Research Article

Antibacterial activity of novel selenium and zinc nanoparticles toward oral *Candida albicans*: An *In vitro* study.

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ABSTRACT

Introduction: For ages, man has tried to find a cure all panaceas for oral maladies caused by microorganisms. Toward this endeavor, many chemicals have been tried and tested, some were successful, but they all have certain side effects on long-term use. Phytotherapy is an emerging field to treat infectious diseases using plant-mediated products which is safe and has very limited side effects. **Aim:** Hence, we wanted to assess the antimicrobial potential of silver and zinc nanoparticles (ZnNPs) synthesized using Triphala against oral candidal organisms. **Materials and Methods:** Selenium and ZnNPs synthesized using green synthesis from Triphala were evaluated for its antimicrobial activity against *Candida albicans* isolated from oral cavity of individuals suffering from dental caries. Various concentrations of the NPs (25, 50, and 100 µl) were used to check the zone of inhibition. The efficacy was tested among 25 samples and data were analyzed using SPSS, V23. **Results:** The mean zone of inhibition for selenium NPs was 10 ± 1.8 mm, 15 ± 2.1 mm, and 31 ± 3.3 mm for 25, 50, and 100 µl concentrations. Similarly, ZnNPs had zone of inhibition of 9.7 ± 1.9 mm, 14 ± 2.8 mm, and 29.9 ± 2.7 mm for 25, 50, and 100 µl concentrations. No statistically significant difference was found between the two groups and both exhibited excellent antimicrobial efficacy against *C. albicans*. **Conclusion:** Selenium and ZnNPs synthesized from Triphala proved to be an excellent antimicrobial agent against oral *C. albicans*. This provides future scope to develop oral antimicrobials using these agents to prevent candidal infections of the oral cavity.

KEY WORDS: Antimicrobial, Candida, Green synthesis, Nanoparticles, Selenium, Zinc

INTRODUCTION

Nanoparticles (NPs) are showing great promise as antimicrobial agents as they have large surface area to volume ratio and a greater number of functional sites [1]. The antimicrobial activity of the NPs is majorly mediated through direct contact with the cell wall and the chance of microbial resistance from the organism toward them is minimal as they cannot penetrate these NPs. Selenium NPs (SeNPs) exhibit positive charge and that allow it to elicit enhanced antimicrobial activity against the negatively charged bacterial cell walls[2]. SeNPs incorporated into titanium nanotubes were found to inhibit *E. coli* and *S. aureus* and modulate macrophages[3]. Similarly, zinc NPs (ZnNPs) have also been very successful as potential antimicrobial agents.

Green synthesis of NPs using plants and microbes has also been used for mass production of NPs and they are economical and require no predefined environment for synthesis[4]. Plant products serve as effective reducing agents which synthesize NPs and stabilize the synthesized NPs[5]. Triphala, an ancient Indian medicine for centuries, an effective antimicrobial agent against oral pathogens, inhibits *S. mutans* at concentrations of 50 µg/ml[6] and had antibiofilm activity against 3- and 6-week-old biofilms in root canals[7]. Further, it is a potent antioxidant due to the presence of essential phytochemicals[8]. Hence, we wanted to assess the antimicrobial efficacy of biogenic SeNPs and ZnNPs against *Candida albicans* isolated from individuals suffering from dental caries.

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MATERIALS AND METHODS

This *in vitro* study was conducted in our institute after obtaining permission and ethical clearance from the Institutional Review Board. The patients who visited our dental institute with complaint of toothache because of dental caries were requested to provide their saliva as part of caries risk assessment and patients were informed regarding the use of their data toward scientific research before consent. The stimulated saliva after chewing paraffin wax for almost 2 min was collected and immediately transported to the lad for culture. The sample was immediately vortexed for 15 s, serially diluted till 10^{-3} and using a micropipette, 0.1 ml (100 µl) was inoculated and plated on CHROMagar to grow *C. albicans*. Different concentrations of NPs (25, 50, and 100 µl) were incorporated through a sterile micropipette into the wells created on the agar plate using sterile cork borer and the plates were incubated at 37°C for 24 h–48 h. Cycloheximide 50 mg was the standard control to test the antimicrobial efficacy of these NPs against *C. albicans.* The antimicrobial efficacy was assessed for 25 samples, obtained from individuals with high caries prevalence (DMFT >7). The zone of inhibition was recorded in millimeters (mm) and the mean values for both the different agents were compared using SPSS, V 21, IL, CH. The level of statistical significance was set at 5% and Student's/ independent t-test was used to evaluate the difference between both the NPs.

RESULTS

The mean age of the participants who provided their saliva was 23 ± 5.6 years and the mean colony-forming units of *C. albicans* were also assessed and those having candidal carriage below 400 CFU/ml were not included. The mean zone of inhibition for *C. albicans* using SeNPs was 10 ± 1.8 mm, 15 ± 2.1 mm, and 31 ± 3.3 mm for 25, 50, and 100 µl concentrations. Similarly, ZnNPs had zone of inhibition of 9.7 ± 1.9 mm, 14 ± 2.8 mm, and 29.9 ± 2.7 mm for 25, 50, and 100 µl concentrations [Figure 1]. There was no statistical significance in the antimicrobial efficacy assessed using zone of inhibition in CHROMagar ($P > 0.05$). Both the agents were found to be at par with regard to their antimicrobial efficacy against *C. albicans.*

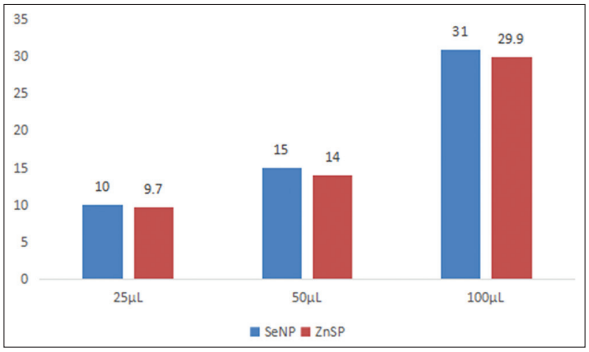

Figure 1: Antimicrobial activity of selenium and zinc nanoparticles against *Candida albicans* (25, 50, and 100 µl)

DISCUSSION

Triphala, an ancient Indian medicine, is a combination of amalaki (*Emblica officinalis*), haritaki (*Terminalia chebula*), and bibhitaki (*Terminalia bellerica*). The phytochemical of Triphala contained a alkaloids, flavonoids, phenols, sterols, resins, quinines, xanthoproteins, and terpenoids and they possess antibacterial and antioxidant properties. These biofunctional groups could have been responsible for reducing selenious acid to SeNPs. Plant flavonols and phenolics are effective reducing agents toward NP synthesis and also function as its stabilizer[9]. Fenugreek extract has been used to synthesize SeNPs and the results were similar to our findings of Triphala extract[4]. The synthesis of NPs was confirmed with color change and ultraviolet spectroscopy and further verified suing Fourier transmission infrared spectroscopy, transmission electron microscopy, and X-ray diffraction analysis. The synthesized NPs for selenium had a mean dimension of 129 nm and zinc had 102 nm.

Only limited evidence exits about the antimicrobial efficacy of biogenic selenium and ZnNPs. Studies have not yet reported antimicrobial efficacy of Se and ZnNPs against yeasts isolated from oral cavity. However, Triphala-conjugated NPs were effective against *C. albicans* tested. The effectiveness of Triphala augmented synthesis of Se/ZnNPs could be considered superior to that of commercial as the concentration of NPs was only 2.5 mg, 5 mg, and 10 mg as compared to 50 mg of commercial cycloheximide. This provided a standard control in our study and results of our study were found to be very superior to that of start drug used for testing. The dose of the drug was in fact significantly lower than commercial drug and at a minimal concentration possible.

There are very limited studies in the literature regarding the efficacy of NPs against candida species, but with respect to isolates of oral candida, there is scarcity of reports. There are several merits of our study. First, we isolated and cultivated the organism from the oral cavity, and we can tell with certainty that the examined antimicrobial activity can be translated in clinical practice too, provided safety and other parameters are assessed in future research. Second, we recruited samples from individuals with very high caries rate and it might be relevant to apply the results to these populations in future therapies for consideration. Third, we used a plant based in house methodology to synthesize the NPs and they were

cost effective and efficient. However, our study is not without limitations. Safety evaluation could have greatly improved the findings, but with the limited scope of time and monetary resources, we could not further our objective.

CONCLUSION

The antimicrobial efficacy of selenium and ZnNPs was found to be at par and was significantly more effective than commercial cycloheximide even at a lower concentration. This could have a paradigm shift in caries treatment or oral microbial diseases caused by *C. albicans* and can prove to be a very efficient therapy with limited side effects and toxicity.

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