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Photosensitizing effects of *In vitro* crystal violet on photodynamic therapy for *Candida albicans*

Efeito fotossenssibilizador *in vitro* da violeta de genciana na terapia fotodinâmica sobre *Candida albicans*

Efecto fotosensibilizador *in vitro* de la violeta de genciana en la terapia fotodinámica sobre *Candida albicans*

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This study aimed at evaluating the action of the in vitro dye crystal violet on Candida albicans, isolated or as a photosensitizing tool, when compared to methylene blue in photodynamic therapy. It was an experimental and quantitative research. The ATCC 1106 Candida albicans strain was inoculated with an overnight103 UFC/ml. 50µl of the fungal suspension (overnight) were added to 18,0ml of Saboraud agar. To this, 0.2ml of the crystal violet dye was added at 1% and plated. A five-minute period of pre-irradiation was conceded to the sample, after which the red laser was applied. The dosage applied was that of 100J/cm^2, with a total energy of 3J. After that, the samples were subcultured in Saboraud agar at 37ºC, for 1 hour. After this period, an aliquot of 1.0 ml was diluted in 9.0 ml of 0.85% sterilized saline solution. 0.1 ml of the diluted result was plated and cultured in saboraud agar. Three plates were incubated in a growth chamber for micro-organisms at 37ºC, and after 48h, the UFCs/ml were counted. In addition to the 1% crystal violet with the laser, the following groups were analyzed: 1% methylene blue with laser, 1% methylene blue, 1% crystal violet, and the use of the laser with no dying. The analyses were conducted in the IBM SPSS (21.0) software. The Kruskal-Wallis (Mann-Whitney U test) was used as well as the Friedman (Wilcoxon) text, with a significance level α=5%. As a result, the 1% crystal violet in conjunction with the laser has an important effect against Candida albicans in the photodynamic therapy.
Descriptors: Photochemotherapy; Candida albicans; Laser therapy; Gentian violet; Methylene blue.

Este estudo tem como objetivo avaliar a ação do corante violeta de genciana, *in vitro*, sobre *Candida albicans*, isolado ou como fotossensibilizante, em comparação ao azul de metileno na Terapia Fotodinâmica. Foi uma pesquisa experimental com abordagem quantitativa. A linhagem de *Candida albicans* ATCC 1106 foi inoculada obtendo-se um *overnight* de 1,37x10^4 UFC/ml. Foram adicionados 50µl da suspensão fúngica (*overnight)* em 18,0ml de caldo Saboraud. A esse conjunto foi adicionado o 0,2ml do corante da violeta genciana a 1%, foi plaqueado, esperou-se o tempo de pré-irradiação de 5 minutos e aplicou-se o laser vermelho. A dose aplicada foi de 100J/cm^2, com energia total de 3J, depois foram subcultivadas a 37°C em caldo Saboraud por 1 hora. Após este período, uma alíquota de 1,0 ml foi diluída em 9,0ml de solução salina 0,85% esterilizada. 0,1 ml dessa diluição foi plaqueada e semeada em ágar saboraud. As placas, em triplicata, foram incubadas em estufa para microrganismos a 37°C, e após 48h foi feita a contagem das UFCs/ml. Além da violeta genciana a 1% combinada ao laser, foram analisados os grupos: azul de metileno a 1% associado ao laser, azul de metileno a 1%, violeta genciana a 1%, e o laser sem adição de corantes. As análises foram realizadas no software IBM SPSS (21.0), utilizou-se o teste de Kruskal-Wallis (Mann-Whitney) e o teste de Friedman (Wilcoxon), adotando-se um nível de significância de α=5%. Como resultado observou-se que a violeta genciana a 1% associada ao laser tem efeito importante contra *Candida albicans* na terapia fotodinâmica.
Descritores: Fotoquimioterapia; Candida albicans; Terapia a laser; Violeta de genciana; Azul de metileno.

Este estudio tiene como objetivo evaluar la acción del colorante violeta de genciana, *in vitro*, sobre *Candida albicans*, aislado o como fotosensibilizante, en comparación al azul de metileno en la Terapia Fotodinámica. Fue una investigación experimental con abordaje cuantitativo. El linaje de *Candida albicans* ATCC 1106 fue inoculado obteniéndose un *overnight* de 1,37x10^4 UFC/ml. Fueron agregados 50µl de la suspensión fúngica (*overnight*) en 18,0ml de caldo Saboraud. A este conjunto fue agregado el 0,2ml del colorante de la violeta genciana a 1%, fue plaqueado, se esperó el tiempo de pre-irradiación de 5 minutos y se aplicó el láser rojo. La dosis aplicada fue de 100J/cm^2, con energía total de 3J, después fueron subcultivadas a 37°C en caldo Saboraud por 1 hora. Después de este período, una alícuota de 1,0ml fue diluida en 9,0ml de solución salina 0,85% esterilizada. 0,1ml de esta dilución fue plaqueada y plantada en agar saboraud. Las placas, en triplicata, fueron incubadas en estufa para microorganismos a 37ºC, y después de 48h fue hecho el conteo de las UFCs/ml. Además de la violeta genciana a 1% combinada a láser, fueron analizados los grupos: azul de metileno a 1% asociado al láser, azul de metileno a 1%, violeta genciana a 1%, y el láser sin adición de colorantes. Los análisis fueron realizados en el software IBM SPSS (21.0), se utilizó el test de Kruskal-Wallis (Mann-Whitney) y el test de Friedman (Wilcoxon), adoptándose un nivel de significancia de α=5%. Como resultado se observó que la violeta genciana a 1% asociada al láser tiene efecto importante contra *Candida albicans* en la terapia fotodinámica.
Descriptores: Fotoquimioterapia; Photochemotherapy; Candida albicans; Terapia a láser; Violeta de genciana; Azul de metileno.

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INTRODUCTION

The incidence of superficial and profound fungal infections has meaningfully grown throughout the last 20 years.

Many reasons are offered to explain this increase, including the use of anti-neoplastic and immunosuppressant drugs, broad spectrum antibiotics, prosthesis, graft and aggressive surgeries[1]. With the development of medicine, in the field of surgery and transplantology, the number of individuals who are immunocompromised and, therefore, are more susceptible to these infections, has been on the rise[2]

Invasive mycoses represent a growing threat to human health due to a possible combination of slow diagnoses and the existence of few available and effective anti-fungal drugs[3]. This combination may lead to more severe systemic or local infections, broader and difficult to treat when the fungus is present[4,5].

Candida albicans (CA), one of the most frequent opportunist micro-organisms in oral microbiota, is the most prevalent species that is responsible for infections in the mucosa and in the skin of patients with compromised immunity, with special importance for human health[6-8], since it causes superficial mycoses and leads to disseminated systemic disease[9].

As a result of the high rate of mortality due to invasive Candida infections, the limited availability of effective anti-fungal agents, and the increase in the resistance to the commercially available drugs, researches are being conducted to find alternative treatments[10,11]. That is why studies are being conducted in the field of photodynamic therapy (PDT), which has been showing bactericidal and fungicidal effects in oral micro-organisms, through low intensity laser therapies (LILT)[11-13].

The PDT was developed to deal with malignant lesions, but it has successfully been used in the treatment of fungal infections, and successfully employed against Candida albicans and other types of Candida[7], diminishing the chances that the CA will cause a systemic infection[14] and showing itself to be an effective option that, since a 1900 research by Raab, who proved that the dye acridine acted against paramecia when combined with the light of lightning bolts, has been scientifically substantiated well.

This therapy is based on the concept that non-toxic dye, known as photosensitizing dye (PSD), is located, preferably, in certain tissues or cells, and subsequently is activated by visible light, producing reactive oxygen species (EROS), which can kill the cells that link to the PSD[15-17].

The multiplicity of targets in the cells (mitochondria, lysosomes and nuclei) of fungi diminishes the risk of resistant or photo-mutant strains and this risk is minimized with the absence of mutagenic effects of PDT[18], which can be repeated many times, apparently without leading to resistance, since DNA is not the main target of the EROS[19].

The risk of damage to the fungi DNA is smaller due to the presence of a membrane that involves the nucleus, acting as a barrier to the penetration of dyes or its high-energy subproducts.

Different types of PSD are proposed in the PDT. The interaction between PSDs, cellular membrane and intracellular structures is very relevant for PDT. Due to the high diversity of micro-organisms, a PSD with distinct physical-chemical properties may be necessary[21].

The crystal violet (CV) is a mixture of triarylmethane dye, used to dye hair, paper or textiles, and is used in micro-biology labs. Derived from coal tar, it has been widely used as an antiseptic product. Its anti-microbial activity is recognized and recommended for candidiasis[22]. The CV mechanism is not related to a primary cytoplasmic membrane lesion. It is probably related to the inhibition of a metabolic pathway[23].

The World Health Organization recommends topic application of 1% VC for the initial treatment of oral candidiasis in patients infected by HIV when resources are limited, since this is a low cost and easy to undergo treatment[24].

However, due to its properties of dying the oral mucosa, the CV is not currently being used to treat CA. A study to evaluate the security and efficiency of CV in different

concentrations showed that this PSD, in concentrations of 0.000165%, does not stain the oral cavity, is stable, well tolerated, and has a potent anti-Candida acitivity[25]. In another research[23], it was found that the CV presents a fungicidal activity for most Candida species, the most susceptible of which are the albicans and C. tropicalies species.

The CV has potential to treat oral candidiasis due to its anti-biolfim and anti-germination activity. The CV mechanism is not related to a primary cytoplasmic membrane lesion. It is probably related to the inhibition of cellular metabolism[23].

It is possible that the production of hydroxyl/peroxide makes the penetration of CV through the matrix easier, leading to an inhibition of the cellular wall of fungi. Clinical studies to determine the efficiency of CV in the treatment of this disease are guaranteed[26].

One difficulty, in PDT, is the administration of the PSDs, which stains the teeth and oral mucosa[10]. However, some in vitro studies have shown that the oral cavity is especially adequate for PDT, since it is relatively sensitive to the application of the light[27,28]. Not to mention, the PSD is easy to manipulate[7].

Knowing the positive results of the actions of CV as an antifungal over CA, this work aims to evaluate the action of the dye crystal violet, in vitro, on Candida albicans, isolated or as a photosensitizer, when compared with the methylene blue in Photodynamic therapy.

METHOD

An in vitro experimental study was conducted, in the Genetics and Micro-organism Lab of the Biology Department from the Center of Exact and Nature Sciences of the Federal University of Paraiba (UFPB), in the city of João Pessoa, from December 2014 to January 2015.

The ATCC 1106 strain of CA was used (international standard), pertaining to the collection of micro-organisms of the Micology Lab of the Pharmaceutic Sciences Department, in the Health Sciences Center at UFPB.

This study was based on the method proposed by Craig; Gudmunson (1991), and modified by Pereira t. al, (2014) [15]. For fungal determination, the ATCC 1106 Candida albicans strain was inoculated in Saboraud agar and incubated under 37ºC for 18-20 hours, with an overnight of 1.37x103 UFC/ml.

50µl of the fungal suspension (overnight) were added to 18,0ml of Saboraud agar; 0.2 ml of the 1% MB dye or of the 1% CV dye were added to the result. After that, the group was lead to the vortex, and then, plated. The pre-irradiation period (PIP) was of five minutes, after which the laser was applied. The dosage applied was that of 100J/cm^2, with a total energy of 3J, with a 100mW potency, from a distance of 1.0cm per point through the entire extension of the petri dish, with the point of the laser 1.0 cm distant from the plate. After that, the samples were subcultured in Saboraud agar at 37ºC, for 1 hour.

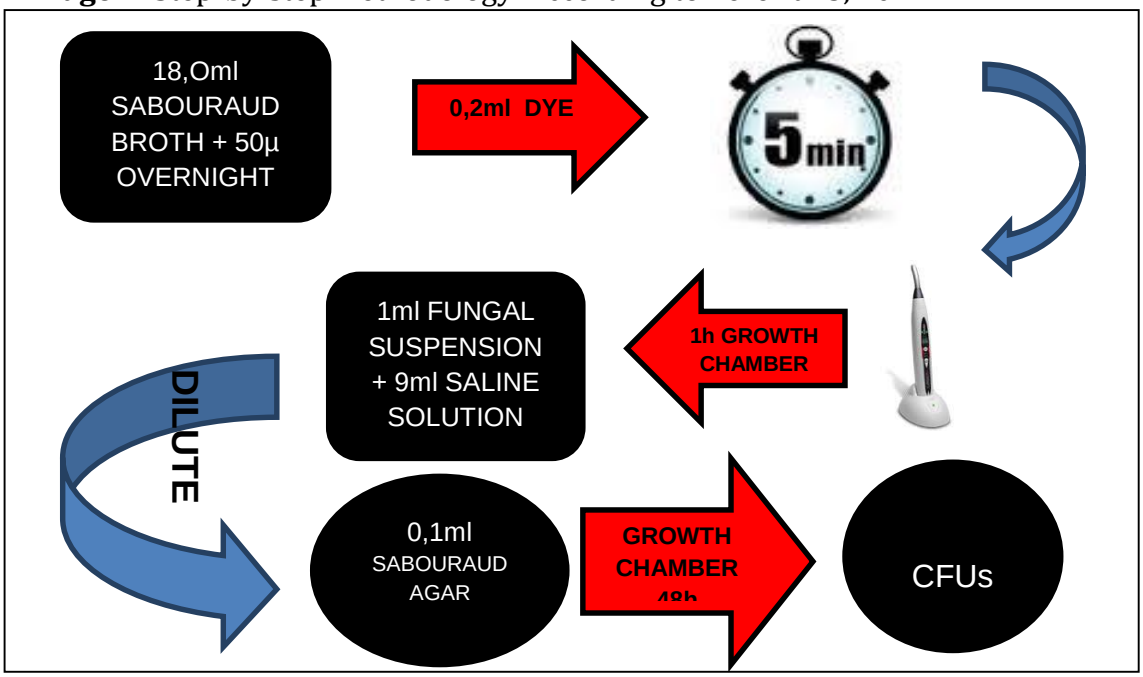
After this period, an aliquot of 1.0 ml was diluted in 9.0 ml of 0.85% sterilized saline solution and taken to the vortex. Then, 0.1 ml of the subculture was plated and cultured in saboraud agar.

The plates were incubated in a growth chamber for the growth of micro-organisms at 37ºC. The number of viable cells, in this tube, was determined and designated as time 0, so that anti-microbial effect could be later determined. The regrowth of the culture was monitored for a period of 0, 24 and 48 hours, through the standard plate counting method. The reading of the plates was conducted after a 48-hour incubation at 37ºC.

There were triplicate experiments. The results of the count of viable cells (UFC/ml) in the culture are described in Table 1.

Sequencing was respected in the manipulation and determination of the fungicidal effect and in the counting of the UFCs - except the PIP, which did not require that, for the dye groups 1% MB and 1% CV without using the laser, for the control group, and for the group of lasers with no PSD.

Image 1. Step-by-step methodology. According to Pereira15, 2014.



A red semi-conductor laser was used (GaA1As e InGaAlP), a device from DUO MMOPTICS São Carlos, SP, Brazil, with a wavelength (λ) of 660 nm. The potency of the device was 100wM, the dosage applied was that of 100J/cm^2, with a total energy of 3J, with a 100mW potency, from a distance of 1.0cm per point through the entire extension of the petri dish, with the point of the laser 1.0 cm distant from the plate. The laser was continuously emitted, in the punctual operation setting.

These specifications were suggested by the producer of the device in case of photodynamic therapy with no optic fiber. The petri dishes selected to be used with the PSD + laser waited the pre-irradiation time of 5 minutes.

The Shapiro-Wilk normality test was conducted to check the distribution of data, and it was found that, for all groups, the distribution according to the UFC count was not normal (P<0.05). In the comparison of UFC means between groups, in the same periods of time, the Kruskal-Wallis test was conducted, and in the comparison between the groups, with two at a time, he Mann-Whitney U test was conducted.

To compare the UFC count within groups, in the different periods of time he Friedman's test was used. The differences were identified with the Wilcoxon text. All analysis adopted a significance level of □=5%. The analyses were conducted with the statistical software IBM SPSS (21.0).

RESULTS

In this research, when the 1% CV was used jointly with the laser, the PDT was conducted, and it was found that in the 0h there was a diminution in the number of UFCs, but at 24h and 48h no colonies were formed, which can be seen in Table 1.

When only the 1% CV was researched, it was found that, at 0h, UFCs diminished, and at 24h and 48h no colonies were formed.

When the 1% MB was used with the laser to conduct the PDT, it was found that at 0h, there was a reduction in UFCs, and at 24h and 48h, no UFCs were developed (Table 1).

In the isolated 1% MB group, there was a gradual increase in the formation of UFCs at 0h, 24h and 48h (Table 1).

In the group where only the laser was used, there was a diminution in the UFCs at 0h, 24h and 48h. These colonies were not generated (Table 1).

Table 1. Mean values for the Colony Forming Units (UFC) obtained for the control group and for the groups treated with laser, violet + laser, methylene blue, and laser + methylene blue, in the 0h, 24h and 48h marks. João Pessoa, PB. 2015.

	Group					
Time	**Control**	**Laser**	**Violet**	**Laser + Violet**	**Methylene blue**	**Laser + Methylene blue**
0h	$3,43 \times 10^3$ A a	$6,10 \times 10^2$ A b	$2,04 \times 10^3$ A c	$1,95 \times 10^3$ A c	$4,9 \times 10^3$ A d	$1,73 \times 10^3$ A c
24h	$1,53 \times 10^6$ B a	0 B b	0 B b	0 B b	$1,64 \times 10^6$ B a	0 B b
48h	$2,45 \times 10^8$ C a	0 B b	0 B b	0 B b	$1,37 \times 10^7$ C c	0 B b

Values in UFC/ml. The same capital letters in columns and the same low-case letters in lines indicate that there is no statistically significant differences (p> 0.05) in the groups. Kruskal-Wallis test. Friedman test.

DISCUSSION

The micro-organism resistances to the medications used to treat infections[10,11,28,21] cause serious damages in immunocompromised and weakened patients[3,6,29,30], possibly worsening blood infections[31] and increasing morbidity and mortality. Studies seeking a type of therapy that leads to cure without causing resistance are increasingly frequent, and thus, the PDT was created.

As it reduces Candida albicans's ability to cause a systemic infection[14,24], the PDT has been considered to be a promising alternative treatment for targeted infections[7,11,28].

It is common for prosthesis users to have candidiasis, an opportunistic and multifactorial infection, resulting from the pathogenic action of the CA fungus, and considered the most prevalent disease in the oral mucosa [3,29,32,33]. Despite guidance regarding hygiene and the recommendation of antifungal medication, this infection takes place very frequently[34].

CV is a traditional fungicide used for the treatment of candidiasis[22,35]. Its use is very common in children and elders, not to mention patients with HIV.

When associating, in this research, the 1% CV with the red laser (660nm), the number of colonies formed, in the first hours, was found to decrease, and, as time went by, no UFCs were verified. A study[36] of in vitro adenosarcoma cells, used 1% CV associated to the Nd:YAG laser, with satisfactory results. In the same research[36], the researcher used 1% MB with the Nd:YAG laser, with satisfactory results.

In this study, as the 1% CV was used with no PDT, there was, at 0h, a diminution of UFCs, and at 24h and 48h, no colonies were formed.

A research tested higher PSD concentrations. The 4µg/ml (0,0004%) CV diminished the biofilm mass in patients with HIV, but found that the higher the CV concentration, the better are the results found[26].

Another study[25], which also tested, with HIV patients, the concentrations of CV that affected the CA treatment, using varied concentrations, showed that the concentration of 0.00165%, the lowest tested, was shown to be table, well tolerated, did not stain the oral mucosa and had strong effects against CA. In spite of the two studies above having good results with lower CV concentrations, since they were trying to treat patients without the discomfort that is staining their mouths, UFCs still formed.

When the 1% BM was tested with the laser, it was found that it, gradually, with time, diminished the UFCs, that is, it has antifungal effects. Another investigation[36], used lower MB concentrations, almost all of which diminished the UFCs, but the 0.045% and 0.05% concentrations were the only ones with similar results to those of this study, that is, with no colony growth.

Corroborating this research, there is a consensus that the MB can be used with excellent restuls[6,13,38,39]. The concentration of PSDs must be determined with caution, since

a higher dosage is necessary for a higher PDT efficiency, still, very high concentrations tend not to be absorbed by the fungi, thus prejudicing the PDT results[13,14].

As the 1% MB was used without the laser, an increase in the number of CA was observed. Although there was a decrease, it was not significant. The use of the MB with no PDT is not viable[40].

In this study, it was found that the laser with no PSD is fatal for the micro-organism. These results are corroborated by another in vivo research[12], which compared the use of a laser with a wavelength of 685nm and 830nm with antifungal oral gel (myconazolum), associated to an antiseptic solution for the prosthesis.

That research[12] stated that it was not possible to conclude whether the fungicidal effect was caused by the biostimulation and the low intensity laser, or due to endogenous chromophores present in the fungi. Disputing these findings, in another study[40], no reduction of CA was found when using the laser alone on the fungi, although the result was found when the laser was applied on Candida tropicalis.

CONCLUSION

The in vitro results indicate that the PDT associated to 1% CV can be used in the treatment of infections caused by CA, but researches with humans are necessary to substantiate the efficacy of this dye in PDT, since the results may be different in vivo.

REFERENCES

1. Donnelly RF, McCarron PA, Tunney MM. Antifungal photodynamic therapy. Microbiol Res. 2008; 163(1):1-12.

2. Karkowska-Kuleta J, Rapala-Kozik M, Kozik A. Fungi pathogenic to humans: molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus. Acta Biochim Pol. 2009; 56(2):211-24.

3. Dai T, Fuchs BB, Coleman JJ, Prates RA, Astrakas C, Denis TGS, et al. Concepts and principles of photodynamic therapy as an alternative antinfungal discovery platform. Front Microbiol. 2012; 3:1-16.

4. Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunocompromised patients. Lancet Infect Dis. 2003; 3(11):685-702.

5. Pupo YM, Gomes GM, Santos EB, Chaves L, Michel MD, Koslowski Jr. VA et al. Susceptibility of Candida albicans to photodynamic therapy using methylene blue and toluidine blue as photosensitizing dyes. Acta Odontol Latinoam. 2011; 24(2):188-92.

6. Mitra S, Haidaris PHD, Snell SB, Giesselman BR, Hupsher SM, Foster TH. Susceptibility of Candida albicans to photodynamic therapy using methylene blue and toluidine blue as photosensitizing dyes. Lasers Surg Med. 2011; 43(4):324-32.

7. Lyon JP, Moreira LM, de Moraes PC, dos Santos FV, Resende MA. Photodynamic therapy for pathogenic fungi. Mycoses. 2011; 54(5):e265-71.

8. Grice EA, Segre JA. The human microbiome: our second genome. Annu Rev. Genomics Hum Genet. 2012; 13:151-70.

9. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20(1):133-63.

10. Calzavara-Pinton PG, Rossi MT, Sala R. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MAL-PDT) in 20 Italian dermatology departments. Part 2: oncologic and infectious indications. Photochem Photobiol Sci. [Internet]. 2013 [cited in 12 jan 2017]; 12(1): 158-65. DOI: 10.1039/c2pp25125f

11. Li DD, Xu Y, Zhang D-G, Quan H, Mylonakis E, Hu DD, et al. Fluconazole assists berberine to kill fluconazole-resistant Candida albicans. Antimicrob Agents Chemother. 2013; 57(12):6016-27.

12. Maver-Biscanin M, Mravak-Stipetic M, Jerolimov V, Biscanin A. Fungicidal effect of diode laser irradiation in patients with denture stomatitis. Lasers Surg Med. 2004; 35(4):259-62.

13. Queiroga AS, Trajano VN, Lima EO, Ferreira AF, Queiroga AS, Limeira FA Jr. In vitro photodynamic inactivation of Candida spp by different doses of low power laser light. Photodiagnosis Photodyn Ther. 2011; 8(4):332-6.

14. Kato IT, Prates RA, Sabino CP, Fuchs BB, Tegos GP, Mylonakis E et al. Antimicrobial photodynamic inactivation inhibits Candida albicans virulence factors and reduces In vivo

pathogenicity. Antimicrob Agents Chemother. 2013; 57(1):445-51.
15. Pereira MSV, Siqueira-Júnior JP, Rodrigues E, Cavalcanti ML, Nascimento AE, Campos-Takaki GM. Evaluation of ultrastrutural changes induced by ofloxacin associated with cephalexin against human and bovine strains of staphylococcus aureus during post antibiotic effect (PAE). Int J Pharmacol Res. 2014; 1(1):15-21.
16. Dai T, Bil de Arce VJ, Tegos GP, Hamblin MR. Blue dye and red light, a dynamic combination for prophylaxis and treatment of cutaneous Candida albicans infections in mice. Antimicrob Agents Chemother. 2011; 55(12):5710-7.
17. Machado-de-Sena RM, Corrêa L, Kato IT, Prates RA, Senna AM, Santos CC et al. Photodynamic therapy has antifungal effect and reduces inflammatory signals in Candida albicans-induced murine vaginitis. Photodiagnosis Photodyn Ther. 2014; 11(3):275-82.
18. Calzavara-Pinton P, Rossi MT, Sala R, Venturini M. Photodynamic antifungal chemotherapy. Photochem Photobiol. 2012; 88(3):512-22.
19. Maisch T. A new strategy to destroy antibiotic resistant microorganisms: antimicrobial photodynamic treatment. Mini Rev Med Chem. 2009; 9(8):974-83.
20. Zeina B, Greenman J, Purcell WM, Das B. Killing of cutaneous microbial species by photodynamic therapy. Br J Dermatol. 2001; 144(2):274-8.
21. Dovigo LN, Pavarina AC, Ribeiro AP, Brunetti IL, Costa CA, Jacomassi DP et al. Investigation of the photodynamic effects of curcumin against Candida albicans. Photochem Photobiol. 2011; 87(4):895-903.
22. Mardh PA, Rodrigues AG, Genç M, Novikova N, Martinez-de-Oliveira J, Guaschino S. Facts and myths on recurrent vulvovaginal candidosis – a review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. Int J STD AIDS. 2002; 13(8):522-39.
23. Gomes-de-Elvas AR, Palmeira-de-Oliveira A, Gaspar C, Gouveia P, Palmeira-de-Oliveira R, Pina-Vaz C. et al. In vitro assessment of gentian violet anti-candida activity. Gynecologic Obstet Invest. 2012; 74(2):120-4.
24. Maley AM, Arbiser JL. Gentian violet: a 19th century drug re-emerges in the 21st century. Exp Dermatol. 2013; 22(12):775-80.
25. Jurevic RJ, Trabolsi RS, Mukherjee PK, Salata RA, Ghanoum MA. Identification of gentian violet concentration that does not stain oral mucosa, possesses anti-candidal activity and is well tolerated. Eur J Clin Microbiol Infect Dis. 2011; 30(5):629-33.
26. Traboulsi RS, Mukherjee PK, Chandra J, Salata RA, Jurevic R, Ghannoum MA. Gentian violet exhibits activity against biofilms formed by oral candida isolates obtained from HIV-infected patients. Antimicrob Agents Chemother. 2011; 55(6):3043-45.
27. Dörtbudak O, Haas R, Bernhart T, Mailath-Pokorny G. Lethal photosensitization for decontamination of implant surfaces in the treatment of peri-implantitis. Clin Oral Implants Res. 2001; 12(2):104-8.
28. Perni S, Prokopovich P, Pratten J, Parkin IP, Wilson M. Nanoparticles: heir potential use in antibacterial photodynamic therapy. Photochem Photobiol Sci. 2011; 10(5):712-720.
29. Sharon V, Fazel N. Oral Candidiasis and Angular Cheilits. Dermatol Ther. 2010; 23(3):230-42.
30. Carvalho GG, Felipe MP, Costa MS. The photodynamic effect of methylene blue and toluidine blue on Candida albicans is dependent on medium conditions. J Microbiol. 2009; 47(5):619-23.
31 Atalay MA, Koc AN, Demir G, Sav H. Investigation of possible virulence factors in Candida strains isolated from blood cultures. Niger J Clin Pract. 2015; 18(1):52-5.
32. Morales DK, Grahl N, Okegbe C, Dietrich LEP, Jacobs NJ, Hogan DA. Control of Candida albicans metabolism and biofilm formation by pseudomonas aeruginosa phenazines. MBio. 2013; 4(1):e00526-12.
33. Ford CB, Funt JM, Abbey D, Issi L, Guiducci C, Martinez DA et al. The evolution of drug resistance in clinical isolates of Candida albicans. Elife. 2015; 4:e00662.
34. Neppelenbroek KH, Machado AL, Pavarina AC, Massucato EM, Colombo AL, Vergani CE. Effectiveness of microwave disinfection of complete dentures on the treatment of Candida-related denture stomatitis. J Rehabil Oral. 2008; 25(3):232-44.
35. Vazquez JA, Sobel JD. Mucosalcandidiasis. Infect Dis Clin North Am. 2001; 16(4):793-820.
36. Teichert MC, Jones JW, Usacheva MN, Biel MA. Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model.

Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002; 93(2):155-60.
37. Oliveira CS, Turchiello R, Kowaltowski AJ, Indig GL, Baptista MS. Major determinants of photoinduced cell death: subcellular localization versus photosensitization efficiency. Free Radic Biol Med. 2011; 51(4): 824-33.
38. Mima EG, Pavarina AC, Ribeiro DG, Dovigo LN, Vergani CE, Bagnato VS. Effectiveness of photodynamic therapy for the inactivacion of candida SSP. on dentures: in vitro study. Photomed Laser Surg. 2011; 29(12):827-33.
39. Khademi H, Torabinia N, Allameh M, Jebreilamtigh HR. Comparative evaluation of photodynamic therapy induced by two different photosensitizers in rat experimental candidiasis. Dent Res J. 2014; 11(4):452-9.
40. Souza SC, Junqueira JC, Balducci I, Koga-Ito CY, Munin E, Jorge AO. Photosensitization of different Candida species by low power laser light. J Photochem Photobiol B. 2006; 83(1):34-8.

CONTRIBUTIONS
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