Annals of Medical Research

DOI: 10.5455/annalsmedres.2019.06.340 2019;26(12):2733-7

Original Article

Evaluation of the effects of various agents on aggregatibacter actinomycetemcomitans, candida albicans, and streptococcus mutans growth

Mehmet Murat Taskan[1], Isa Karaman[2], Hatice Balci Yuce[1], Gozde Isiker Kara[1]

[1]Tokat Gaziosmanpasa University, Faculty of Dentistry, Department of Periodontology, Tokat, Turkey
[2]Tokat Gaziosmanpasa University, Faculty of Engineering and Natural Science, Tokat, Turkey

Abstract

Aim: Oral environment hosts many microorganisms and even symbiotic microorganisms can cause diseases under appropriate conditions and antimicrobial therapy might be required. The aim of the present study was to evaluate the effect of various antioxidants on Aggregatibacter actinomycetemcomitans, Candida albicans, and Streptococcus mutans as pathogenic microorganisms of the oral environment.

Material and Methods: In present research, the antibacterial effects of various antioxidants on oral bacteria, Aggregatibacter actinomycetemcomitans (A.actinomycetemcomitans, ATCC 33384), Streptococcus mutans (S.mutans, ATCC 35668), and fungus Candida albicans (C.albicans, ATCC 90819) were tested. The tested antioxidants were curcumin, gallic acid, and vanillic acid. The concentrations of the antioxidants were adjusted to 5% and appropriate solvents were used for the agents. The solvents were distilled water for gallic acid, ethanol for vanillic acid and dimethyl sulphoxide (DMSO) for curcumin. Antibiotics ciprofloxacin, penicillin, tetracycline, and chlorhexidine were used as positive control agents. The antibacterial effect of the antioxidants were tested with disc-diffusion method.

Results: Positive control agents provided significant antibacterial efficacy compared to the test agents and negative controls. The most significant effect was observed on ciprofloxacin against all tested microorganisms. On the other hand, penicillin provided similar effects with the chlorhexidine and tetracycline. All antioxidants tested in the present study was found ineffective against all tested microorganisms. Topical use of antioxidants did not provide efficacy.

Conclusion: Antioxidants have significant effects on preventing oxidative stress and decreasing inflammation-driven pathologies. However, the tested antioxidants did not provide an antibacterial effect against A.actinomycetemcomitans, S.mutans, and C.albicans.

Keywords: A. actinomycetemcomitans; antioxidants; C.albicans, S.mutans

INTRODUCTION

The oral environment is a natural habitat that hosts many different species and numbers of microorganisms and is in a delicate balance with host defense systems (1). When this balance is disturbed, different infectious diseases such as periodontal disease, caries, and fungal infection might occur (2,3). Treatment of infectious diseases requires the removal of the causative microorganism. Furthermore, regular use of chemotherapeutics damages host tissues and organs and is not recommended as a long-term treatment option (4). In this respect, natural compounds with antimicrobial efficacy which are consumed on a daily basis might be useful without affecting host tissue and organs.

The most common infectious diseases of the oral cavity are caries and periodontal diseases (5). Certain microorganisms such as Streptococcus mutans which causes dental caries and Aggregatibacter actinomycetemcomitans which causes rapid destruction of the periodontal tissues are responsible for those diseases (5). However, neither of them can be cured with any chemical agents and the patients would have to suffer from the diseases throughout their lives. Yet the development of the diseases and progress can be

Received: 21.06.2019 **Accepted:** 24.09.2019 **Available online:** 17.12.2019
Corresponding Author: Mehmet Murat Taskan, Tokat Gaziosmanpasa University, Faculty of Dentistry, Department of Periodontology, Tokat, Turkey **E-mail:** mmtaskan@gmail.com

prevented at least slowed down with appropriate therapies and oral hygiene instructions (6). In addition, antibiotics do not have a place in the routine clinical treatments as long-term use of these agents cause adverse effects like antibiotic resistance, suppression of regular oral microbiota and fungus superinfection (7).

Another common infectious disease in the oral environment is the fungal infections which are mostly associated with immunosuppression or overuse of antibiotics (7). Furthermore, senile patients who use removable acrylic prosthesis might also suffer from fungal infections (8). Antiseptics and antibiotics are frequently used in the treatment of infectious diseases (7). The effect of antibiotics occurs through disruption of genetic materials, cell wall integrity or metabolism (9). On the other hand, the most potent antiseptic used in dentistry is chlorhexidine (CHX) and it binds cationic molecules on the cell wall and disrupts cell wall integrity (10).

Antioxidants are natural compounds with various effects such as modulating host defense system, reducing inflammation, increasing bone formation and decreasing apoptosis of the host cells (11). Numerous biological activity such as antioxidant, antimicrobial, cell protective activities were also reported (12-20). Potent antimicrobial efficacy in skin wounds and decreased ROS production was demonstrated (13,21). Antioxidants exhibit significant preventive efficacy against various fungi such as Candida species, gram positive bacteria such as Staphylococcus aureus, and gram negative bacteria such as Eschericia coli (21-23).

The viability of microorganisms depends on the environment and severe alteration on the environment cause cell death (24). The effects of antioxidants on microorganisms occur through reactive oxygen species (ROS) (25). Based on the previous studies reporting antimicrobial effects of potent antioxidants (21,26), the aim of the present study was to evaluate the effect of various antioxidants on oral microorganisms A. Actinomycetemcomitans, S. mutans, and C.albicans on cell culture.

MATERIAL and METHODS

Penicillin, ciprofloxacin, tetracycline, CHX, and nystatin were used as positive controls and distilled water, ethanol and dimethyl sulphoxide were used as negative controls. The tested antioxidants were curcumin, gallic acid, and vanillic acid. The solvents were distilled water for gallic acid, ethanol for vanillic acid and dimethyl sulphoxide (DMSO) for curcumin. All agents were tested as 5% concentration.

For positive controls; 0.12% CHX, 5% concentration dissolved in distilled water for ciprofloxacin, 10 µg/disc for penicillin, 30 µg/disc for tetracycline and 10 µg/ disc for nystatin were used. Nystatin was not tested for Aggregatibacter actinomycetemcomitans and Streptococcus mutans. Tetracycline and ciprofloxacin were not tested for Candida albicans also. Antibacterial and antifungal efficacy of test materials tested via Kirby-Bauer (Disc-diffusion) method.

Disc-diffusion method

Disc-diffusion method is an effective method and frequently used to investigate the efficacy of various agents against microorganisms (26-28). The bacterial species used in this study was Aggregatibacter actinomycetemcomitans (A.actinomycetemcomitans, ATCC 33384), Streptococcus mutans (S.mutans, ATCC 35668), and fungus Candida albicans (C.albicans, ATCC 90819). The antimicrobial activity was determined with the disc-diffusion method previously described in the literature (27,29). Firstly, nutrient agar (NA) was prepared and 108 CFU/mL of bacteria was added to 100 mL NA solution. Then, bacteria were inoculated to the petri dish containing Mueller-Hinton agar (MHA) medium which does not include any indicator or inhibitor. 38.0 g/L MHA was sterilized by autoclave (121°C, 15 min). After cooling to 45-50 °C 5% de-fibrinated sheep blood was added. 20 mL of blood-enriched MHA was poured to sterile petri dishes. The blank discs (6 mm diameter, Oxoid) were impregnated with 20 mL of each test compound dissolved in distilled water (105 µg/disc) and placed on the inoculated agar. The inoculated plates were incubated at aerobic conditions with 36°C for 24 h. After incubation, the growth inhibition zones were measured via a millimetric scale. The procedure was repeated thrice and the arithmetic mean of three measurements were recorded as one inhibition zone. The agents tested were shown in (Table 1).

RESULTS

The widest inhibition zones were observed on positive control group ciprofloxacin, against A. Actinomycetemcomitans and S.mutans. Negative control groups; both distilled water, ethanol and dimethyl sulphoxide did not exhibit any inhibition zones. No inhibition zones were observed in antioxidants; curcumin, gallic acid, and vanillic acid tested against A. Actinomycetemcomitans and S.mutans. Tetracycline provided wider zone compared to the penicillin and CHX. Positive controls penicillin and CHX provided similar inhibition zone (Table 2).

Table 1. The agents used in the study

Distilled water	
Ethanol	50% concentration
Dimethyl sulphoxide	50% concentration
Penicillin	10 µg/disc
Tetracycline	30 µg/disc
Ciprofloxacin	5% concentration dissolved in distilled water
Nystatin	10 µg/disc
Chlorhexidine	0.12%
Curcumin	5% dissolved in dimethyl sulphoxide
Gallic acid	5% dissolved in distilled water
Vanillic acid	5% dissolved in ethanol

Table 2. Inhibition zones of the tested agents.

Microorganism/ Tested agents	Aggregatibacter actinomycetemcomitans	Streptococcus mutans	Candida albicans
Distilled water	0	0	0
Ethanol	0	0	0
Dimethyl sulphoxide	0	0	0
Penicillin	10	18	3
Tetracycline	17	17	Not tested
Ciprofloxacin	40	34	Not tested
Nystatin	Not tested	Not tested	20
Chlorhexidine	11	15	20
Curcumin	0	0	0
Gallic acid	0	0	0
Vanillic acid	0	0	0

Against C.albicans, the most effective agents were tetracycline and nystatin which provided similarly medium antimicrobial effect with 20 mm inhibition zone. Penicillin exhibited 3 mm wide inhibition zone also. Negative controls did not exhibit antimicrobial effect (Table 2). Antioxidants also did not create any inhibition zones against C.albicans.

DISCUSSION

Periodontitis is the irreversible chronic inflammatory disease triggered by dysbiotic oral microbiota causing soft and hard periodontal tissue destruction (2). Many pathogenic bacteria are involved in the content of dysbiotic flora (2). Aggregatibacter actinomycetemcomitans plays a critical role in the development of periodontal disease due to its virulence factors (30). There are several studies in the literature that have been investigated the effectiveness of various plant extracts and agents on this microorganism (26,30,31). Dental caries is one of the most common preventable diseases; people are susceptible to the disease throughout their lifetime (5). S. mutans is the primary microorganism responsible for the formation of dental caries (6,32). In addition to daily oral hygiene habits, the use of certain agents has been proposed to reduce S. mutans levels, especially in individuals with a high incidence of caries (33). C. albicans is the most common cause of oral fungal infections and it may occur as a result of flora deterioration due to long- term antibiotic use (20). And the use of natural products instead of antifungals has been the subject of many studies before (20,21,34). The present study evaluated the antimicrobial effect of curcumin, gallic acid, and vanillic acid on oral microorganisms A. actinomycetemcomitans,

S. mutans, and C. albicans. The results have revealed that antioxidants did not exhibit any antimicrobial effect on the tested microorganisms. Positive controls as CHX and penicillin provided acceptable efficacy and the most significant effect was observed with ciprofloxacin.

The oral environment is a complex milieu, rich in microorganism species in both diversity and number (5). Bacteria in the oral flora have normally a symbiotic relationship, and disruption of this equilibrium leads to infection and related diseases (1). However, unlike other infectious diseases, oral diseases such as caries and periodontal diseases are not mono-infections which could be treated with antibiotic use (3). Antiseptics can also be used however they could cause adverse effects such as suppressing existing oral microbiota, discoloration in the teeth, and systemic side effects such as gastrointestinal problems or kidney problems (35). Furthermore, in patients with immunosuppression, in situations such as pregnancy or lactation, chemotherapeutics also are not recommended for oral infections (36). In this regard, natural compounds with low side effects can be used on daily basis and aid treatment of infectious diseases of the oral environment (31).

Curcumin is a polyphenolic compound which was demonstrated to exhibit potent antibacterial, antiviral, antifungal, and anti-malarial effect (37). Mahdizade-Ari et al. recently reported that A. actinomycetemcomitans cell viability, metabolic activity, and quorum sensing ability significantly decreased after curcumin expose (30). Alalwan et al. showed that curcumin was also effective against C. albicans adhesion and biofilm formation (34). In contrast, no inhibition zone was observed in the present study in A. actinomycetemcomitans, S. mutans, and C. albicans cultures in response to curcumin treatment.

Antioxidant molecules have diverse activity spectrum as they can up-regulate anabolic activities, reduce inflammation, apoptosis, and prevent tumor growth (11,26,38). Gallic acid is a potent antioxidant and anti-inflammatory agent which was recently shown to prevent periodontopathogenic bacteria cell growth (26, 31). Nakamura et al. Suggested that gallic acid might be a potent disinfectant as gallic acid implementation exterted significant antibactericidal activity against A actinomycetemcomitans and S. mutans (31). It was also effective against Porphyromonas gingivalis (26). As for

C. albicans, Teodoro et al. recently found that gallic acid exhibited potent antifungal effect and can be considered as a novel antifungal treatment agent along with its anti-inflammatory activity (39). However, the present results did not reveal any inhibitory effect of gallic acid with 5% concentration which was lower than the doses used in the aforementioned studies (10%) (26,31). According to these results, the concentrations of antioxidants included in this study may be considered to be lower than the minimum inhibitory concentration (MIC) value. And for future studies, it should be noted that doses below the MIC values will not be effective to these species.

Vanillic acid is another strong antioxidant agent which was reported to reduce major pathogenic bacteria in the oral environment. Bodet et al. showed that vanillic acid and its derivatives significantly reduced S.mutans biofilm

formation and P. gingivalis cell growth (40). In contrast, Panyo et al. reported that vanillic acid did not exhibit inhibitory effects against C.albicans and S. mutans. Currently, there is no published study regarding the effect of vanillic acid on A. actinomycetemcomitans.

CONCLUSION

Among tested molecules, positive control agents provided significant antimicrobial efficacy, however antioxidants did not exhibit any effect. That might be the result of used concentrations in the study. Future studies with different agents and bacterial strains, or high concentrations might be beneficial in searching natural antimicrobial compounds.

ACKNOWLEDGEMENTS

The authors declare that this study has never been submitted/published to any other journals. There is no funding for this research. The authors also declare that there is no conflict of interest for this study.

Competing interests: The authors declare that they have no conflict of interest.
Financial Disclosure: There are no financial supports.
Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

Mehmet Murat Taskan ORCID: 0000-0002-4830-0075
Isa Karaman ORCID: 0000-0002-0709-9563
Hatice Balci Yuce ORCID: 0000-0003-3574-9751
Gozde Isiker Kara ORCID: 0000-0002-6011-6043

REFERENCES

1. Ruby J, Goldner M. Nature of symbiosis in oral disease. J Dent Res 2007;86:8-11.
2. Honda T, Domon H, Okui T, et al. Balance of inflammatory response in stable gingivitis and progressive periodontitis lesions. Clin Exp Immunol 2006;144:35-40.
3. Graves DT, Jiang Y, Genco C. Periodontal disease: bacterial virulence factors, host response and impact on systemic health. Curr Opin Infect Dis 2000;13:227-32.
4. Brandt M, Warnock D. Epidemiology, clinical manifestations, and therapy of infections caused by dematiaceous fungi. J Chemother 2003;15:36-47.
5. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett 2014;162:22-38.
6. Tonetti MS, Bottenberg P, Conrads G, et al. Dental caries and periodontal diseases in the ageing population: call to action to protect and enhance oral health and well-being as an essential component of healthy ageing–Consensus report of group 4 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. J Clin Periodontol 2017;44:135-44.
7. Verdugo F, Laksmana T, Uribarri A. Systemic antibiotics and the risk of superinfection in peri-implantitis. Arch Oral Biol 2016;64:39-50.
8. Hayran Y, Sarikaya I, Aydin A, et al. Determination of the effective anticandidal concentration of denture cleanser tablets on some denture base resins. J Appl Oral Sci 2018;26:20170077.
9. Chambrone L, Vargas M, Arboleda S, et al. Efficacy of local and systemic antimicrobials in the non-surgical treatment of smokers with chronic periodontitis: A systematic review. J Periodontol 2016;87:1320-32.
10. Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. J Clin Periodontol 1988;15:415-24.
11. Yuce HB, Karatas O, Turkal HA, et al. The effect of melatonin on bone loss, diabetic control, and apoptosis in rats with diabetes with ligature-induced periodontitis. J Periodontol 2016;87:35-43.
12. Lim H-J, Jeon Y-D, Kang S-H, et al. Inhibitory effects of Euphorbia supina on Propionibacterium acnes-induced skin inflammation in vitro and in vivo. BMC Comp Alt Med 2018;18:263.
13. Park H-H, Ko S-C, Oh G-W, et al. Characterization and biological activity of PVA hydrogel containing chitooligosaccharides conjugated with gallic acid. Carbohydrate Polym 2018;198:197-205.
14. Albouchi F, Avola R, Dico G, et al. Melaleuca styphelioides sm. Polyphenols modulate interferon gamma/histamine-induced inflammation in human nctc 2544 keratinocytes. Molecules 2018;23:2526.
15. Fischer N, Seo E-J, Efferth T. Prevention from radiation damage by natural products. Phytomedicine 2017;47:192-200.
16. Ola-Davies OE, Olukole SG. Gallic acid protects against bisphenol A-induced alterations in the cardio-renal system of Wistar rats through the antioxidant defense mechanism. Biomed Pharmacother 2018;107:1786-94.
17. Rong Y, Cao B, Liu B, et al. A novel Gallic acid derivative attenuates BLM-induced pulmonary fibrosis in mice. Int Immunopharmacol 2018;64:183-91.
18. Harikrishnan H, Jantan I, Haque MA, et al. Anti-inflammatory effects of phyllanthus amarus Schum. & Thonn. through inhibition of NF-κB, MAPK, and PI3K-Akt signaling pathways in LPS-induced human macrophages. BMC Comp Alt Med 2018;18:224.
19. Setayesh T, Nersesyan A, Mišík M, et al. Gallic acid, a common dietary phenolic protects against high fat diet induced DNA damage. Eur J Nutr 2019;58:2315-26.
20. Teodoro GR, Gontijo AV, Salvador MJ, et al. Effects of acetone fraction from buchenavia tomentosa aqueous extract and gallic acid on candida albicans biofilms and virulence factors. Front Microbiol 2018;9:647.
21. Li ZJ, Liu M, Dawuti G, et al. Antifungal activity of gallic acid in vitro and in vivo. Phytotherapy Res : PTR 2017;31:1039-45.
22. Li X, Liu Y, Jia Q, et al. A systems biology approach to investigate the antimicrobial activity of oleuropein. J Indust Microbiol Biotech 2016;43:1705-17.
23. Wang Q, Leong WF, Elias RJ, et al. UV-C irradiated gallic acid exhibits enhanced antimicrobial activity via generation of reactive oxidative species and quinone. Food Chem 2019;287:303-12.

24. Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. Nature Rev Immunol 2017;17:151.
25. Van Acker H, Gielis J, Acke M, et al. The role of reactive oxygen species in antibiotic-induced cell death in Burkholderia cepacia complex bacteria. PloS one 2016;11:0159837.
26. Bali EB, Acik L, Akca G, et al. Antimicrobial activity against periodontopathogenic bacteria, antioxidant and cytotoxic effects of various extracts from endemic Thermopsis turcica. Asian Pac J Trop 2014;4:505-14.
27. Singh P, Misra R, Prasad K. Reliability of kirby-bauer disk diffusion method for detecting doripenem susceptibility in oxidase positive non-fermenting gram negative bacilli. Int J Health Sci Res 2016;6:395-7.
28. Direkel S, Uzunoglu E, Uzalp C, et al. Determination of piperacillin/tazobactam and ticarcillin/clavulanate susceptibilities in pseudomonas aeruginosa isolates in hospitalised patients by e-test gradient method and comparison of results with disk diffusion tests. Clin Microbiol 2017;6:2.
29. Bauer A, Kirby W, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
30. Mahdizade-Ari M, Pourhajibagher M, Bahador Changes of microbial cell survival, metabolic activity, efflux capacity, and quorum sensing ability of Aggregatibacter actinomycetemcomitans due to antimicrobial photodynamic therapy-induced bystander effects. Photodiag Photodyn Ther 2019;26:287-94.
31. Nakamura K, Ishiyama K, Sheng H, et al. Bactericidal activity and mechanism of photoirradiated polyphenols against gram-positive and -negative bacteria. J agric Food Chem 2015;63:7707-13.
32. Loesche WJ. Role of streptococcus mutans in human dental decay. Microbiol Rev 1986;50:353.
33. Fine D, Furgang D, Barnett M, et al. Effect of an essential oilcontaining antiseptic mouthrinse on plaque and salivary streptococcus mutans levels. J Clin Periodontol 2000;27:157-61.
34. Alalwan H, Rajendran R, Lappin DF, et al. The anti-adhesive effect of curcumin on candida albicans biofilms on denture materials. Front Microbiol 2017;8:659.
35. Sajadi FS, Moradi M, Pardakhty A, et al. Effect of fluoride, chlorhexidine and fluoride-chlorhexidine mouthwashes on salivary Streptococcus mutans count and the prevalence of oral side effects. J Dent Res Dent Clin Dent Prospects 2015;9:49.
36. Leslie KK, Koil C, Rayburn WF. Chemotherapeutic drugs in pregnancy. Obstet Gynecol Clin 2005;32:627-40.
37. Moghadamtousi SZ, Kadir HA, Hassandarvish P, et al. A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Res Int 2014;2014:186864.
38. A Puertollano M, Puertollano E, Alvarez de Cienfuegos G, et al. Dietary antioxidants: immunity and host defense. Curr Top Med Chem 2011;11:1752-66.
39. Teodoro GR, Gontijo AVL, Borges AC, et al. Gallic acid/ hydroxypropyl-beta-cyclodextrin complex: Improving solubility for application on in vitro/ in vivo Candida albicans biofilms. PloS one 2017;12:0181199.
40. Bodet C, Epifano F, Genovese S, et al. Effects of 3-(4'- geranyloxy-3'- methoxyphenyl)- 2-trans propenoic acid and its ester derivatives on biofilm formation by two oral pathogens, Porphyromonas gingivalis and Streptococcus mutans. Eur J Med Chem 2008;43:1612-20.