ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 2, April - 2021, Page No. : 441 - 446

Evaluation of antimicrobial efficacy of 1% and 2% arginine containing toothpaste against enterococcus faecalis and candida albicans – An In Vitro Study

[1]Dr Anisha Jenny, Post graduate student, Department of Paediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, International Airport Road, Bangalore, 562157

[2]Dr Pallavi Urs, Reader, Department of Paediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, International Airport Road, Bangalore 562157

[3]Dr Priya Nagar, Head of the Department, Department of Paediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, International Airport Road, Bangalore 562157

[4]Dr Vatsala N, Post graduate student, Department of Paediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, International Airport Road, Bangalore 562157

[5]Dr Richa Lakhotia, Post graduate student, Department of Paediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences. Hunasamaranahalli, International Airport Road, Bangalore 562157

Corresponding Author: Dr Anisha Jenny, Post graduate student, Department of Paediatric and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, International Airport Road, Bangalore, 562157

Citation of this Article: Dr Anisha Jenny, Dr Pallavi Urs, Dr Priya Nagar, Dr Vatsala N, Dr Richa Lakhotia, "Evaluation of antimicrobial efficacy of 1% and 2% arginine containing toothpaste against enterococcus faecalis and candida albicans – An In Vitro Study", IJDSIR- April - 2021, Vol. – 4, Issue - 2, P. No. 441 – 446.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Dental caries is one of the most common oral health problems experienced by the population across the globe. Despite significant efforts to prevent and treat caries, including the prophylactic use of fluoride, it still remains a major oral health issue. The purpose of this study was to evaluate the antimicrobial efficacy of 1% and 2% arginine containing toothpaste against Enterococcus faecalis and Candida albicans and compare it with the commercially available toothpastes.

Methods: An attempt was made to develop a toothpaste containing arginine by trituration method. A colony of Enterococcus faecalis and Candida albicans was prepared using BHI agar and Sabaroud Dextrose broth respectively. The antimicrobial efficacy of the prepared toothpaste was compared with the commercially available toothpastes by measuring the inhibition zones by the agar well diffusion method.

Results: The results of the present study suggested that the arginine containing toothpaste had significant antimicrobial activity against Enterococcus faecalis and

Candida. Albicans. The arginine containing toothpaste showed greater antimicrobial activity than the commercially available toothpastes. The toothpaste containing 2% arginine showed higher antimicrobial activity than the toothpaste containing 1% arginine, which was statistically significant when tested with ANOVA test.

Conclusion: Arginine containing toothpaste can be considered as promising formulations for oral diseases caused by Enterococcus faecalis and Candida albicans with regard to its significant antimicrobial activity.

Keywords: Dental caries, Arginine, Toothpaste, Enterococcus Faecalis, Candida Albicans

Introduction

Dental caries is one of the most common oral health problems experienced by all age groups of the population but in the recent years, there has been a significant increase in the number of early childhood caries in different parts across the globe. Dental caries remains a major health issue, despite the significant efforts to prevent and treat it, including the use of fluoride. Most importantly, it can lead to long term effects on the general health of children by affecting their overall growth, development and nutrition.[1]

Caries progression involves a series of events involving acid production from bacterial fermentation of the dietary food, especially carbohydrates, shifting the neutral pH to a more acidic pH favouring the growth of acid-producing and acid-resistant microflora leading to loss of tooth mineral. Caries preventive measures include ways to prevent the initial dissolution of the dental enamel and also arrest the progression of early lesions. This is achieved by maintaining the pH of the oral cavity, clearance and buffering capacity of saliva within the physiological limits and counteracting the acidification of biofilms.

The use of fluoride has always been the priority when it comes to caries prevention and its further progression. Its widespread use has led to a dramatic decline in the incidence of caries worldwide: daily use of fluoride toothpaste while brushing is considered as the most cost-effective method in preventing caries, but several questions have been raised regarding its safety, especially in children. Recent studies on fluoride have turned out to be controversial in terms of its use in children and pregnant women.[3, 4] these disadvantages of fluoride can be dealt with a suitable substitute with similar antimicrobial properties and minimal side-effects.

Arginine has been lately introduced as an additive to toothpaste and other fluoride-containing dental care products. It was initially marketed for the treatment of sensitivity of exposed necks of teeth; however, arginine now is being promoted as a caries-preventive agent. It is a semi-essential amino acid that occurs naturally in a range of food products and in the saliva. It is metabolized by arginolytic bacteria, Streptococcus sanguinis, Streptococcus parasanguinis and Streptococcus gordonii via the Arginine Deiminase System (ADS) to ornithine, citrulline, ammonia, CO2, and ATP, which modulates the cariogenic biofilms via alkali production, which leads to an increase in the pH in the oral biofilm..[5]

The ammonia which is produced from the arginine metabolism of oral bacteria inhibits the tooth demineralization by shifting the pH making it more alkaline and neutralizing the glycolytic acids and also favors the growth of a desirable microflora that is compatible with dental health.[1]

Incorporation of Arginine in toothpaste can be beneficial, keeping in mind its anti-caries property and desensitizing effect. The present study aims to evaluate the antimicrobial efficacy of two different concentrations of arginine containing toothpaste against Enterococcus

feacalis and Candida Albicans and compare it with two commercially available toothpastes for children.

Materials and Methods

Preparation of the Bacterial/Fungal Culture and Agar

A colony of E. faecalis (ATTC 29292) and C. Albicans was prepared using BHI and Sabaroud's Dextrose broth respectively. The inoculums were prepared and adjusted to 0.5 McFarland turbidity standards. Then Mueller Hinton agar plates were inoculated with broth cultures of each isolate. After the plates were dry, wells of 6 mm in diameter were punched using sterile tips in each plate.

Preparation of toothpaste

An attempt was made to prepare toothpaste containing 1% and 2% L- arginine (Hi- media laboratories Pvt. Ltd) using trituration method. The liquid base was prepared first with humectants, preservatives and water. To this base binder was added, trituarated well and kept aside for 15 min to allow the binding agent to swell. Next, powder ingredients except detergent were mixed together and were added gradually to the aqueous mucilaginous mixture with slow but continuous stirring. After addition of all powders, flavouring agent was added. The finished product thus obtained was allowed to stand for 24 h. (Table 1)

The toothpastes were then divided into 4 groups:

Group 1: Kedodent, Group 2: Cherio, Group 3: 1% Arginine containing toothpaste, Group 4:- 2% Arginine containing toothpaste

Antimicrobial evaluation

The antimicrobial activity of the different toothpastes was determined by modified agar well diffusion technique. Two commercially available toothpaste (Cherio® – Dr Reddy's and Kedodent® - 0.38% w/w gel) for children were used to compare the antimicrobial efficacy of the arginine containing toothpaste. 50µl of each of the four groups of toothpaste were placed on predesignated area of 6 mm in diameter onto the surface of the plates and incubated at 35°C for 16 to 18 hours. After incubation, zone of inhibition was examined around the each well that contained the dentifrice. The diameters of the zones of inhibition around the well were measured using a vernier caliper.

Ingredients	Quantity	
Arginine	1%	2%
Calcium carbonate	5.2gm	5.2gm
Glycerine	2ml	2ml
Sodium carboxy methylcellulose	1.2gm	1.2gm
Sodium sachharine	0.2gm	0.2gm
Methyl Salicylate	0.2gm	0.2gm
Menthol	1ml	1ml
Water	5ml	5ml

Table 1: Composition of toothpaste containing 1% and 2% Arginine

Results

The results of the antimicrobial effect by the modified agar well diffusion method showed that all the formulations had significant activity against Enterococcous Faecalis and Candida albicans. The toothpaste containing 2% arginine was most effective against Enterococcous Faecalis and Candida Albicans with a zone of inhibition of 20.95 mm (Table no.2) and 16.95 mm (Table no.3) diameter respectively. The toothpaste containing 1% arginine was more effective against C. Albicans and had similar effects when compared with the commercially available Cherio® toothpaste.

Table 2: Comparison of mean Zone of Inhibition (in mm) for E. Faecalis between different study groups using One-way ANOVA test

Groups	N	Mean	SD	P-Value
Group 1	3	10.13	0.05	<0.003
Group 2	3	12.98	0.15	
Group 3	3	14.68	0.18	
Group 4	3	16.13	0.03	

Table 3: Comparison of mean Zone of Inhibition (in mm) for C. Albicans between different study groups using One-way ANOVA test

Groups	N	Mean	SD	P-Value
Group 1	3	15.13	0.29	<0.003*
Group 2	3	19.11	0.48	
Group 3	3	17.33	0.27	
Group 4	3	20.95	0.43	

Discussion

Oral bacteria that colonize the teeth form dental plaque, a multispecies microbial biofilm associated with the development of dental caries. A caries lesion is the result of acids produced by bacterial glycolysis of dietary carbohydrates causing demineralization of the tooth enamel. Continuous acidification of oral biofilms results in increase in the proportions of acid producing and acid-tolerant organisms, a selective process that alters dental plaque pH homeostasis and shifts the demineralization-remineralization balance toward loss of tooth minerals. Thus, while caries is known as a multi-factorial disease, low pH is its primary determinant.[6]

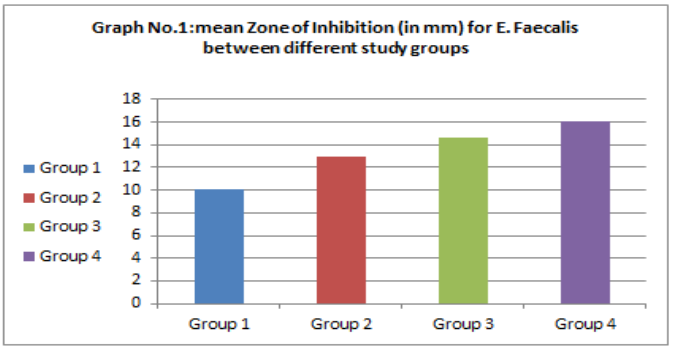
Arginine is found free in saliva in micromolar concentrations and is also abundant in salivary peptides and proteins. In oral biofilms, Arginine is mainly metabolized by the arginine deiminase system (ADS) of oral bacteria, which yields citrulline, ornithine, CO2, ATP, and ammonia contributing to the pH rise following the ingestion of carbohydrates and during fasting periods. Arginine metabolism has also been shown to significantly increase plaque pH in vitro and in vivo, even in the presence of carbohydrates, thereby inhibiting the progression of dental caries.[7]

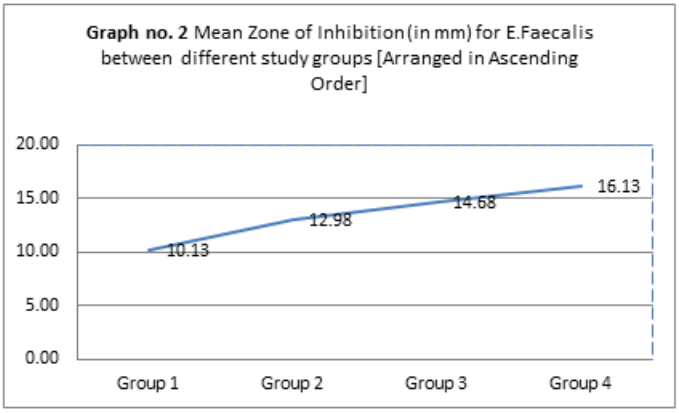
A variety of products containing arginine have been introduced in the market which includes dentrifices, mouthrinses, soft chew candies etc. Studies have shown that arginine containing products significantly reduces the plaque pH in the oral cavity and is proven to be beneficial in inhibiting the progression of caries.[8]

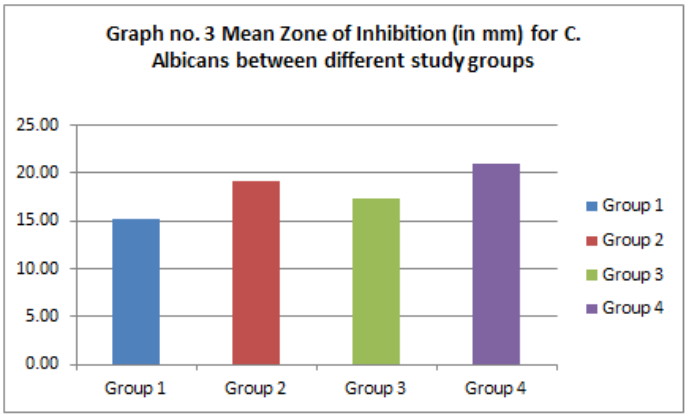
The present study evaluated the antimicrobial efficacy of prepared 1% and 2% arginine containing toothpaste against Enterococcous Faecalis and Candida Albicans (Table no.1 and no.2) and compared it with two commercially available fluoride containing toothpaste for children. The results of the study revealed that the arginine containing toothpaste had better antimicrobial activity than the commercially available toothpaste. Group 1 showed a mean Zone of Inhibition of 10.13 ± 0.49, Group 2 with 12.98 ± 0.15, Group 3 with 1468 ± 0.54 and Group 4 with 16.13 ± 0.46 against E. Faecalis between different study groups which was statistically significant at P<0.003. (Graph no.1 and 2) (Fig no. 1)

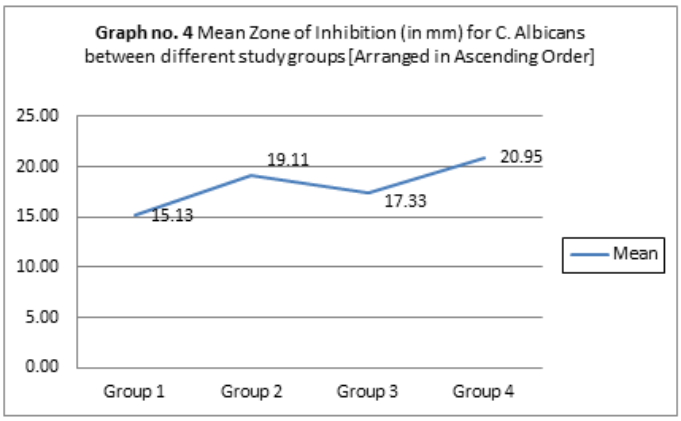
The antimicrobial activity against C. Albicans demonstrated that Group 1 showed a mean Zone of Inhibition of 15.13 ± 0.29, Group 2 with 19.11 ± 0.48, Group 3 with 17.33 ± 0.27 and Group 4 with 20.95 ± 0.43 which was statistically significant at P<0.003. (Graph no 3 and 4) (Fig no. 1)

Future in vivo studies are needed to establish the antimicrobial efficacy of different formulations of arginine on various microbial species and compare it with fluoridated toothpastes in different caries-related individuals to decipher the heterogeneity in the arginolytic capacity of plaque biofilms and their role in caries risk assessment and interventions. These arginine based formulations can be a promising solution to combat the possible side effects of fluoride in children.









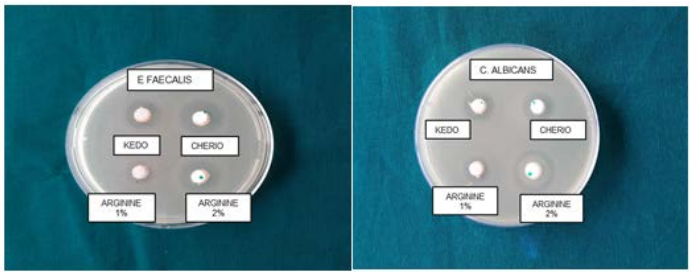


Fig 1: Zones of inhibition against E. faecalis and C. Albicans by the different toothpastes

Conclusion

The present study concludes that the 2% arginine containing toothpaste can be considered as promising formulations for oral diseases caused by Enterococcus faecalis and Candida albicans with regard to its significant antimicrobial activity. These arginine containing toothpaste can be used as an alternative to fluoridated toothpaste in children to reduce their possible side-effects in near future.

References

1. Nascimento MM, Liu Y, Kalra R, Perry S, Adewumi A, Xu X, Primosch RE, Burne RA. Oral arginine metabolism may decrease the risk for dental caries in children. Journal of dental research. 2013 Jul;92(7):604-8.
2. Al-Shahrani MA. Microbiology of dental caries: A literature review. Annals of Medical and Health Sciences Research. 2019.
3. Yu X, Chen J, Li Y, Liu H, Hou C, Zeng Q, Cui Y, Zhao L, Li P, Zhou Z, Pang S. Threshold effects of moderately excessive fluoride exposure on children's health: a potential association between dental fluorosis and loss of excellent intelligence. Environment international. 2018 Sep 1;118:116-24.
4. Green R, Lanphear B, Hornung R, Flora D, Martinez-Mier EA, Neufeld R, Ayotte P, Muckle G, Till C. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. JAMA pediatrics. 2019 Oct 1;173(10):940-8.
5. Nascimento MM, Burne RA. Caries prevention by arginine metabolism in oral biofilms: translating science into clinical success. Current Oral Health Reports. 2014 Mar 1;1(1):79-85.
6. Wolff, M.S.; Schenkel, A.B. (2018). The Anticaries Efficacy of a 1.5% Arginine and Fluoride Toothpaste.

Advances in Dental Research, 29(1), 93–97. doi:10.1177/0022034517735298

7. Ástvaldsdóttir Á, Naimi-Akbar A, Davidson T, Brolund A, Lintamo L, Attergren Granath A, Tranæus S, Östlund P. Arginine and Caries Prevention: A Systematic Review. Caries Res. 2016;50(4):383-93.
8. Ahsan, Haseeb (2019). Potential benefits of arginine formulation in oral health care products. Oral Science International, osi2.1021.