The effect of curcumin on growth and adherence of major microorganisms causing tooth decay

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Abstract

Background and objective: Streptococcus mutans and Lactobacillus acidophilus are bacteria producing tooth decay which by adhering to tooth surfaces contribute to its pathogenesis. By increasing bacterial resistance to antibiotics, along with other high-cost of treatment, the use of natural antibacterial agents is essential. In this regard, the role of curcumin on the adhesion process of cariogenic pathogens has been studied.

Materials and Methods: The bacterial strains of Streptococcus mutans (PTCC1683) and Lactobacillus acidophilus (PTCC1643) were obtained from the Iranian Research Organization for Science and Technology). The early growth of the bacteria was carried out in BHI medium, and then the concentration of microorganisms reached the half-MacFarland standard, and using different concentrations of curcumin (1, 2, 4, 8, 16, 31, 25, 52, 62, 125, 250, 500 µg/ml) obtained by serial dilution method the substances were mixed. BHI medium without bacteria was considered as negative control. Then samples were incubated for 24 hours at 37°C in anaerobic conditions for determining the minimum inhibitory concentration. Inhibitory concentrations determined in the previous step were used to determine adhesion using the amount of light absorbance determination method.

Results: The minimum inhibitory concentration of growth was determined in both bacterial strains of 250 µg/ml. The results showed a decrease in light absorption with increasing curcumin concentration ($P<0.001$), which indicates a high correlation (correlation coefficient of -0.93) of curcumin concentrations with reverse adhesion.

Conclusion: Adhesion is the most important factor in tooth decay and its reduction is an effective solution in preventing the disease. Considering the inhibitory role of curcumin on growth and binding of bacterial strains, this curcuminoid agent is considered as a potent anti-decay agent.

Key words: Adhesion, Curcumin, Tooth decay

Please cite this article as: Please cite this article as:
Helalat L. et al. The effect of curcumin on growth and adherence of major microorganisms causing tooth decay. *World Family Medicine.* 2017; 15(9):214- 220
DOI: 10.5742/MEWFM.2017.93128

Introduction

The oral cavity diseases, especially tooth decay, are common diseases with high prevalence in human societies (1). 95% of people in each society suffer from dental caries and periodontal diseases (2). However, our country's state of health in terms of oral hygiene and the teeth of children aged 6 to 12 in the Middle East region are the best, but unfortunately we (Iranian) are the worst in the 20-25 age group among the countries of the region. There is no exact data on the rate of caries of Iranian teeth, but what is certain is that we do not have a good situation in this age group (3).

Dental caries is a multi-factorial infectious disease, mainly due to the reduction in pH following carbohydrate fermentation, due to the action of two bacterial strains of streptococci and lactobacillus (1 and 4).

The formation of dental biofilms has been associated with the onset and development of dental caries as the extracellular matrix surrounding the bacteria (7-5). The first step in the formation of dental biofilms is bacterial adhesion to the dental surfaces (8). Oral and oral diseases have a negative effect on the quality of life, general health, nutritional status and social function of individuals. The pain, infection, sleep disorders and difficulty in chewing and eating and talking are considered as adverse outcomes of oral and dental illnesses in individuals (9). Dental diseases such as dental caries and gum disease may also significantly affect the health of the individual. Dental caries are associated with the development of chronic diseases such as diabetes and heart disease (9, 27).

Considering the high cost of treatment in oral cavity diseases (1) as well as increased bacterial resistance to commonly used antibiotics for treatment, new strategies for the prevention and treatment of dental caries and the fight against pathogens are needed (10). Nutrition is an effective factor in the prevention and treatment of dental caries and primary prevention programs at community level, including nutritional control, have played an important role in reducing tooth decay (11). Today, the use of natural antimicrobial agents in the form of alternative therapies has expanded due to the low and effective nature of the treatment and even the prevention of many diseases, including oral and dental diseases (13-12). Therefore, finding these natural and low risk compounds and studying the effect of these compounds on growth and adhesion of cariogenic bacteria, as the first step in the onset of tooth decay is considered necessary (5.8).

Tumeric is a yellow spice of the family Zingiberaceae (14), which grows mainly in the southern and tropical regions of Asia, such as China, India and Malaysia. The root and stem of this plant mainly contains a yellow curcuminoid compound (15). Turmeric is one of the most popular plants with extensive therapeutic properties in traditional medicine (16).

Curcumin (diferuloylmethane), as the main constituent of the main color of yellow in turmeric (17), due to its various antioxidant, anti-inflammatory, anti-carcinogenic, antimicrobial and antiparasitic effects can be used in various parts of the body, including the oral cavity (18-20). Most studies in the field of oral cavity diseases, such as the study by Izui et al. (29), have focused on periodontal disease due to the anti-inflammatory effects of curcumin, or in the form of mouthwash containing curcumin in the study of Mali et al. (22) and in the form of a curcumin-based gel by Bhatia et al (21), curcumin has been shown to be effective on periodontal disease, but the effect of curcumin on dental caries is less marked. On the other hand, studies such as Mandroli and Bhat (23) have mainly focused on the growth and microbiological counting of decay pathogens. Therefore, studying the effect of curcumin on growth of major microorganisms of dental caries as a biological factor along with their adhesion, which is one of the main causes of their pathogenicity, is one of the goals to be considered in this study.

Materials and Methods

Analysis method

This study is an experimental study approved by the Ethics Committee of the Medical University of Ahvaz number 1395.590IR.AJUMS.REC. The curcumin used in this study was from the German Merck company with a purity of about 97%. DMSO (Dimethyl sulfoxide) solution was used to increase solubility and the final concentration of 1mg/ml solution was prepared from curcumin solution, from which was obtained a uniform solution of curcumin using shaker. A bacterial filter was used to remove contaminants.

Microorganisms

The bacterial strains of Streptococcus mutans (PTCC1683) and Lactobacillus acidophilus (PTCC1643) were obtained from the Iranian Research Organization for Science and Technology). BHIB (Brain heart infusion broth) containing 1% sucrose was used for early growth of the microorganisms. The culture media was incubated in anaerobic conditions after inoculation of bacteria for 24 hours at 37°C.

Preparation of bacterial suspension: A linear culture of the initial BHI medium was performed on a Blood Agar culture medium under sterile conditions and resumed for 24 hours at 37°C incubation. The selective harvesting of microbial colonies was performed from Blood Agar level (surface) under sterile conditions and the colonies were transferred to a culture medium (BHIB) (Brain heart infusion broth) containing 1% sucrose and then incubated for 24 hours under anaerobic conditions at 37°C. The growth concentration was adjusted to 5×106 organism/ml by using 0.5 McFarland's turbidity standard.

To prepare McFarland's standard, 0.6 ml of 1% sodium chloride solution was dissolved in 100 ml of sulfuric acid in a volume of 100 ml and uniform solution of bacterial suspension was obtained by shaker.

Using an ultraviolet spectrophotometer (UV-2802 United States Unicode), the OD (optical absorption) at a wavelength of 620 nm was set at a pH of about 0.1 to achieve a concentration of 5×106 CFU (24).

The method of exposure to bacterial strains with different concentrations of curcumin

Different concentrations of curcumin solution were obtained by serial dilution method. In such a way 12 sterile tubes were considered. In all tubes, 1ml of bacterial solution was added at a concentration equivalent to the 0/5 McFarland's turbidity standard. Then in the first tube containing 1 ml bacterial solution, 1 ml of the curcumin solution was added. After mixing well, 1 ml was transferred to the second tube, this was continued till the last (10th) tube. From the last tube 1ml of final solution was discarded. By following this serial dilution, the concentration of, 500, 250, 125, 5.62, 25.31, 16, 8, 4, 2, 1µg/ml, respectively was acheived) (23).

BHI medium without bacteria was considered as a negative control and non-curcumin bacterial suspension as a positive control, and then the test and control samples were incubated at 37°C for 24 hours under anaerobic conditions.

Determination of MIC
(Minimum inhibitory concentration)

Considering the generation of turbidity from bacterial growth, examination of the transparency of tubes indicated the inhibition of growth in tubes.

The the tubes were incubated for 24 hours at 37°C after the incubation, the MIC values were determined by visual inspection of tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the tube indicated growth of the bacteria implying that the bacteria are resistant to curcumin. All steps for each bacterial strain were repeated 2 times and the mean of measurements was expressed in 2 replicates (23.31).

Assessment of adhesion

2.5 ml of inhibitory concentrations of curcumin, which did not inhibit the growth of bacteria in the previous stage, was added to 2.5 ml of BHI medium containing 1% sucrose and 250µl of bacterial suspension prepared at a concentration of 5×106 CFU. In positive control sample, instead of the curcumin solution, DMSO alone was used and in the negative control sample, BHI medium containing 1% sucrose without bacteria was considered. Then, at 37°C, for 24 hours, with Angle 30, the incubation was performed under anaerobic conditions. The tubes were examined externally, and the lowest concentration without attachment of visible cell to tubes wall was determined as Total Bacterial Adherence Inhibition (TBAI) (25). After removing the contents of the tubes containing suspended cells for isolation of the cells that were attached to the tubes, 3ml of KPB 0.5M buffer with Ph=6.8 was used and 0.25ml trypsin. The amount of cells suspended by optical density was measured by a spectrophotometer (UV -2802 Unico) at a wavelength of 490nm. All tests were repeated for 2 bacterial strains and the mean of measurements was expressed in 2 replicates (26).

Statistical analysis

In this study, data analysis and statistical analysis of data were performed using SPSS22 software. Spearman statistical test to examine the correlation between quantitative variables and Independent sample-T test to compare the mean of quantitative variables in two groups and One Way ANOVA test followed by a Sidak test to compare the mean of quantitative variables in the control group with each of the test groups were used.

Results

MIC

After 24 hours of incubation, tubes containing different concentrations of curcumin and control tubes in both bacterial strains were examined externally, and as shown in Figure 1, in the Lactobacillus acidophilus strain examination, negative control with a completely transparent coating without any turbidity and positive control with a similar turbidity with 1µg/ml curcumin solution, and with increasing curcumin concentration, the turbidity of the tubes was reduced and in the concentrations of 250 and 500µg/ml, completely transparent procedure with a transparency equal to the negative control tube was observed. In the strain of Streptococcus mutans, a negative control with a perfectly transparent procedure without any turbidity and positive control with a turbidity similar to that of 2 and 1µg/ml of curcumin solution and by increasing the concentration of curcumin, the turbidity of the tubes was reduced and at 250 and 500µg/ml concentrations, completely transparence with a transparency equal to negative control tube, was detected. The lowest concentration with transparent procedure was determined as MIC. In the study, MIC in both samples of Lactobacillus acidophilus and Streptococcus mutans was obtained in 250µg/ml.

Evaluation of adhesion

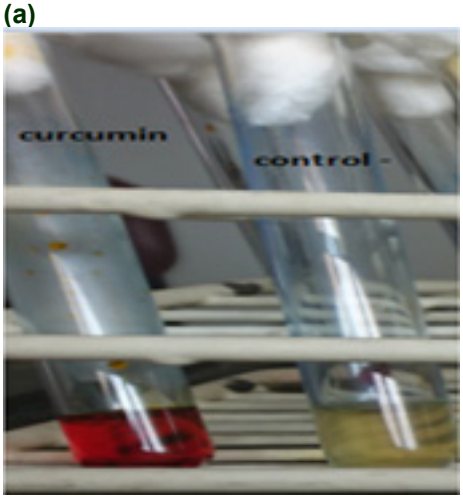
In the apparent examination (Figure 2) of the Lactobacillus acidophilus strain, no visible cells were observed in the tube wall after about 24 hours at a concentration of 31.25µg/ml and higher of curcumin. In the apparent inspection (evaluation) of Streptococcus mutans strain, in curcumin concentrations of less than 125µg/ml, visible cells adhered to the tube wall were clearly and significantly higher than lactobacillus acidophilus.

In the method to evaluate the adherence (adhesion) by determining the optical absorption, in both bacterial strains, there was a high inverse correlation coefficient (-0.93) between the adhesion and curcumin concentration and with increasing the curcumin concentration, the optical absorption was significantly reduced which indicates a significant reduction in adhesion ($p<.001$).

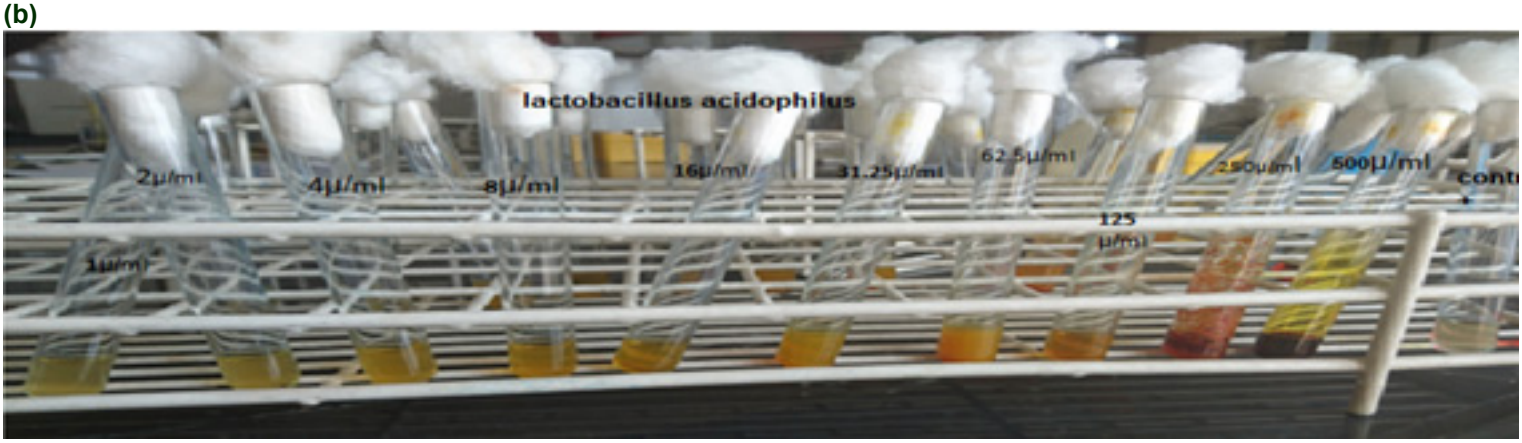
In the comparison between the control tube and the other tubes, it was observed that in both bacterial strains, adhesion in the tube walls was significantly less than the

Figure 1: Determination of MIC at different concentrations of curcumin prepared by serial dilution method. In curcumin concentrations of 250µg/ml and above, the transparent layer is clearly visible (a) negative control and curcumin solution; (b) Lactobacillus acidophilus strain; (c) Streptococcus mutans

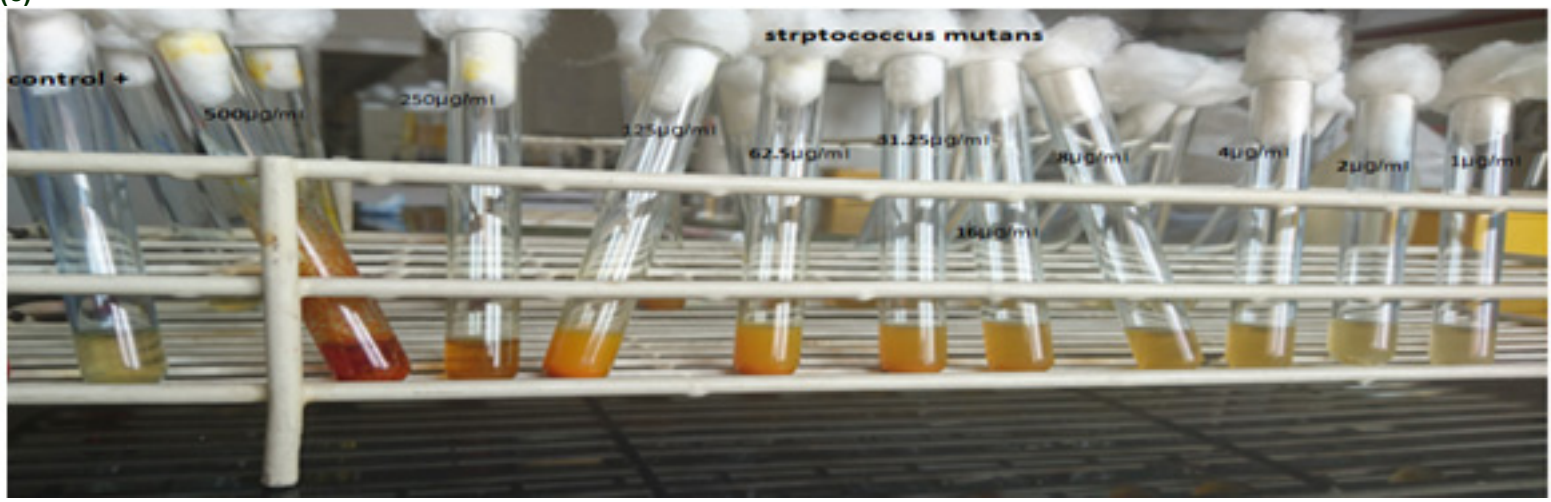
(a)



(b)



(c)

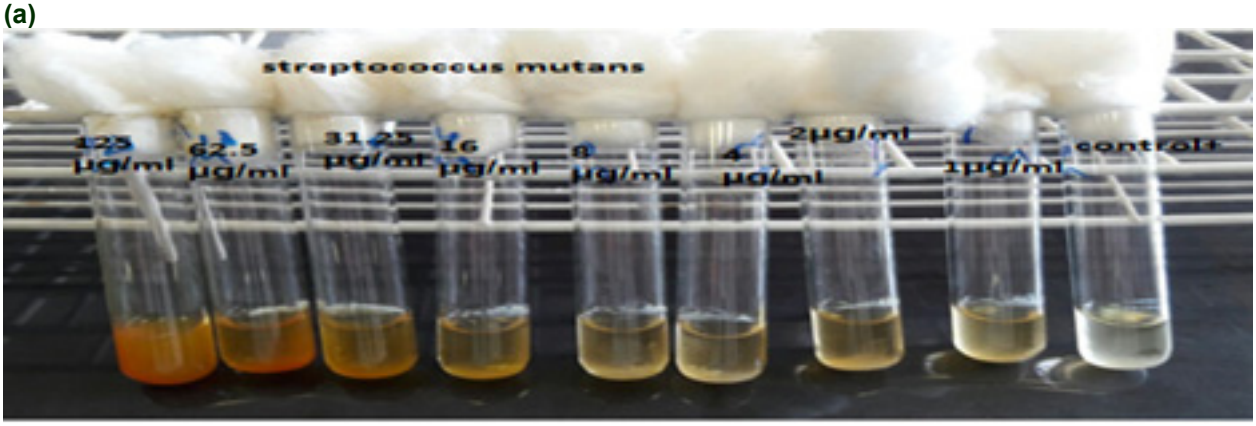


adhesion in the positive control tube and more than the cells adhering to the negative control wall. In The Lactobacillus acidophilus strain, the mean of optical absorption of cells adhering to the test tube wall (Figure 1), showed a significant statistical difference in the concentrations of less than 4 µg/ml of the curcumin solution with the negative control group ($p<0.001$). While there was no significant difference ($p<0.05$) comparing the concentrations equal to and above the concentration of 4 µg/ml with the negative control group. Concentrations of less than of 2µg/ml curcumin solutions showed no significant statistical difference ($p<0.05$) compared to positive control, indicating a significant decrease in the binding of bacterial cells by increasing the concentration of curcumin.

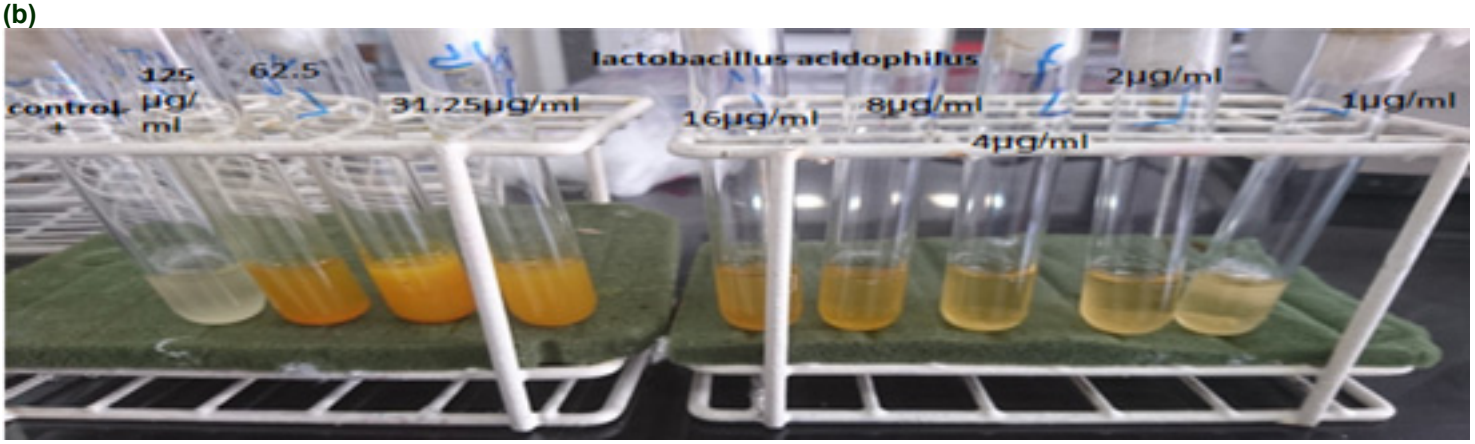
In Streptococcus mutans strain, the comparison of curcumin concentration and control group at concentrations less than 125µg/ml showed significant differences with the negative control group, and the difference was significant ($p<0.001$) at concentrations ranging from 4 to 1 µg/ml, and with increasing concentrations, there is less difference. The difference in

Figure 2: The adhered (sticking) cells in test tubes wall in various concentrations of curcumin. (a) Streptococcus mutans: Curcumin at a concentration of 125 µg/ml and above caused adhesion inhibition on the surfaces of the test tube (b) Lactobacillus acidophilus: Curcumin at a concentration of 31.25 µg/ml and more caused adhesion inhibition tested on the test tube surfaces.

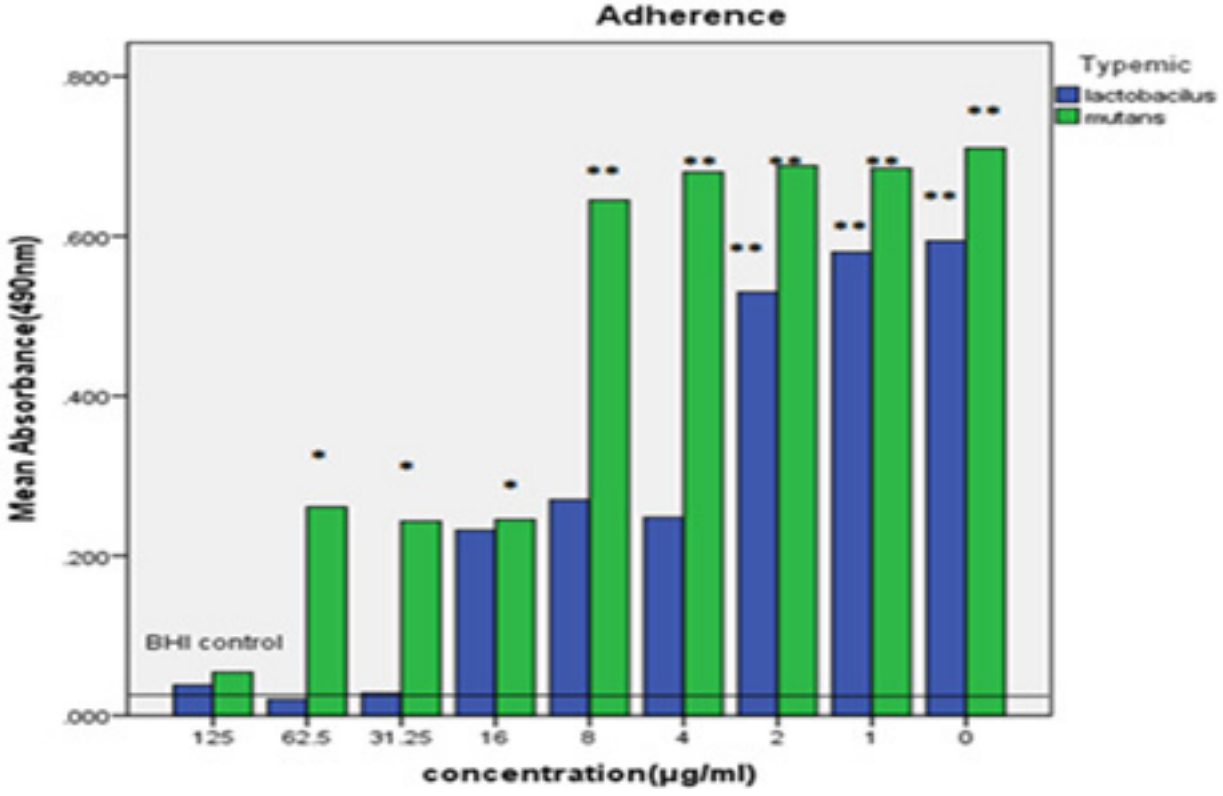
(a)



(b)



Graph 1: The comparison of the adhesion based on the mean optical absorption in Streptococcus mutans and Lactobacillus acidophilus. The horizontal line shows the mean optical absorption in the negative control group. * Significant difference with negative control group with ($p<0.05$) ** : Significant difference with negative control group with ($p<0.001$) in curcumin concentration less than 8 µg/ml and less than 2 µg/ml in strains of Streptococcus mutans and Lactobacillus acidophilus, the absorption rate was significantly higher than the negative control group.



concentrations of 8 to 62.5µg/ml of curcumin solution with negative control group was significant ($p<0.05$).

Concentrations of 8-1 µg/ml of curcumin solution ($p<0.001$) showed a significant difference compared to the positive control group, while at a concentration of 16 to 125 µg/ml of curcumin, this difference wasn't statistically significant ($p<0.05$) which is indicative of a significant reduction in the binding of bacterial cells by increasing the concentration of curcumin.

The comparison between Streptococcus mutans and Lactobacillus acidophilus showed no significant difference ($p<0.05$) between the two bacterial strains.

Discussion

Considering the polymicrobial nature of the dental caries (27) and the major role of two bacterial strains of Streptococcus and Lactobacillus acidogenesis in its pathogenesis (4), the study of pathogenicity of these microbial species is of particular importance. Adhesion of microbial cells to surfaces and aggregation of these cells are a key step in the formation of multilayer cell clusters (biofilms). In this regard, adhesion is considered as one of the major factors of pathogenicity (28). Based on the results of this study, curcumin was found to be effective at certain concentrations as a polyphenolic agent with anti-bacterial and antioxidant effects (8) on inhibiting growth and adherence inhibition, Lactobacillus acidophilus and Streptococcus mutans. This inhibitory growth in both bacterial strains has increased with curcumin concentration increase. Curcumin inhibitory effect was not statistically significant in comparison with the two bacterial species.

Most studies on oral cavity diseases have focused on the pathogens of periodontal disease, given the curcumin-based anti-inflammatory nature. In this regard, Izui et al., (2005) examined the effect of curcumin on bacteria producing periodontal disease and showed that curcumin had a dose-dependent inhibitory effect on pathogen microorganisms at all concentrations (29).

In the year 2015, Shahzad et al. investigated the effects of a number of polyphenols, including curcumin, on the growth and biofilm formation of a number of periodontal microorganisms producing diseases, and it was found that polyphenols, including curcumin, can be considered as inhibitors of growth and the biofilm formation in these diseases (30). Similarly, in the same year, Savita and colleagues showed a strong anti-bactericidal effect of curcumin on aggregatibacter actinomycetemcomitans bacteria (31).

In relation to dental caries, Hu and colleagues investigated the effect of curcumin on inhibiting the activity of sortase A produced by mutants as an enzyme involved in adhesion and biofilm formation, and finally, an inhibitory effect on adhesion was demonstrated through inhibition of enzyme (32), as well as Mandroli and Bhat, who investigated the effect of curcumin on a string of oral bacterial bacteria. In this study, the inhibitory effect of curcumin on all studied microorganisms was shown except for enterococcus faecalis with different minimal inhibitory concentrations (23).

In relation to the effect of curcumin on dental caries, studies have focused on inhibiting growth or microbiological counting cariogenic microorganisms as a factor associated with bacterial bioactivity. In this study, along with inhibition of growth the evaluation of curcumin role on adhesion as one the main mechanisms involved in the pathogenesis of decay pathogens has been considered, as well as the effects of various concentrations of curcumin have been investigated to determine effective concentrations. In most studies, the protective effect of polyphenols against mutans has been demonstrated through effects on metabolic activity and virulence factors, and less effect on bioavailability has been detected (33). However, in this study, a significant inhibitory effect on growth inhibition as a viability factor with adhesion as a pathogenic factor was observed.

Conclusion

The present study showed that growth inhibitory and binding of cariogenic pathogens in culture media (in vitro) with increase in curcumin concentration, and this inhibitory effect is reported statistically significant at some concentrations.

In the end, the question remains what effects curcumin have on decay (cariogenic) pathogens in the oral cavity? And what practical solutions are available to determine the duration of contact with pathogens with curcumin in the oral cavity, and that effective concentrations on pathogens in the culture medium, so the oral cavity will also be safe and effective.

Acknowledgment
From here, the Deputy Director of the Jundishapur University of Ahwaz, who has been involved in the financing of the research project, as well as the Vice-Chancellor of the Faculty of Para-medicine and Laboratory of the Faculty of Paramedical Sciences of Ahvaz is commended.

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