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Comparison of the effect of photodynamic therapy with curcumin and methylene Blue on streptococcus mutans bacterial colonies

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ABSTRACT

Background and Aim: Streptococcus mutans (S. mutans) is a bacterium that colonizes in the mouth and is a common cause of dental caries and periodontal diseases. This bacterium comprises 70% of the bacteria in the dental plaque. Although tooth decay is a multifactorial complication, S. mutans biofilms are the main cause of cavitated carious lesions. Considering the importance of this microorganism, we aimed at investigating the effect of photodynamic therapy (PDT) using curcumin (CUR) and methylene blue (MB) photosensitizers on S. mutans.
Materials and Methods: In this in-vitro experimental study, first, samples of S. mutans were prepared in 110 test tubes and were randomly assigned to 11 groups after colony counting: 1) Positive control group, 2) Negative control group, 3) CUR extract group, 4) 460-nm laser group, 5) 460-nm continuous laser + CUR group, 6) 460-nm discontinues 50% duty cycle (DC) laser + CUR group, 7) 660-nm laser group, 8) 660-nm laser + MB group, 9) MB group, 10) dental light-curing group, and 11) chlorhexidine (CHX) group. After the intervention, cultivation was performed again in blood agar medium, and the bacterial colony-forming units per milliliter (CFU/ml) were counted again. Data were analyzed using analysis of variance (ANOVA) and Tukey's test.
Results: CHX and 460-nm low-level continuous laser + CUR had the highest and most significant effect on inhibiting the growth of S. mutans bacterial colonies and showed significant differences with other groups ($P < 0.001$).
Conclusion: According to the results, MB- and CUR-mediated PDT can significantly eradicate S. mutans colonies.

1. Introduction

Streptococcus mutans (S. mutans) is a selective anaerobic gram-positive coccus, comprising the oral cavity flora in humans. This bacterium is the most important cause of tooth decay. S. mutans damages dental enamel through sucrose fermentation and lactic acid production. It also uses sucrose to form dental plaque. Although tooth decay is a multifactorial complication, S. mutans biofilms are its main cause. The accumulation of streptococci on the dental plaque surface creates a solid foundation for the bond of other cariogenic bacteria [1,2]. To reduce the number of Streptococci, various methods, such as tooth brushing and irrigation with mouthwashes, can be used; however, continuous use of mouthwashes can be associated with side effects such as a sense of bitterness in the mouth and staining of dental surfaces. These side effects limit the use of mouthwashes. Chlorhexidine (CHX) is one of the most well-known mouthwashes, which despite having a wide-spectrum antimicrobial effect, it has several side effects, including dysgeusia and discoloration of dental surfaces and restorations [3].

Photodynamic therapy (PDT) is a new therapeutic strategy based on an interaction between a light-sensitive substance or a non-toxic photosensitizer and a harmless light source. The combination of these two factors in the presence of oxygen leads to a specific sequence of biological events through the production of various active oxygen species, resulting in apoptosis and cell death [4]. PDT was first used in 1903 for treatment of tuberculosis, and its role in the treatment of skin cancer was discovered in 1975. Recently, it has been shown that PDT can eradicate bacteria with low-level laser light mediated by coloring agents, such as Methylene blue (MB) and Indocyanine green (ICG) [5].The application of this method has been considerably addressed in dentistry, in the treatment of bacterial and fungal infections as well as oral cancer, and in the diagnosis of malignant transformations in oral lesions. Today's research is mostly being directed towards producing

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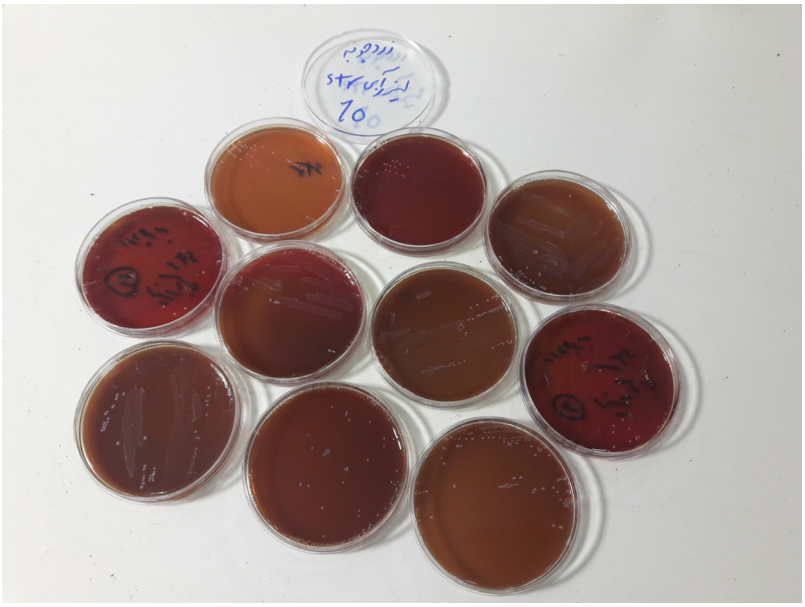

Fig. 1. The group of 460-nm/100-mW continuous laser (60 s) + curcumin (CUR) solution.

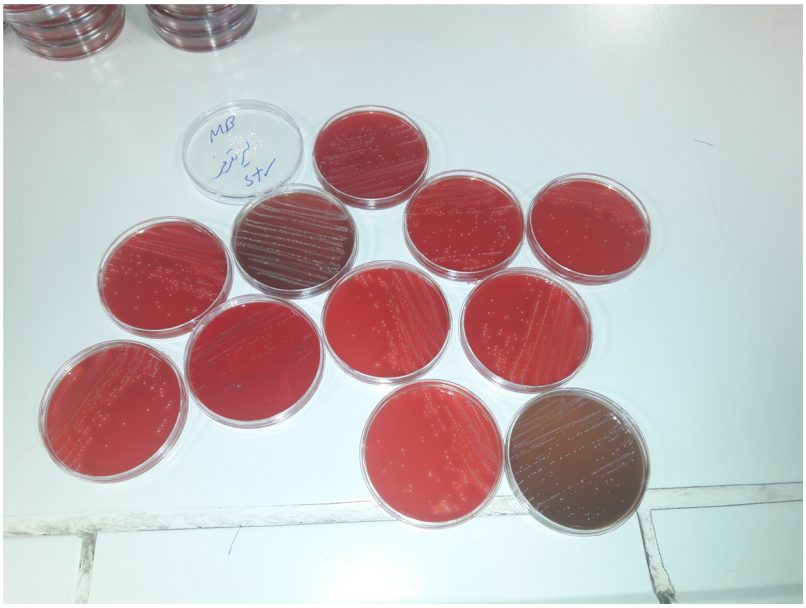

Fig. 2. The group of 660-nm/100-mW laser (100 s) + 0.02% methylene blue (MB).

light-sensitive materials with selective inhibition property to reduce opportunistic infections [6]. Turmeric, with the scientific name of Longa Curcumin, is an herb and spice that is one of the most effective ingredients in preventing carcinogenesis in body cells due to its strong antioxidant properties. Also, the active ingredient of turmeric, i.e. curcumin (CUR), has anti-inflammatory and analgesic properties, similar to conventional analgesics, as well as antibacterial, antiviral, and antifungal activities [7].

The effects of PDT on fungi and other microorganisms have been proven in some studies [8].

Studies by Azizi et al have shown the positive effect of MB- and ICG-mediated PDT with red lasers and an 810-nm laser in reducing Candida albicans (C. albicans), S. mutans, and Lactobacillus colonies [9–12].

Despite the recent arrival of 460-nm blue lasers in the market, their high efficacy in soft tissue surgeries has led to their extensive use. Since CUR extract is the mediating agent in PDT with blue lasers, and to date, the effect of PDT with blue lasers and CUR extract on microorganisms has not been investigated, in this study, it was decided to evaluate the antimicrobial effect of PDT using blue lasers and CUR extract on S. mutans.

2. Materials and methods

In this in-vitro study, first, S. mutans bacterial suspensions (ATCC str.m 1683) were procured from the Iranian Research Organization for Science and Technology (IROST), were cultured on blood agar culture medium, and were incubated for 48 h at 37 °C with 10% CO2. The S. mutans bacteria present in blood agar culture medium were diluted to 0.5 McFarland standard (approximately 1.5 × 10 [8] bacteria/ml), and after ensuring purity, culturing was performed again in blood agar culture medium.

460-nm and 660-nm lasers (Hamerz Co., Tehran, Iran) with a 100-mW power were used in this study. The laser beam was irradiated at a distance of 1 cm from the surface of the suspension in all specimens.

Fig. 3. Irradiation of curcumin (CUR) solution in the culture medium containing *Streptococcus mutans* (S. mutans) by the light of a dental light-curing unit.

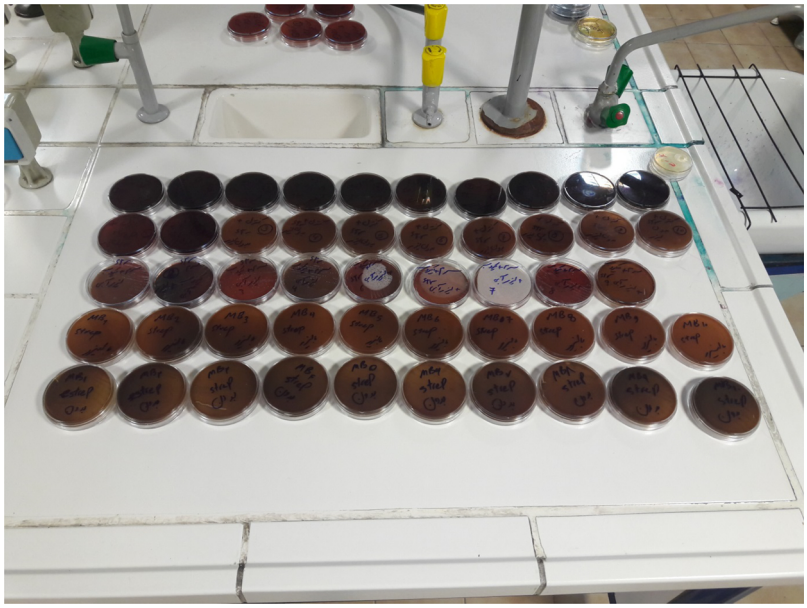

Fig. 4. Plates examined after incubation in order to recount the Streptococcal colonies.

The S. mutans samples were divided into the following groups:

1 A positive control group with 0.5 McFarland turbidity, holding 1.5×10 [8] colony-forming units (CFU), without using any light- or laser-sensitive materials and routine treatment.
2 A negative control group (pure physiologic serum in pure culture medium without the presence of S. mutans bacteria).
3 Use of CUR photosensitizer at the concentration of 10.2 g/100 cc (Adonis Gol-Darou Co., Tehran, Iran) without laser irradiation.
4 Continuous irradiation of the 460-nm/100-mW laser plus CUR solution.
5 Irradiation of the 460-nm/100-mW laser with 50% duty cycle (DC) for 120 s plus CUR solution.
6 Irradiation of the 660-nm/100-mW laser for 100 s plus 0.02% MB (Merck KGaA, Darmstadt, Germany; Fig. 1).
7 Irradiation of the 460-nm/100-mW laser for 60 s.
8 Irradiation of the 660-nm/100-mW laser for 100 s.
9 Use of MB photosensitizer without laser radiation (Fig. 2).
10 Dental lighting-curing (light-emitting diode (LED) light-curing machine, RIXI HK Medical Equipment Industry Co. Ltd., Hong Kong) for 120 s plus CUR solution (Fig. 3).
11 Addition of 0.1 ml of 0.2% CHX (Shafa Pharmaceuticals, Tehran, Iran) to the bacterial suspension.

Ten samples from each therapeutic group were examined (a total of 220 specimens). CUR extract, which is sensitive to blue light and is activated at a 460-nm wavelength, was used in the present study at the concentration of 10.2%. Also, MB at the concentration of 0.02% was used, which is a photosensitizer that is activated at a wavelength of 660 nm.

The bacterial colonies were kept at a temperature of −70 °C before testing and were then cultured in blood agar culture medium for 48 h at 37 °C.

After the intervention, the samples were again cultured in blood

Table 1
The number of *Streptococcus mutans* (S. mutans) samples in each studied group.

NEGATIVE CONTROL	CHX	LIGHT-CURE	POSITIVECONTROL	CUR	MB	LASER 660	Laser460	LASER660 + MB	LASER460DC(50%) + CUR	LASER460 + CUR	GroupSample
0	0	250000	231000	105000	104000	11000	104000	68000	51000	22000	1
0	0	270000	250000	94000	100000	88000	75000	70,000	73000	14,000	2
0	0	240000	180000	110000	88000	70,000	75000	54,000	34000	19000	3
0	0	268000	175000	125000	97000	94000	92000	80000	57000	40000	4
0	0	225,000	162000	96000	110000	81,000	55000	95000	38000	37000	5
0	0	248000	212000	103000	85000	110000	100000	93000	30,000	51000	6
0	0	228000	235000	90000	115000	95000	65000	76000	42000	18000	7
0	0	239000	222000	81,000	91,000	96000	78000	80000	45,000	30,000	8
0	0	242000	198000	110000	104000	86000	70,000	77000	46000	27000	9
0	0	253000	138,000	117000	105000	80000	73000	65000	40000	35000	10

CUR = curcumin, MB = methylene blue, DC = duty cycle.

agar culture medium, were incubated at 37 °C for 48 h, and the streptococcal colonies per milliliter (CFU/ml) were counted and recorded (Fig. 4).

The numbers of streptococcal colonies in different samples after the intervention were analyzed using analysis of variance (ANOVA) and SPSS 18 software (SPSS Inc., Chicago, IL, USA). Tukey's post-hoc test was used for pairwise comparisons of the groups.

3. Results

In this study, we investigated the effects of the application of 460-nm and 660-nm lasers with a power of 100 mW mediated by MB and CUR laser-sensitive agents. The separate effect of MB and CUR photosensitizers, without laser irradiation, was also investigated.

In the present study, two types of investigations were carried out in relation to the 460-nm low-level laser such that in one group, the irradiation was continuous, whereas in the other group, the irradiation was discontinuous and with 50% DC. In the continuous 460-nm laser + CUR group, the colonies were significantly destroyed.

In Table 1, the overlapping of the groups is reported by P-value in four subgroups. According to this table, 460-nm low-level laser groups (both continuous irradiation and pulsed irradiation) exhibited a significant difference with the other studied groups ($P < 0.0001$), indicating no overlapping or similar performance. According to Table 1, in the mean P-values of 0.067 and 0.076, the overlapping of the three groups of 460-nm laser, 660-nm laser, and MB is evident, which shows that these groups are not significantly different ($P > 0.05$); however, each of these groups has a significant difference with the control group ($P < 0.0001$) (Tables 2 and 3).

Also, the antimicrobial property of MB and CUR photosensitizers was proven in the present research. Both groups had a significant difference with the control group ($P < 0.05$) but there was no significant difference between the two groups in pairwise comparisons and they showed relatively similar performance ($P > 0.05$).

According to the below tables, 460-nm and 660-nm laser irradiation in combination with photosensitizers led to a significant decrease of bacterial colonies such that the continuous 460-nm laser + CUR group exhibited the greatest antimicrobial effect in comparison with the positive control group ($P < 0.0001$), whereas the dental light-curing group exhibited the least antimicrobial efficacy ($P = 1.000$).

After the intervention, the numbers of CFUs of Streptococci in the studied groups were as follows:

1 CHX = > zero CFU.
2 The 460-nm/100-mW laser (60 s) plus CUR solution = > 29,300 CFUs.
3 The 460-nm/100-mW laser with 50% DC and irradiation time of 120 s plus CUR solution resulted in 45,000 CFUs.
4 The 660-nm/100-mW laser (60 s) plus MB = > 75,800 CFUs.
5 The 460-nm/100-mW laser (60 s) = > 78,700 CFUs.
6 The 660-nm/100-mW laser (100 s) = > 91,000 CFUs.
7 MB = > 99,900 CFUs.
8 CUR = > 103,100 CFUs.
9 Positive control = > 246,300 CFUs.
10 Dental light-curing (120 s) plus CUR = > 200,300 CFUs.

4. Discussion

In this in-vitro study, the susceptibility of S. mutans to PDT was investigated using CUR and MB photosensitizers and a diode laser.

In several studies, PDT has been introduced as a method to eliminate various microorganisms, including S. mutans, and it can be used as a substitute for CHX mouthwash.

In 2016, Azizi et al investigated the antimicrobial effect of PDT mediated with ICG and MB photosensitizers on S. mutans [10]. The results of the study showed that ICG- and MB-mediated PDTs are able to

Table 2
Central dispersion indexes of the number of *Streptococcus mutans* (S. mutans) colonies in different groups.

Group	Minimum	Maximum	Mean	Std. Deviation
LASER460 + CUR	14,000.00	51000.00	29,300.0000	11566.71470
LASER460DC(50%) + CUR	30,000.00	73000.00	45600.0000	12429.35593
LASER660 + MB	54,000.00	95000.00	75,800.0000	12416.83445
LASER460	55000.00	104000.00	78,700.0000	15477.94129
MB	70,000.00	110000.00	91,000.0000	12771.49604
LASER660	85000.00	115000.00	99,900.0000	9666.09194
CUR	81,000.00	125000.00	103,100.0000	13186.27232
LIGHT-CURE	138,000.00	250000.00	200,300.0000	35947.64712
POSITIVE CONTROL	225,000.00	270000.00	246,300.0000	14885.11561

CUR = curcumin, MB = methylene blue, DC = duty cycle.

eliminate all S. mutans colonies, which is in line with the results of the present research.

In 2016, Azizi et al examined the antibacterial effect of ICG- and MB-mediated PDT on *Lactobacillus acidophilus* (L. acidophilus) in laboratory conditions [11]. The results of the mentioned study indicated that the use of MB and red laser significantly inhibited Lactobacillus growth compared to the control group, which is consistent with the results of the present study.

In 2014, Hakimiha and colleagues examined the sensitivity of S. mutans to PDT with Toluidine blue (TB) and Radachlorin photosensitizers at proper wavelengths of a diode laser [13]. The cited study demonstrated that PDT with these two photosensitizers significantly

decreased S. mutans colonies, and there was no significant difference between the two groups [13]; these results are in agreement with that of the present study.

In 2013, Fekrazad et al confirmed the specific effects of PDT using ICG, TB, and Radachlorin photosensitizers against S. mutans [14]. There was no significant difference between the three groups in reducing the number of colonies. Also, the study showed that ICG in combination with 810-nm laser irradiation has efficient bactericidal effects against S. mutans [14], which is in line with the results of our research.

In 2012, Rolim et al examined the exclusive effects of PDT with different laser wavelengths and MB, TB, malachite green (MG), erythrosine, eosin, and rose bengal (RB) photosensitizers [15]. In the cited study, it was reported that 660-nm laser radiation plus MB has no significant bactericidal effect compared to the control group, which is not consistent with our research results.

Research has shown that the bactericidal effect of CUR solution increases in combination with light in the blue spectrum. Depending on the amount of blue light penetration into superficial tissues, CUR solution can be used to treat superficial local infections. CUR or turmeric extract has extensive medicinal effects. CUR is the active ingredient in turmeric extract, which has been reported to have a variety of activities including antibacterial, anti-inflammatory, antioxidative, and wound healing effects. CUR is an anionic photosensitizer that binds to a positively charged bacterial cell membrane and destroys the membrane. In addition, CUR combined with laser radiation removes bacteria through the photothermal effect [16].

In 2017, Lee et al investigated the effect of CUR and ginger on S. mutans strains and concluded that S. mutans showed a significant reduction in the presence of CUR and 405-nm light radiation [17], which is agreement with the results of the present study.

In 2015, Tyagi et al assessed the antibacterial effect of CUR on four types of bacteria [18]. The results of the study indicated that CUR exhibited a strong bactericidal effect against all microorganisms, and the bactericidal effect of CUR increased with increasing the dosage and the duration of use [17]. Also, Rudrappa and Bais (2008) examined the effect of CUR on *Pseudomonas aeruginosa* genes and reported that CUR reduces pathogenicity and controls viral agents in biofilms [19].

MB is an alkaline photosensitizer and is a formal derivative of phenothiazine. It is a dark green powder that yields a blue solution in water that passes through the bacterial cell membrane and affects the bacterial genome, causing bacteria to eradicate. Also, MB in combination with laser irradiation produces free oxygen that eliminates bacteria. [9].Curcumin is a diarylheptanoid,belonging to the group of curcuminoids,which are natural phenols responsible for turmeric 's yellow color. [20].

It should be noted that when a photosensitizer is stimulated by its optimal wavelength, it transforms from the low-energy state to a highly energized, high half-life triple state, resulting in a reaction between the photosensitizer, environmental molecules, and intratissue oxygen, which leads to the production of singlet oxygen and other free radicals that cause tissue damage [21].

Overall, the bactericidal effect of PDT can be explained by two mechanisms:

1 Damage to DNA
2 Damage to the cytoplasmic membrane of the bacterium through cytotoxic agents produced by PDT, which inactivate the membrane's transfer system and enzymes. As a result, membrane permeability increases [5,6].

The advantages of PDT include:

3 This method is non-invasive and does not damage the surrounding tissues.
4 There is low resistance against PDT.
5 There is no need for antibiotics or local anesthetic administration.
6 The bacteria are destroyed in a short time.

Moreover, PDT mediated with photosensitizers exhibits its effects by altering the activity of ion channels and superficial receptors. Studies have shown that PDT inhibits the dihydrofolate reductase enzyme. Bacteria synthesize purines and pyrimidines using this enzyme, which thicken their cell wall; therefore, the inhibition of this enzyme causes bacterial cell death. The exudation of these types of ions results in the disruption of vital processes in the cell, leading to the excretion of main cellular elements, water imbalance, membrane potential damage, prevention of adenosine triphosphate (ATP) synthesis, and finally, cell death [22].

Table 3
Results of pairwise comparisons of changes in the number of colony-forming units (CFU) of *Streptococcus mutans* (S. mutans).

Group (I)		Mean Difference	Standard Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
LASER460 + CUR	LASER460DC(50%) + CUR	−.21035*	.04015	.000	−.3383	−.0824
	LASER660 + MB	−.43896*	.04015	.000	−.5669	−.3110
	LASER460	−.45318*	.04015	.000	−.5811	−.3252
	MB	−.51986*	.04015	.000	−.6478	−.3919
	LASER660	−.56241*	.04015	.000	−.6904	−.4345
	CUR	−.57471*	.04015	.000	−.7027	−.4468
	LIGHT-CURE	−.85967*	.04015	.000	−.9876	−.7317
	POSITIVE CONTROL	−.95546*	.04015	.000	−1.0834	−.8275
LASER460DC(50%) + CUR	LASER460 + CUR	.21035*	.04015	.000	.0824	.3383
	LASER660 + MB	−.22862*	.04015	.000	−.3566	−.1007
	LASER460	−.24283*	.04015	.000	−.3708	−.1149
	MB	−.30951*	.04015	.000	−.4375	−.1816
	LASER660	−.35206*	.04015	.000	−.4800	−.2241
	CUR	−.36436*	.04015	.000	−.4923	−.2364
	LIGHT-CURE	−.64932*	.04015	.000	−.7773	−.5214
	POSITIVE CONTROL	−.74511*	.04015	.000	−.8731	−.6172
LASER660 + MB	LASER460 + CUR	.43896*	.04015	.000	.3110	.5669
	LASER460DC(50%) + CUR	.22862*	.04015	.000	.1007	.3566
	LASER460	−.01421	.04015	1.000	−.1422	.1137
	MB	−.08090	.04015	.538	−.2088	.0471
	LASER660	−.12345	.04015	.067	−.2514	.0045
	CUR	−.13574*	.04015	.029	−.2637	−.0078
	LIGHT-CURE	−.42071*	.04015	.000	−.5487	−.2928
	POSITIVE CONTROL	−.51649*	.04015	.000	−.6444	−.3885
LASER460	LASER460 + CUR	.45318*	.04015	.000	.3252	.5811
	LASER460DC(50%) + CUR	.24283*	.04015	.000	.1149	.3708
	LASER660 + MB	.01421	.04015	1.000	−.1137	.1422
	MB	−.06668	.04015	.768	−.1946	.0613
	LASER660	−.10923	.04015	.157	−.2372	.0187
	CUR	−.12153	.04015	.076	−.2495	.0064
	LIGHT-CURE	−.40649*	.04015	.000	−.5344	−.2785
	POSITIVE CONTROL	−.50228*	.04015	.000	−.6302	−.3743
MB	LASER460 + CUR	.51986*	.04015	.000	.3919	.6478
	LASER460DC(50%) + CUR	.30951*	.04015	.000	.1816	.4375
	LASER660 + MB	.08090	.04015	.538	−.0471	.2088
	LASER460	.06668	.04015	.768	−.0613	.1946
	LASER660	−.04255	.04015	.978	−.1705	.0854
	CUR	−.05485	.04015	.907	−.1828	.0731
	LIGHT-CURE	−.33981*	.04015	.000	−.4678	−.2119
	POSITIVE CONTROL	−.43560*	.04015	.000	−.5635	−.3076
LASER660	LASER460 + CUR	.56241*	.04015	.000	.4345	.6904
	LASER460DC(50%) + CUR	.35206*	.04015	.000	.2241	.4800
	LASER660 + MB	.12345	.04015	.067	−.0045	.2514
	LASER460	.10923	.04015	.157	−.0187	.2372
	MB	.04255	.04015	.978	−.0854	.1705
	CUR	−.01230	.04015	1.000	−.1402	.1157
	LIGHT-CURE	−.29726*	.04015	.000	−.4252	−.1693
	POSITIVE CONTROL	−.39305*	.04015	.000	−.5210	−.2651
CUR	LASER460 + CUR	.57471*	.04015	.000	.4468	.7027
	LASER460DC(50%) + CUR	.36436*	.04015	.000	.2364	.4923
	LASER660 + MB	.13574*	.04015	.029	.0078	.2637
	LASER460	.12153	.04015	.076	−.0064	.2495
	MB	.05485	.04015	.907	−.0731	.1828
	LASER660	.01230	.04015	1.000	−.1157	.1402
	LIGHT-CURE	−.28496*	.04015	.000	−.4129	−.1570
	POSITIVE CONTROL	−.38075*	.04015	.000	−.5087	−.2528
LIGHT-CURE	LASER460 + CUR	.85967*	.04015	.000	.7317	.9876
	LASER460DC(50%) + CUR	.64932*	.04015	.000	.5214	.7773
	LASER660 + MB	.42071*	.04015	.000	.2928	.5487
	LASER460	.40649*	.04015	.000	.2785	.5344
	MB	.33981*	.04015	.000	.2119	.4678
	LASER660	.29726*	.04015	.000	.1693	.4252
	CUR	.28496*	.04015	.000	.1570	.4129
	POSITIVE CONTROL	−.09579	.04015	.306	−.2237	.0322

(*continued on next page*)

Table 3 (*continued*)

Group (I)		Mean Difference	Standard Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
POSITIVE CONTROL	LASER460 + CUR	.95546*	.04015	.000	.8275	1.0834
	LASER460DC(50%) + CUR	.74511*	.04015	.000	.6172	.8731
	LASER660 + MB	.51649*	.04015	.000	.3885	.6444
	LASER460	.50228*	.04015	.000	.3743	.6302
	MB	.43560*	.04015	.000	.3076	.5635
	LASER660	.39305*	.04015	.000	.2651	.5210
	CUR	.38075*	.04015	.000	.2528	.5087
	LIGHT-CURE	.09579	.04015	.306	−.0322	.2237

CUR = curcumin, MB = methylene blue, DC = duty cycle.
* The mean difference is significant at the 0.05 level.

Researchers have argued that low-level 660-nm laser irradiation plus TB or MB would eliminate bacterial cell membrane proteoglycans. MB is a reductase that is activated by laser irradiation (depending on the intensity of the laser), and it inhibits the development of influenza by degradation of the membrane proteoglycans. Also, the photoactive compounds inhibit bacterial growth through their main antimicrobial activity i.e. denaturation and coagulation of proteins inside the bacterial cell wall [22].

Low-level 445-460-nm blue lasers have special applications in dentistry such as use in soft tissue surgeries and hemostasis as well as periodontics and endodontics. The extremely high antibacterial effects of these lasers have been specially considered in dentistry. Blue lasers have short wavelengths and much higher energy than red lasers; this extremely high energy efficiently eliminates pathogenic bacteria in the mouth. These lasers are widely used in soft tissue surgeries [23].

S. mutans was evaluated in this study and some other studies because it is the main cariogenic bacterium [10]. In addition, CHX was selected for comparison of the efficacy of the treatment due to its proven bactericidal effects and the prevalence of its use for reduction of oral microbial load. To reduce S. mutans in high risk individuals with a high rate of caries, CHX mouthwash is used once a day for two weeks; however, its long-term use can lead to some complications such as changes in the taste buds, oral metallic taste, and discoloration of teeth and tooth-colored restorations, which lead to patient dissatisfaction.

Therefore, achieving a modality with suitable efficacy against S. mutans but without these complications is very important, for which purpose, the effects of MB- and CUR-mediated PDT plus a diode laser were evaluated in the present study. Nevertheless, this research was performed in-vitro, and it should be noted that there are significant differences between clinical and laboratory conditions.

5. Conclusion

The results of the present research indicated that the use of lasers plus MB and CUR photosensitizers decreases S. mutans bacterial colonies; however, CHX still remains the golden standard of antibacterial therapy in oral health, despite the formation of stains on dental surfaces.

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