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Original Research Article

Evaluation of preliminary anti-microbial efficacy of curcumin and chitosan nanoparticles with and without photoactivation against *candida albicans* – An invitro study

Aishwarya S. Tamase[1], Sheetal Ghivari[1,*], Kritika K Kadam[1]

[1] *Dept. of Conservative Dentistry and Endodontics, Maratha Mandals Nathajirao Halgekar Institute of Dental Sciences and Research Centre, Belgaum, Karnataka, India*

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ABSTRACT

Context: The success of endodontic treatment lies in adequate debridement of the root canal. There are various microorganisms responsible for the failure of root canal treatment, one among them is *Candida albicans*. Sodium hypochlorite is considered as the gold standard. Since it has various disadvantages newer irrigants like Curcumin and Chitosan nanoparticles have been tried.
Aim: The aim of this study was to evaluate the preliminary anti-microbial efficacy of Curcumin and Chitosan nanoparticles with and without photoactivation against *Candida albicans.*
Materials and Methods: The antimicrobial activity of Curcumin, Chitosan nanoparticles with and without Photoactivation, Saline and Sodium hypochlorite was assessed. MIC was done with serial dilution method, incubated for 48-72 hours andobserved for turbidity.MBC was done from the MIC dilutions tubes, first 3 tubes were plated, incubated for 24 hrs and the colony was counted. For Time kill assay broth with the compound was mixed, plated and inoculated at 1hr, 2hrs and 4hrsinterval and colony count was noted.
Statistical analysis: Results were tabulated and statistical analysis was done using Analysis of Variance (ANOVA) and Tukey multiple comparison test.
Results: Photoactivated Curcumin has shown the best antimicrobial results as compared to Curcumin, Chitosan nanoparticles (photoactivated and nonphotoactivated) and 3% Sodium hypochlorite.
Conclusion: Curcumin with and without photo activation and Chitosan nanoparticles can be considered as an alternate endodontic irrigants against sodium hypochlorite.

1. Introduction

The success of root canal treatment in contemporary endodontic therapy mainly depends upon three important aspects like debridement, cleaning and shaping followed by three-dimensional obturation.[1] The first step in root canal treatment is to eliminate the microorganisms which are considered to be the primary source of endodontic infections. Endodontic microflora is diverse containing different species of bacteria, fungi and viruses.

The most commonly found fungi in root canal infections are *Candida albicans* with a prevalence ranging from 0.5%–55%.[2] The *Candida albicans* biofilms possess virulence factors that may play a role in the onset of endodontic pathologies.

As the root canal anatomy is complicated complete removal of these microorganisms is not possible from all areas which are difficult to reach by instruments. Root canal irrigants are important adjuvants in root canal debridement and disinfection which can reach all parts

* *Corresponding author.*
E-mail address: kubasadsheetal@yahoo.in (S. Ghivari).

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of the root canal.[3] Sodium hypochlorite is considered as an ideal root canal irrigant, however, this popular irrigant has various side effects such as unpleasant taste and odour, tissue toxicity, inability to remove the smear layer, inability to fully eradicate microbes from the infected canals, allergic potential, risk of emphysema on overfilling, and discolourations of clothes.[4] To overcome these disadvantages various newer irrigants having antimicrobial activity have been tried in endodontics.

Most herbal alternatives researched are Neem, Tulsi, Green tea, Propolis, Triphala and Curcumin. Curcuma longa (commonly called as turmeric) belonging to the Zingiberaeceae family has been used as a traditional medicine from ancient times. Curcumin (diferuloylmethane) is the main yellow bioactive component of turmeric and has been shown to have a wide spectrum of actions like anti-inflammatory, antioxidant, antibacterial, antifungal, antiprotozoal and antiviral activities.[5]

Antimicrobial efficacy can also be enhanced by using Chitosan Nanoparticles, which are particles sized less than 100 nm, have demonstrated broad-spectrum antimicrobial activity. Nanoparticles have the ability to exert their biological activity without causing adverse reactions neither local nor systemic in patients receiving the therapy.[6]

Because of the increasing incidence of fungal infections, coupled with the growing resistance against antifungal agents, an alternative technique of root canal disinfection involves photodynamic therapy (PDT). Photodynamic inactivation of micro-organisms is based on the combination of a drug, known as photosensitizer, and the delivery of visible light of the appropriate wavelength to excite the photosensitizer molecule.

Hence the aim of this study was to evaluate the preliminary anti-microbial efficacy of Curcumin and Chitosan nanoparticles with and without photoactivation against *Candida albicans*.

2. Materials and Methods

Pure strain of *Candida albicans* ATCC strain 10231 was obtained from the Department of Microbiology, MMNGH Institute of Dental Sciences and Research Centre, Belagavi.

Ethanolic extract of turmeric was prepared by adding 200mg of Curcumin in 20ml ethanol for preparation of irrigant.

Chitosan nanoparticle solution was prepared by dissolving commercially procured Chitosan in dilute acetic acid (2 % v/v), the pH was adjusted to 5.0 using 4M NaOH. This solution was stirred at 600 rpm on a magnetic stirrer. Afterwards, sodium tripolyphosphate (HiMedia Laboratories Pvt. Ltd, Mumbai, India) (TPP, 0.1 % w/v) solution was added to the above mixture. The suspension obtained was stirred further for 30 min at room temperature and centrifuged at 12857 × g at 20 °C for 20 min to pelletize the prepared nanoparticles. The prepared pellet was subsequently redispersed in deionized water and stored at 4^{o}C.

Other irrigants used were commercially available 3%Sodium hypochlorite, Normal saline

2.1. Studied groups

Group 1— Curcumin, Group 2— Photoactivated Curcumin, Group 3— Chitosan nanoparticles, Group 4— Photoactivated Chitosan nanoparticles, Group 5— Sodium hypochlorite 3% (Positive control), Group 6— Sterile saline (Negative control).

2.1.1. Minimum inhibitory concentration

9 dilutions of each drug was done with Thioglycollate broth for MIC. In the initial tube 20microliter of drug was added into the 380microliter of Thioglycollate broth. For dilutions, 200microliter of Thioglycollate broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200 microliters of Thioglycollate broth. This was considered as 10-1 dilution. From 10-1 diluted tube 200microliter was transferred to the second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of Thioglycollate broth. In each serially diluted tube, 200microliter of above culture suspension was added. The tubes were incubated for 48-72 hours in an anaerobic jar at 37°C and observed for turbidity.

2.1.2. Minimum bactericidal concentration

From the MIC dilutions tubes, first 3 or 5 tubes were plated (which was sensitive in MIC) and incubated for 24 hrs then next day the colony count was taken. MBC is done to see whether there was a bacteriostatic or bactericidal effect of the extract (Drug) against the organism. If there is no growth then - it's a bactericidal effect. If there is growth then – it's a bacteriostatic effect.

2.1.3. Time kill assay

An equal quantity of the broth with organism and compound was mixed then immediately it was plated, which was considered as a baseline and inoculated at time intervals of 1hr, 2hrs and 4hrs and incubated in an anaerobic jar. After 48-72 hrs of incubation, the plates were removed and the colony count was noted.

2.2. Statistical analysis

Results were tabulated and statistical analysis was done using Analysis of Variance (ANOVA) and Tukey multiple comparison test using graphite prism software.

3. Results

3.1. And MBC values in triplicates

Table 1: MIC values in triplicates and mean value

Groups	1	2	3	Mean
Nonphotoactivated Curcumin	0.4 μg/ml	0.8 μg/ml	0.8 μg/ml	0.66
Photoactivated Curcumin	0.2 μg/ml	0.2 μg/ml	0.4 μg/ml	0.26
Nonphotoactivated Chitosan nanoparticles	0.8 μg/ml	0.8 μg/ml	0.8 μg/ml	0.80
Photoactivated Chitosan nanoparticles	1.6 μg/ml	0.4 μg/ml	0.8 μg/ml	0.66
Sodium hypochlorite 3% (Positive control)	3.12 μg/ml	3.12 μg/ml	12.5 μg/ml	4.24
Sterile saline (Negative control)	12.5 μg/ml	100 μg/ml	100 μg/ml	70.83

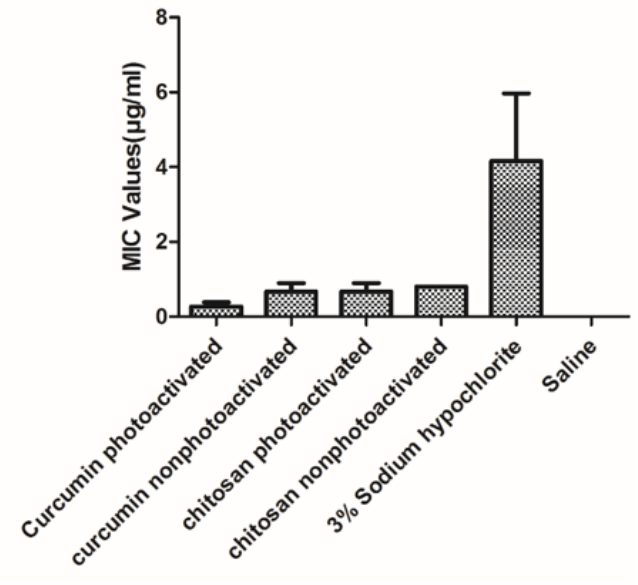

Fig. 1: Comparison of six groups with MIC values against *Candida albicans*.

3.2. MBC

Mean MIC values of all 6 groups were compared. Photoactivated Curcumin with mean value of (0.26 μg/ml) showed better antimicrobial efficacy followed by Curcumin without photoactivation(0.66 μg/ml), Chitosan nanoparticles with photoactivation(0.66 μg/ml), Chitosan nanoparticles without photoactivation (0.80 μg/ml), NaOCl(4.24 μg/ml) and Sterile saline (70.83 μg/ml).

Table 2: MBC values in triplicates andmean

Groups	1	2	3	Mean
Nonphotoactivated Curcumin	1.6μg/ml	0.8 μg/ml	0.8 μg/ml	1.0
Photoactivated Curcumin	0.8 μg/ml	0.4 μg/ml	0.4 μg/ml	0.53
Nonphotoactivated Chitosan nanoparticles	1.6 μg/ml	0.4 μg/ml	0.8 μg/ml	0.66
Photoactivated Chitosan nanoparticles	1.6 μg/ml	0.4 μg/ml	0.8 μg/ml	0.93
Sodium hypochlorite 3% (Positive control)	6.25 μg/ml	1.6 μg/ml	12.5 μg/ml	4.7
Sterile saline (Negative control)	25μg/ml	100 μg/ml	100 μg/ml	75

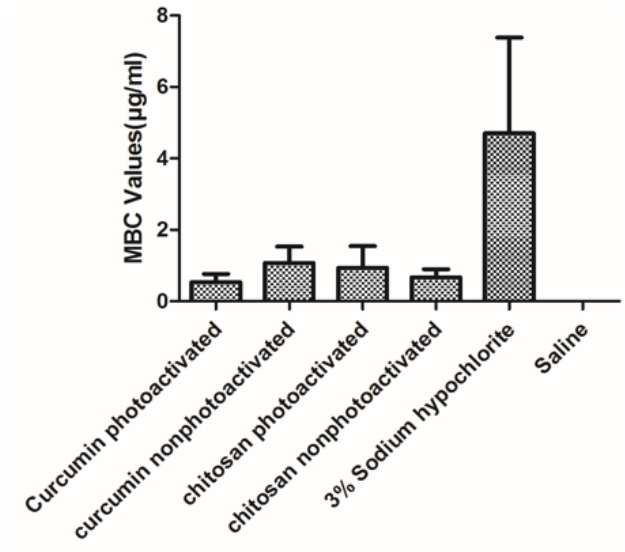

Fig. 2: Comparison of six groups with MBC values against *Candida albicans*.

Mean MBC values of all 6 groups were compared. Photoactivated Curcumin with mean value of (0.53μg/ml) showed better antimicrobial efficacy followed by Chitosan nanoparticles without photoactivation(0.66μg/ml), Chitosan nanoparticles with photoactivation(0.93 μg/ml)Curcumin without photoactivation(1μg/ml), NaOCl(4.7 μg/ml) and Sterile saline (75 μg/ml).

4. Discussion

Endodontic treatment success, to great extent, depends on the depletion or elimination of microorganisms from the root canal system. The use of irrigating solutions with proper anti-microbial and anti-fungal properties during canal debridement and preparation are considered enormously important.

Candida albicans was chosen for this study as it is the most common fungus seen in the root canals with a prevalence of 21% in primary infections and 18% in cases of retreatments. The portal of entry of yeasts into an infected endodontic eco-niche is likely to be through cracks and leakage around faulty restorations. They may also enter through dentinal tubules of deep carious lesions and invade frank pulpal exposures. They survive harsh environmental conditions, thereby making the penetration of antimicrobial agents and irrigants difficult and thus adding to the virulence of microorganisms.[7]

Nara et al reported Sodium hypochlorite (NaOCl) as a gold standard irrigant. As it has high antimicrobial action and the ability to dissolve organic material. But it has deleterious effects like tissue toxicity, unpleasant taste and odour, and inability to remove the smear layer. In the present study to overcome these disadvantages, we have used Curcumin and Chitosan nanoparticles with and without photoactivation to check for antimicrobial efficacy.

Curcuma longa a member of the ginger family possesses anti-inflammatory, antioxidant, antimicrobial and anticancer activity. In an in vitro study conducted by Neelakantan P et al. (2011), it has been shown that curcumin has significant antibacterial activity against E.faecalis and can be used as an alternative to sodium hypochlorite for root canal irrigation.[8]

Mean MIC values of all 6 groups were compared. Photoactivated Curcumin with mean value of (0.26 μg/ml) showed better antimicrobial efficacy followed by Curcumin without photoactivation (0.66 μg/ml), Chitosan nanoparticles with photoactivation (0.66 μg/ml), Chitosan nanoparticles without photoactivation(0.80 μg/ml), NaOCl (4.24 μg/ml) and Sterile saline(70.83 μg/ml).

Mean MBC values of all 6 groups were compared. Photoactivated Curcumin with mean value of (0.53μg/ml) showed better antimicrobial efficacy followed by Chitosan nanoparticles without photoactivation (0.66μg/ml), Chitosan nanoparticles with photoactivation(0.93 μg/ml)Curcumin without photoactivation (1μg/ml), NaOCl (4.7 μg/ml) and Sterile saline (75 μg/ml).

There are various modes to activate or increase the efficacy of irrigants. One among them is photodynamic therapy. Photodynamic therapy (PDT) is a novel therapeutic strategy based on the interaction between a nontoxic photosensitizer and a safe source of visible light at a low intensity; the combination of these two leads to the development of reactive oxygen species (ROS) which are toxic and cause oxidative damage to microorganisms and tumour cells. Cells treated with photo-sensitizers in PDT are susceptible to eradication by exposure to light.

This study clearly demonstrates the efficacy of curcumin and chitosan nanoparticles with and without light activation using a blue light source on the *Candida albicans*. In this study, special attention was given to the protocol of light exposure by the blue light device during photoactivation. The PDT was able to promote anti-fungal effects against C. Albicans and was able to produce significant reductions in viable Candida counts when compared to other groups. Although the statistical analysis did not find significant differences between photoactivated curcumin and nonphotoactivated curcumin groups.

In the present study, Curcumin without photoactivation also showed good results. It may be because of the main yellow bioactive component of turmeric as it has shown to have a wide spectrum of antifungal action. Which causes leakage of potassium ions from the fungal cytosol, increase in membrane permeability and disruption of membrane.

In a study Jing Ma et al used one standard strain ATCC 90028 and two clinical isolates from HIV (CCA1) and oral lichen planus (CCA2) patients' oral cavities. Biofilms were photosensitized with 60 μM Curcumin and irradiated by a light emitting diode (LED) under the wavelength of 455 nm and energy densities of 2.64, 5.28, 7.92, 10.56, 13.2 J/cm^2. Then the antifungal effects of Curcumin-photodynamic therapy were evaluated by XTT reduction assay and confocal light scanning microscopy (CLSM) observations. The study concluded that the combination of curcumin with blue LED light was effective against *Candida albicans*.[9]

In dentistry, Chitosan has been used due to its antibacterial characteristics for the prevention of dental caries. Chitosan nanoparticles have superior activities, including antimicrobial effects, drug, gene and/or vaccine delivery systems, and antitumor effects.[10] Louise et al studied the effect of Chitosan nanoparticles (ChNPs) on the inhibition of Candida spp. biofilm on the denture base surface. Results showed the ChNPs inhibited C. Albicans biofilm and reduced Candida biofilm on resin.[10]

The results of our study were in accordance with Fabio et al who showed that combining photodynamic therapy (PDT) and chitosan reduced the endodontic infection caused by E. faecalis compared to Chitosan without photoactivation.[11]

Therefore, within the limitation of this study, it can be concluded that photoactivated Curcumin has shown the best antimicrobial results as compared to Curcumin, Chitosan nanoparticles (photoactivated and nonphotoactivated) and 3% Sodium hypochlorite. Further studies should concentrate on the evaluation of the antimicrobial activity of photoactivated Chitosan nanoparticles on different species of microorganisms and also evaluate the effect of Curcumin with photoactivation in invivo situations using animal models and on humans.

5. Source of Funding

None.

6. Conflict of Interest

None.

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Author biography

Aishwarya S. Tamase, Private Practitioner

Sheetal Ghivari, Professor https://orcid.org/0000-0003-2968-6133

Kritika K Kadam, Private Practitioner

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