were lesser than that of chemically synthesised SeNPs.(25) also found similar results in their study wherein naturally synthesized SeNps had lesser cytotoxicity than selenium dioxide.Yet another study by (26) establishes that selenium compounds like selenite induce cytotoxicity causing apoptosis but Selenium has no cytotoxic effect which is synonymous with our study and thus adds evidence to the present study.

However the study by (27) found results contradictory to our study.In their study it was found that Chitosan stabilized SeNPs exhibited higher toxicity than polyvinyl alcohol stabilized SeNPs against MTT cell lines which is contrary to the cytotoxicity exhibited by SeNP and Chitosan based herbal mouthwash.

No literature is available based on the cytotoxicity of Selenium nanoparticle based mouth rinse There is a scope for the future that further studies should be carried out on Selenium nanoparticle based herbal mouthwashes and their cytotoxicity.They also need to be compared with the commercially available mouthwashes as well as other nanoparticle based mouthwashes to be incorporated into clinical practice.

Limitations

Cytotoxicity testing was done at aquatic level. Further toxicity tests should be employed prior to employing its use in orthodontic patients.

5. Conclusion

This study has shown that a novel herbal mouthwash containing selenium nanoparticles had no toxic effect on the brine shrimps upto 10 µL concentration. There is a mild toxic effect elicited by the mouthwash at 40 µL and toxicity increased as the concentration of the mouthwash is increased.Hence, it is a potential alternative to commercially available mouthwashes in orthodontic patients at 5-10µL concentration.

Abbreviations

SeNPs: Selenium Nanoparticles,µL : Microliter; IACUC: Institutional Animal Care and Use Committees.

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No non-author contributions.

Conflict of interest

The authors declare no conflict of interest.

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