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Photoinactivation Effects of Curcumin, Nano-curcumin, and Erythrosine on Planktonic and Biofilm Cultures of *Streptococcus mutans*

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Abstract

Introduction: This in vitro study was conducted to assess the phototoxic effects of curcumin, nano-curcumin, and erythrosine on the viability of *Streptococcus mutans (S. mutans)* in suspension and biofilm forms.

Methods: Various concentrations of curcumin (1.5 g/L, 3 g/L), nano-curcumin (3 g/L), and erythrosine (100 µM/L, 250 µM/L) were examined for their impact on planktonic and biofilm cultures of *S. mutans*, either individually or in conjunction with light irradiation (photodynamic therapy or PDT). A blue light-emitting diode (LED) with a central wavelength of 450 nm served as the light source. The results were compared to 0.12% chlorhexidine digluconate (CHX) as the positive control, and a solution containing neither a photosensitizer (PS) nor a light source as the negative control group. The dependent variable was the number of viable microorganisms per experiment (CFU/mL).

Results: Antimicrobial PDT caused a significant reduction in the viability of *S. mutans* in both planktonic and biofilm forms, compared to the negative control group ($P<0.05$). The highest cell killing was observed in PDT groups with curcumin 3 g/L or erythrosine 250 µmol/L, although the difference with PDT groups using curcumin 1.5 g/L or erythrosine 100 µmol/L was not significant ($P>0.05$). Antimicrobial treatments were more effective against planktonic S. mutans than the biofilm form.

Conclusion: PDT with either curcumin 1.5 g/L or erythrosine 100 µmol/L may be suggested as an alternative to CHX to inactivate the bacteria in dental plaque or deep cavities. Nano-curcumin, at the selected concentration, exhibited lower efficacy in killing *S. mutans* compared to Curcumin or erythrosine.

Keywords: Photodynamic therapy; *Streptococcus mutans*; Photosensitizer; Dental caries; curcumin

Introduction

Dental caries is a prevalent, chronic, and infectious disease, resulting from the consumption of fermentable carbohydrates by cariogenic bacteria, which produces an acidic environment to dissolves tooth structure.[1-5] *Streptococcus mutans (S. mutans)* plays a pivotal role in dental caries development and is usually colonized with other species of microorganisms in the form of biofilm on teeth and oral soft tissues.[6,7] Various strategies have been proposed to reduce the load of pathogenic organisms in the oral cavity. Although the mechanical disruption of microbial biofilms is considered the most effective approach, it is not desirably achieved in noncompliant or disabled patients.[8,9] The use of antimicrobial mouthwashes or antibiotics presents alternative options for treating diseases originating from biofilm infections,

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but these approaches can lead to adverse effects such as alterations in taste, discoloration of oral tissues and restorative materials, and the emergence of drug-resistant microorganisms.[7-9]

Photodynamic therapy (PDT) is increasingly being used in both medicine and dentistry for eradicating bacterial or fungal infections and for diagnosing and treating malignant lesions.[10] PDT employs a combination of a dye or photosensitizer (PS) and a low-intensity light source that corresponds to the absorption band of the applied PS. In the presence of oxygen molecules, this combination generates reactive oxygen species (ROS), which are toxic to microbial or mammalian cells.[4,11] Antimicrobial PDT can be used to control the formation of oral biofilm in subjects who show a high risk for the development of dental caries. It can also be applied to inactivate remaining bacteria on the axial and pulpal surfaces during cavity preparation, thereby helping preserve maximum tooth structure.[2,10-12]

Curcumin is a natural yellow pigment extracted from the rhizomes of the *Curcuma longa* (turmeric) plant, a member of the ginger family. Curcumin offers multiple therapeutic benefits, including antioxidant, anti-inflammatory, anti-tumour, and antimicrobial effects,[4] and it can be effectively employed as a dye in PDT.[13-17] It shows strong absorption in the blue light range with a peak at 430 nm.[4] Traditionally, curcumin powder is dissolved in dimethylsulfoxide (DMSO) and ethanol before dilution with aqueous solutions.[7, 17] However, the therapeutic potential of curcumin is limited by factors such as low water solubility, low bioactive absorption, rapid metabolization, and physicochemical instability.[7,17,18] The use of curcumin in bioadhesive and biocompatible formulations (nano-curcumin) has been suggested to enhance its solubility and bioactive properties.[7] Nevertheless, there is limited research on the effectiveness of nano-curcumin in eliminating oral microorganisms.

Erythrosine is a red fluorescein dye belonging to a group of cyclic compounds called xanthenes. Erythrosine has long been employed in dentistry as a plaque-disclosing agent and exhibits antibacterial potential against gram-positive and gram-negative bacteria, especially when combined with light irradiation.[19-22] Erythrosine absorbs light in the green range (500–550 nm),[10,22] with maximum absorbance occurring at a wavelength of 532 nm.[23]

Previous studies demonstrated the effectiveness of PDT with different PSs and light sources for plaque-related diseases.[2,4,7,17,24] However, there is a scarcity of studies comparing the antibacterial effects of different dyes activated by conventional light curing units in dental practice on planktonic and biofilm cultures of cariogenic *S. mutans*. Therefore, this study was conducted to assess the impact of curcumin, nano-curcumin, and erythrosine, either alone or activated by a high-intensity light-emitting diode (LED) device (PDT effect), on reducing the viability of *S. mutans* organized in suspension or biofilm forms.

Methods and Materials

Bacterial Strain and Growth Conditions

Approval for this research protocol was obtained from the ethics committee of Mashhad University of Medical Sciences and assigned the code IR.MUMS.DENTISTRY.REC.1398.102. The study used *Streptococcus mutans* (ATCC 35668 strain), which was purchased from the Iranian Research Organization for Science and Technology (IROST). The bacteria were reactivated in a brain heart infusion (BHI) culture medium under aerobic conditions at 37 °C for 16-24 hours until they reached the logarithmic growth phase. The optical density of the suspensions was measured at a wavelength of 650 nm using a spectrophotometer. The concentration of the material was then adjusted by the BHI medium to reach the optical density of 0.5 McFarland standard bacterial suspension, corresponding to the content of 1.5×10^8 colony forming units per millilitre (CFU/ml).

Photosensitizers and the Light Source

Curcumin (CUR), nano-curcumin (N-CUR), and erythrosine (ER) were used as PSs in this study. Curcumin (Sami Labs Ltd., Bangalore, India) was dissolved in sterile distilled water containing 4% DMSO to achieve solutions at concentrations of 1.5 g/L or 3 g/L. The nano-curcumin (SinaCurcumin®; Exir Nano Sina Company, Tehran, Iran) and erythrosine (Sigma Ltd, Poole, UK) solutions were prepared by dissolving the dyes in distilled water to reach the concentrations of 3 g/L for nano-curcumin and 100 µM/L or 250 µM/L for erythrosine. The solutions were sterilized after filter purification and stored in tubes wrapped with aluminum foil at 4 °C. Fresh solutions were prepared before each experiment.

A high-intensity LED unit (X-cure, Woodpecker, Guilin, China) served as the light source to excite the PSs. The device had an output intensity of 1200 mW/cm^2 and emitted light in the range of 385-515 nm, with a maximum intensity at 450 nm. In this study, the LED unit was held at a distance of approximately 7 mm from the specimens, and thus the final irradiance delivered to the samples was calculated as 738 mW/cm^2. Light activation was carried out in six periods of 20 seconds each, separated by dark intervals of 60 seconds (total period of dark and light cycles = 7 minutes).

The Treatment Groups

The experiments were conducted with a total of 12 groups, as detailed in Table 1. Different concentrations of curcumin (1.5 g/L, 3 g/L), nano-curcumin (3 g/L), and erythrosine (100 µM/L, 250 µM/L) were assessed for their antibacterial effects, either alone or in combination with light irradiation (PDT effect), on planktonic and biofilm cultures of *S. mutants*. The results were compared with

0.12% chlorhexidine digluconate (CHX) solution as the standard positive control group, and a solution containing neither the PS nor the light source as the negative control group. The anti-bacterial effect of light irradiation was not assessed in this study, as several studies demonstrated no significant bacterial reduction after the isolated light treatment.[4,25,26] Five independent experiments were conducted for each group in planktonic or biofilm cultures. The response variable was the number of *S. mutans* colonies per experiment (CFU/mL).

Photodynamic Therapy of Streptococcus mutans in Planktonic Suspensions

In all the study groups, 10 µL of cultured *S. mutans* bacteria (1.5×10^8 CFU/mL) was transferred into each microtube containing 490 µL of BHI medium. Subsequently, 500 µL of the PS solution, at a concentration twice that of the desired value, was added to the bacterial suspension. Therefore, the volume of the resulting material reached 1 mL, and the number of bacteria reduced to 1.5×10^6 CFU/mL. In the negative control group (group 11), 500 µL of phosphate-buffered saline (PBS) was put into the microtubes instead of the PS to maintain the same volume of solution, whereas, in the positive control group, CHX at the final concentration of 0.12% was added. The samples were put on a vortex mixer for 10 seconds to ensure even suspension and then kept in a dark environment at room temperature for 5 minutes (pre-irradiation time). Afterwards, the samples in PDT groups (groups 6 to 10) underwent illumination at a distance of 7 mm according to the previously mentioned protocol (six periods of 20 seconds each, with 60-second dark intervals), whereas the other groups were maintained in the dark place for an equivalent duration (7 minutes).

Induction of In Vitro Biofilm Caries Model on Dentin Slabs

Sixty-three dentin slabs were prepared from the roots of the bovine lower incisors, following the procedure described by Lamarque et al.[27] For this purpose, the roots were separated from the crowns using a diamond disk attached to a low-speed handpiece under water cooling. Then, the roots were divided mesiodistally into two halves (buccal and lingual). Each root half was ground flat and polished with sandpaper disks to reach a smooth and uniform surface with a 1 mm thickness. Two square dentin samples, measuring 5 mm × 5 mm, were obtained from each half by the diamond disk, as illustrated in Figure 1. The dentin slabs were washed for 20 minutes and stored at 4 °C under 100% humidity. The slabs were autoclaved at 121 °C for 15 minutes before the experiments.

To establish biofilms, dentin slabs were distributed into three 24-well plates. A volume of 10 µL cultured *S. mutans* (1.5×10^8 CFU/mL) was put into each well and supplemented with a 990 µL BHI medium containing 1% (wt/vol) sucrose. Subsequently, the plates were

Figure 1. Preparation of Dentin Specimens From Bovine Lower Incisor Roots

Table 1. The Study Groups and Respective Treatments

Groups	Abbreviation	Definition	Photosensitizer	Light
Group 1	CUR1.5	Curcumin 1.5 g/L	+	_
Group 2	CUR3	Curcumin 3 g/L	+	_
Group 3	N-CUR3	Nano-curcumin 3 g/L	+	_
Group 4	ER100	Erythrosine 100 µmol/L	+	_
Group 5	ER250	Erythrosine 250 µmol/L	+	_
Group 6	CUR1.5 + L	Curcumin 1.5 g/L + light	+	+
Group 7	CUR3 + L	Curcumin 3 g/L + light	+	+
Group 8	N-CUR3 + L	Nano-curcumin 3 g/L + light	+	+
Group 9	ER100 + L	Erythrosine 100 µmol/L + light	+	+
Group 10	ER250 + L	Erythrosine 250 µmol/L + light	+	+
Group 11	PBS (Negative control)	Phosphate-buffered saline	_	_
Group 12	CHX (Positive control)	0.12% Chlorhexidine digluconate	_	_

incubated at 37 °C with 5% CO_2 for five days to allow biofilm formation and maturation. The culture medium was refreshed with BHI supplemented with 1% sucrose after 48 and 96 hours. After 120 hours, scanning electron microscope (SEM) images were obtained from three random samples to confirm biofilm formation (Figure 2). The remaining 60 slabs were submitted to the experimental and control groups (n = 5).

Photodynamic Therapy of Streptococcus mutans Biofilms

The bovine slabs containing 120-hour biofilms were rinsed three times with 1 ml PBS solution to remove non-adherent bacteria. The slabs were then transferred to new 24-well plates, with six dental slabs evenly distributed into each plate to prevent overlapping light exposure. The well was covered with 1 mL of the PS solution. After 5 minutes of contact between the dye and biofilms in the dark (pre-irradiation time), the PDT groups (groups 6-10) received light irradiation following the previously described protocol, whereas the other groups (groups 1-5) were maintained for the same duration (7 minutes) without light exposure. For the positive and negative control groups, 0.12% CHX and PBS were employed, respectively, and kept for the same period.

Following the treatments, the dentin slabs were removed from the wells, rinsed with PBS, and inserted into microtubes containing 1 mL of PBS. The microtubes containing the biofilms were sonicated at 35°C for a total of 30 minutes (three 10-minute sonication periods with 10-minute intervals between them) to detach the biofilm from the dentin slabs.

Determination of CFU Counts

After the treatments described on suspension and biofilm cultures, 50 µL of the final samples were submitted to serial dilutions (ranging from 1/10 to 1/10 000) with 450 µL of PBS medium. For each dilution, 50 µL aliquots were spread on blood agar plates using the streaking technique by a sterile spreader. The plates were incubated under aerobic conditions at 37 °C for 24 hours. and then photographed to count the number of viable microorganisms (CFU/mL) by colony count software.

Statistical Analysis

The normal distribution of the data was confirmed by the Shapiro-Wilk test ($P > 0.05$). One-way analysis of variance (ANOVA) was applied to detect any difference in colony counts between the groups in planktonic and biofilm cultures. Pairwise comparisons were assessed using Games-Howell and Tukey tests for planktonic and biofilm samples, respectively. Data analysis was conducted by SPSS software (Statistical Package for the Social Sciences, version 16.0; SPSS Inc., IL, USA), and the significance level was set at $P < 0.05$.

Results

Table 2 presents the mean, standard deviation (SD), percentage of reduction relative to the control group, and statistical significance regarding the number of living *S. mutans* bacteria in suspension and biofilm cultures.

Antimicrobial treatments were more effective in the planktonic form than in the biofilm form of *S. mutans*, as evidenced by the difference in the percentage of bacterial reduction.

Photoinactivation of Streptococcus mutans in the Planktonic Phase

Table 2 presents the results of various treatments against planktonic *S. mutans*. The positive control group (0.12% CHX) exhibited no bacterial growth. Among the experimental groups, the highest antibacterial activity was observed in the PDT group using curcumin 3 g/L (CUR3 + L), followed by the PDT group with erythrosine 250 µmol/L (ER250 + L), while the lowest activity was observed in the samples treated with nano-curcumin 3

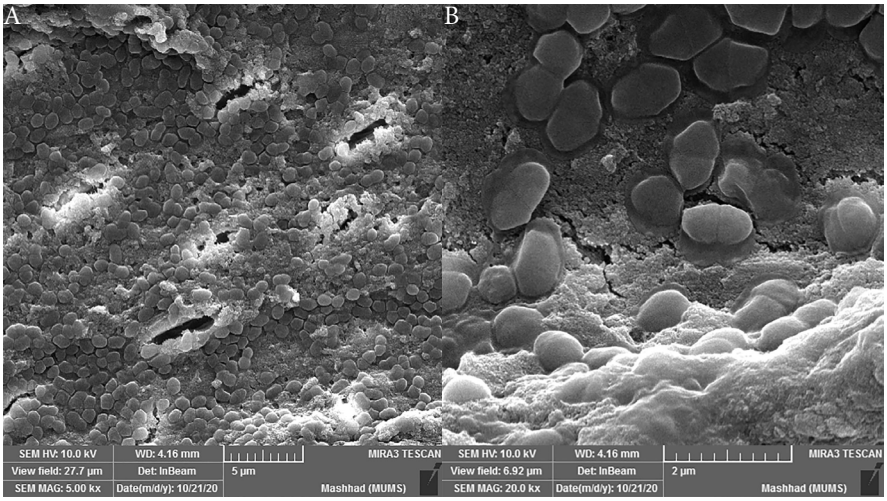

Figure 2. SEM Images of *Streptococcus mutans* Biofilms Formed *In Vitro* at 5.00 kX (A) and 20.0 kX (B) Magnifications

Table 2. The Number of *Streptococcus mutans* Colonies (CFU/mL × 10^3) After Different Treatments in Planktonic and Biofilm Cultures

		Planktonic Form		Biofilm Form	
		Mean ± SD (CFUs/mL × 10^3)*	% Of Reduction Compared to the Negative Control	Mean ± SD (CFUs/mL × 10^3)	% Of Reduction Compared to the Negative Control
1	CUR1.5	134 ± 17 [a]	96.6%	252 ± 33 [e,f]	9.0%
2	CUR3	77 ± 15 [a]	98.1%	213 ± 25 [d,e]	23.1%
3	N-CUR3	2384 ± 326 [c]	40.0%	232 ± 37 [e,f]	16.2%
4	ER100	118 ± 33 [a]	97.0%	231 ± 30 [e,f]	16.6%
5	ER250	62 ± 19 [a]	98.4%	168 ± 19 [c,d]	39.3%
6	CUR1.5 + L	56 ± 15 [a]	98.6%	107 ± 13 [a,b]	61.4%
7	CUR3 + L	13 ± 2 [a]	99.7%	65 ± 21 [a]	76.5%
8	N-CUR3 + L	708 ± 290 [b]	82.3%	125 ± 20 [b,c]	54.9%
9	ER100 + L	60 ± 19 [a]	98.5%	96 ± 18 [a,b]	65.3%
10	ER250 + L	46 ± 12 [a]	98.8%	57 ± 15 [a]	79.4%
11	PBS (Negative control)	4000 ± 448 [d]	-	277 ± 14 [f]	-
12	CHX (Positive control)	0.00 ± 0.00 [a]	100%	79 ± 10 [a,b]	71.4%
P value		< 0.001		< 0.001	

SD: Standard deviation
The groups that have been defined by different letters denote significant differences at $P < 0.05$.
CUR1.5 = Curcumin 1.5 g/L; CUR3 = Curcumin 3 g/L; N-CUR3 = Nano-curcumin 3 g/L; ER100 = Erythrosine 100 µmol/L; ER250 = Erythrosine 250 µmol/L; L = light; PBS = Phosphate buffered saline; CHX = 0.12% Chlorhexidine.

g/L without light radiation (N-CUR3). The ranking of bacterial counting after different treatments in *S. mutans* suspensions was as follows:

CHX < CUR3 + L < ER250 + L < CUR1.5 + L < ER100 + L < ER250 < CUR3 < ER100 < CUR1.5 < N-CUR3 + L < N-CUR3 < Negative control

The statistical analysis revealed a significant difference in the number of *S. mutans* colonies between the study groups in the planktonic phase ($P < 0.001$; Table 2). According to pairwise comparisons, the positive control group, curcumin (both concentrations, with or without light irradiation) and erythrosine (both concentrations, with or without light irradiation) exhibited comparable bacterial counts (a reduction range of 96.6% to 99.7% in the number of bacteria) ($P > 0.05$), which were significantly lower than those of the nano-curcumin (with or without light irradiation) and negative control groups ($P < 0.05$; Table 2).

Increasing the concentration of curcumin from 1.5 g/L to 3 g/L and erythrosine from 100 µmol/L to 250 µmol/L enhanced the inhibition of bacterial growth, but the difference between the two concentrations was not significant, whether the dye was used with or without light irradiation ($P > 0.05$).

Figure 3 illustrates the percentage of reduction in *S. mutans* colonies after different treatments relative to the negative control group in the planktonic phase.

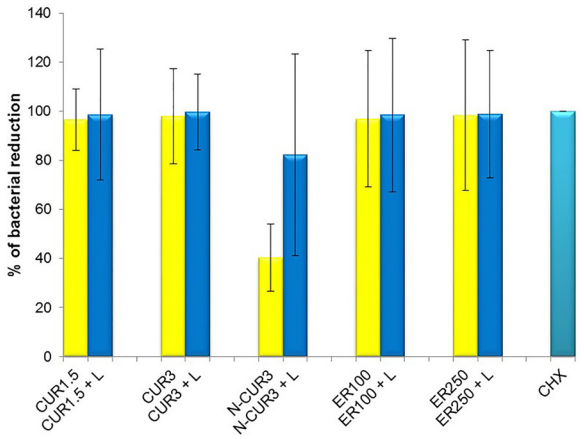

Figure 3. Percentage of Reduction in *Streptococcus mutans* Colonies After Different Treatments Relative to the NNegative Control Group (Phosphate buffered saline) in in the Planktonic Phase. CUR1.5 = Curcumin 1.5 g/L; CUR3 = Curcumin 3 g/L; N-CUR3 = Nano-curcumin 3 g/L; ER100 = Erythrosine 100 µmol/L; ER 250 = Erythrosine 250 µmol/L; L = light; CHX = 0.12% Chlorhexidine. Error bars represent standard deviations

Photoinactivation of Streptococcus mutans biofilms

Table 2 presents the results of different treatments against *S. mutans* within the preformed biofilm. The maximum antibacterial activity was observed in the PDT group using erythrosin 250 µm/L (ER250 + L), followed by the PDT group with curcumin 3 g/L (CUR3 + L). These effects were even more pronounced than those of the CHX group (positive control). The ranking of bacterial counting of *S. mutans* in the biofilm culture was as follows:

ER250 + L < CUR3 + L < CHX < ER100 + L < CUR1.5 + L < N-CUR3 + L < ER250 < CUR3 < ER100 < N-CUR3 < CUR1.5 < Negative control

ANOVA displayed a significant difference in the

number of *S. mutans* colonies between the study groups in the biofilm form ($P < 0.001$; Table 2). Pairwise comparisons revealed that the positive control group and PDT groups with both concentrations of curcumin (CUR1.5 + L, CUR3 + L) and erythrosine (ER100 + L, ER250 + L) exhibited comparable bacterial counts ($P > 0.05$), which were significantly lower than those of the other study groups ($P < 0.05$; Table 2). On the other hand, curcumin 1.5 g/L (CUR1.5), nano-curcumin 3 g/L (N-Cur3), and erythrosine 100 µmol/L (ER100) exhibited a comparable number of bacteria to the negative control group ($P > 0.05$; Table 2).

Increasing the concentration of curcumin from 1.5 g/L to 3 g/L and erythrosine from 100 µmol/L to 250 µmol/L enhanced the inhibition of bacterial growth, but the difference was only significant between the ER100 and ER250 groups without light irradiation ($P < 0.05$; Table 2).

Figure 4 demonstrates the percentage of reduction in *S. mutans* biofilms after different treatments relative to the negative control group.

Discussion

The present study investigated the impact of PDT with three PSs including curcumin, nano-curcumin, and erythrosine on the viability of *S. mutans* in suspension and biofilm cultures. Bovine incisors were used for biofilm induction because of their availability, structural similarity to human teeth, and flat and extensive surfaces to prepare dentin slabs.[28,29] The findings revealed that the best antibacterial activity occurred in the PS groups activated by light irradiation (PDT groups). Nonetheless, the use of PSs alone also demonstrated efficacy against cariogenic *S. mutans*, compared to the negative control group. Previous studies indicated no toxic effect of light application without dyes on microbial cultures.[4,25,26]

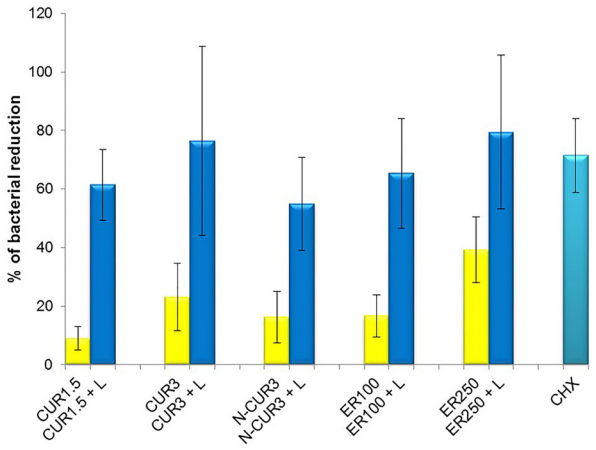

Figure 4. Percentage of Reduction in *S. mutans* Colonies After Different Treatments Relative to the Negative Control Group (Phosphate buffered saline) in the Biofilm Form. CUR1.5 = Curcumin 1.5 g/L; CUR3 = Curcumin 3 g/L; N-CUR3 = Nano-curcumin 3 g/L; ER100 = Erythrosine 100 µmol/L; ER 250 = Erythrosine 250 µmol/L; L = light; CHX = 0.12% Chlorhexidine. Error bars represent standard deviations

Therefore, the antibacterial effect of light irradiation was not assessed in the present investigation. Overall, the experimental treatments proved to be more effective against *S. mutans* in the planktonic phase compared to the biofilm phase.

A pre-irradiation (incubation) time of 5 minutes was selected in this study to allow the dye to bind and penetrate the cell layers. Previous studies employed pre-irradiation periods ranging from 1 to 20 minutes before light activation.[4,30] While longer pre-irradiation times may enhance cell-PS interaction and singlet-oxygen production, they are not practical in clinical settings.

A high-power LED unit with an emission range of 385–515 nm was used to excite the PS. Blue light sources are commonly used in dentistry for the polymerization of composite resins and they can also be applied for exciting PSs like curcumin. The use of a blue LED unit along with readily available PSs makes PDT an affordable and feasible technique against pathogenic microorganisms in the oral cavity. Although the emission range of the LED unit used in this study was well matched with the absorption spectrum of curcumin (430 nm), it provided moderate coverage for the region of absorption of erythrosine (500–550 nm). The use of green light sources that align better with the maximum absorption peak of erythrosine (532 nm) may lead to more prominent effects against *S. mutans*, but this should be evaluated in future studies. The light activation protocol consisted of 2 minutes of irradiation that was divided into 6 periods of 20 seconds each, with dark intervals of 1 minute. This approach was chosen to prevent hyperthermic conditions and allow the recovery of oxygen supply during the dark periods, maintaining the photodynamic reaction.[10]

An ideal PS should be biocompatible with human tissues and possess a high capacity to produce ROS to impact oral microorganisms. Both curcumin and erythrosine are non-toxic materials, approved for use in food products. Erythrosine, additionally, is used as a plaque-disclosing agent in the oral environment, and its therapeutic concentrations are considered safe.[19] It is noteworthy that even daily consumption of curcumin at doses as high as 12 g has shown no clinical side effects.[31] An important concern when using dyes in the oral cavity is the possible staining of tooth surfaces. Both curcumin and erythrosine are easily incorporated with dental pellicles and plaque, whereas the tooth surfaces do not get stained easily.[32] Therefore, the cosmetic appearance of the teeth would not be compromised after PDT with routine concentrations of these PSs.

In the present study, two concentrations of curcumin (1.5 g/L and 3 g/L) and two concentrations of erythrosine (100 µmol/L and 250 µmol/L) were tested in this study. The antimicrobial activity of nano-curcumin was assessed at a concentration of 3 g/L.

By increasing the concentration of curcumin from

1.5 g/L to 3 g/L and erythrosine from 100 µmol/L to 250 µmol/L, the anti-bacterial effects improved to some extent, but the difference between the two concentrations of each PS was not significant. The only exception was the toxic effect of erythrosine without light activation on the biofilm cultures of *S. mutans*, which was significantly greater at the higher concentration. Previous studies demonstrated a greater cell-killing effect after increasing the concentration of PS.[10,33,34] However, the outcomes of this study reveal that PDT with curcumin 1.5 g/L or erythrosine 100 µmol/L is capable of providing optimal antibacterial effects on planktonic and biofilm cultures of *S. mutans*. It is worth mentioning that very high concentrations of PSs may lead to self-shielding, where light absorption mainly occurs within the PS, thus limiting light penetration into the cellular membrane.

The encapsulation of curcumin in polymeric nanoparticles and phospholipids has been suggested as a strategy to enhance its solubility, stability, and biological availability.[7,35] The nanomicelle-curcumin applied in this study contains a hydrophilic part to enhance the solubility and facilitate the transport of curcumin across biological barriers.[18] The core-shell structure of micelles inhibits water penetration into the inner core and provides high stability for curcumin.[18] However, in the present study, the nanomicellar formulation of curcumin was less effective than curcumin dissolved in DMSO in reducing the viability of *S. mutans*. This finding aligns with the results of Santezi et al[7] who evaluated the photodynamic effect of curcumin in three different bioadhesive formulations against the biofilms of various microorganisms, and found that the antimicrobial potential of curcumin in DMSO was substantially higher than in other formulations. They argued that the bioadhesive formulations are more viscous than the liquid formulations, which may cause some difficulty in penetrating the microbial biofilm.[7] Furthermore, the release rate of the drug is limited in bioadhesive formulations, which inevitably decreases the amount of the drug within the cells.[7]

In the planktonic phase, the highest reduction in the number of *S. mutans* colonies was observed in CHX, PDT with curcumin 3 g/L (CUR3 + L), and PDT with erythrosine 250 µmol/L (ER250 + L), which showed 100%, 99.7% and 98.8% reduction in the viability of *S. mutans* relative to the untreated control group, respectively. PDT with curcumin 1.5 g/L and erythrosine 100 µmol/L also proved effective in reducing bacterial counts, with reduction rates of 98.6% and 98.5%, respectively. Interestingly, the use of curcumin or erythrosine alone, without light irradiation, showed satisfactory antibacterial efficacy against *S. mutans*. In contrast, nano-curcumin was significantly less effective than other treatments in inactivating cariogenic bacteria, whether applied with or without light irradiation.

For *S. mutans* biofilms, the maximum antibacterial activity was observed in PDT groups with erythrosine 250 µmol/L (ER250 + L), curcumin 3 g/L (CUR3 + L), and CHX, leading to the cell death rates of 79.4, 76.5% and 71.4%, respectively, compared to the negative control group. PDT with erythrosine 100 µmol/L (ER100 + L) and curcumin 1.5 g/L (CUR1.5 + L) also demonstrated reduction rates of 65.3% and 61.4% in the counting of *S. mutans*, which were statistically comparable to the values obtained with CHX and PDT groups using higher concentrations of these PSs. Nano-curcumin-mediated PDT resulted in lower microbial inactivation (54.9% reduction) than erythrosine-mediated PDT and curcumin-mediated PDT, although the difference was significant only when compared to PDT groups with higher concentrations of curcumin (3 g/L) or erythrosine (250 µmol/L). It is believed that the possible mechanism of action of PDT against gram-positive bacteria like *S. mutans* involves lipid peroxidation and the disruption of cell wall integrity.[11,22]

The outcomes of this study corroborate several reports in the literature that approved the efficacy of PDT in inactivating various microbial species.[4,17,36-40] The bacterial reduction observed in these studies, however, showed a wide range, possibly due to the differences in the type and concentration of PSs, the wavelength and exposure protocol of light sources, and the specific microbial species studied. Araújo et al[13] assessed the effect of PDT with different concentrations of curcumin (0.75, 1.5, 3.0, 4.0, and 5.0 g/L) on dentine carious lesions and recommended the use of a maximum concentration of the drug to gain a significant toxic effect on microorganisms. Merigo et al[37] indicated that the application of the KTP laser (532 nm) with erythrosine dye resulted in complete growth inhibition (100%) of *S. mutans* at different fluence values (10, 20, and 30 J/cm^2). The reduced microbial inactivation in biofilm compared to planktonic cultures has also been exhibited in previous studies[11,41-43] and may be related to the complex physical structure of biofilms, which impedes the penetration and distribution of dyes into the deepest cell layers.[11,41] In contrast to the outcomes of this study, several investigations reported no inactivation of microbial species after isolated PS application.[4,13,17,25,44,45] Araújo et al[13,44,45] reported no significant toxicity on cariogenic microorganisms following the use of different concentrations of curcumin in the dark.

CHX served as the positive control group and demonstrated no bacterial growth in the planktonic form of *S. mutans*, whereas in the biofilm cultures, it was slightly less effective than PDT with curcumin 3 g/L or erythrosine 250 µmol/L. CHX has proven effects on a wide range of gram-positive and gram-negative bacteria and is considered the gold standard for dental plaque control.[27] The prolonged use of CHX, however, leads to cytotoxic effects and discoloration of the tongue and tooth surfaces.[8,9,27,46] It is assumed that the use of CHX to

inactivate the remaining bacteria in deep cavities will be associated with toxic effects on pulpal tissues and should be avoided. Alternatively, PDT with either curcumin or erythrosine can be applied as a safe, non-aggressive, and effective strategy to inactivate cariogenic bacteria in prepared cavities and oral surfaces, while minimizing the chance of raising microbial resistance in the oral cavity.[4,22]

One limitation of this study is its in vitro nature and assessment of a monoculture laboratory strain, which may not fully replicate the complexity of the oral environment. Further studies are suggested to ascertain the clinical efficacy and possible staining of tooth surfaces after PDT with different concentrations of curcumin or erythrosine. Future investigations should also explore the effects of various concentrations and formulations of nano-curcumin and irradiation doses to determine the optimal protocol for killing the multi-species biofilms in the oral cavity.

Conclusion

Under the conditions used in this study, the highest cell killing in both planktonic and biofilm cultures of *S. mutans* was observed in PDT groups with curcumin 3 g/L or erythrosine 250 µmol/L, although PDT with curcumin 1.5 g/L or erythrosine 100 µmol/L showed comparable effectiveness. Therefore, PDT with either curcumin 1.5 g/L or erythrosine 100 µmol/L could be recommended for inactivating *S. mutans* in dental plaque or deep cavities.

Nano-curcumin-mediated PDT was significantly less effective than erythrosine- or curcumin-mediated PDT in both planktonic and biofilm cultures of *S. mutans*.

Increasing the concentration of curcumin from 1.5 g/L to 3 g/L and erythrosine from 100 µmol/L to 250 µmol/L enhanced the percentage of bacterial cell death, but the difference was not significant in most comparisons.

Antimicrobial treatments were more effective in the planktonic form than in the biofilm form of *S. mutans*.

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Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

This research protocol was approved by the ethics committee of Mashhad University of Medical Sciences and coded as IR.MUMS.DENTISTRY.REC.1398.102.

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