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Research Article

Formulation and antimicrobial evaluation of toothpastes containing arginine and proline

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ABSTRACT
Oral health is a component of general health and is important in the normal development of the child. Dental caries is the most prevalent dental disease affecting human race. Saliva acts as a protective factor against dental caries development by providing the main defense system for the host. Saliva contains large amount of proteins and amino acids that help to maintain the homeostasis of oral cavity. Arginine and proline are among the 20 amino acids that were identified in human saliva. So in the presnt work, an attempt was made to develop toothpastes containing arginine and proline by trituration method. The formulated toothpastes were evaluated as per standards specified in Bureau of Indian Standards. The antimicrobial efficiency of the prepared toothpastes were determined. Antimicrobial study showed that the formulations have significant activity against *Candida albicans* and *Streptococcus mutans.*

Keywords: Dental caries, arginine, proline, toothpaste*, Candida albicans, Streptococcus mutans.*

INTRODUCTION

Dental caries is an infectious microbiological disease of the teeth that cause localized dissolution and destruction of the calcified tissues[1]. Many attempts have been made to develop a medication using either fluorides or herbal constituents for this dental disease. Studies have shown that amino acids which are found in saliva arginine and proline have anticariogenic properties[2].

Dental caries is an infection usually bacterial in origin that causes demineralization of the hard tissues (enamel, dentin and cementum) and destruction of the organic matter of the tooth. It occurs usually by production of acid by hydrolysis of the food debris accumulated on the tooth surface. Dental caries is a common cause of tooth loss irrespective of age, sex, caste, creed or geographic location. It causes severe pain in the late stages, expensive to treat and leads to loss of precious man-hours. However, it is preventable to a certain extent. The prevalence of dental caries in India is 50% – 60%.Oral health problems adversely affect the quality of life[3].

Arginine can produce large amounts of base to effectively counter any cariogenic acid that is present, while favoring the emergence of arginolytic over non-arginolytic acidogens. This results in a less cariogenic plaque microflora. It favors an increase in the arginolytic bacterial component of the oral microflora, which in turn will favor an increase in alkalinity when arginine is available. Significantly higher ammonia production from arginine in saliva from caries free subjects affects the plaque ecology by suppressing the manifestation of a cariogenic microbiota[4].

The proline binds with calcium, aids in pellicle formation and in maintaining supersaturation of ionic calcium in relation to phosphate ions in saliva. Therefore, the proline may be of biological significance in maintaining the calcium homeostasis of saliva and in preventing the formation of salivary stones. In addition, proline inhibits apatitic crystal growth, suggesting that, when adsorbed on the tooth surface, they block specific mineral growth sites.

Proline is involved in typical oral processes like mineral homeostasis and neutralization of toxic substances in the diet. Certain basic proline rich protein alleles which bind oral streptococci and neutralize biofilm acids[2]. Hence this study has been designed to prepare the toothpastes containing arginine and proline, characterization of toothpastes and to find out antimicrobial efficacy.

MATERIALS AND METHODS

L- arginine was obtained from Merck Specialities Private Limited, Mumbai and L-proline was from Hi-media laboratories Pvt. Ltd., Mumbai. Calcium carbonate, glycerin, gum tragacanth, sodium alginate, sodium carboxy methyl cellulose, sodium lauryl sulphate, sodium saccharine, menthol, titanium dioxide, methyl paraben were procured from the college laboratory. Nutrient agar, Mutans Sanguis Agar, Sabroud Dextrose Agar, Mueller Hinton agar and Hi Antibiotic Zone Scale were from Hi-media laboratories Pvt. Ltd., Mumbai. All the chemicals and materials used were of analytical grade. Electronic balance (Ohaus corporation, Japan), $_p{}^H$ meter (Eutech instrument), hot air oven (KEMI- HUHS-2) were used in this study.

Preparation of the toothpaste with drug

Laboratory preparation of toothpaste was done by trituration method. A 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 sifted together and were added gradually to the aqueous mucilaginous mixture with slow but continuous stirring. After addition of all powders, flavouring agent(s) was/were added. Surface active agent was added at the end and mixed slowly and thoroughly to prevent aeration or foaming. Mixing was continued till all constituents were evenly distributed. The finished product thus obtained was allowed to stand for 24 h. The paste was finally filled into collapsible tubes, stored and used for further studies[5].

Evaluation of toothpaste

Determination of hard and sharp edged abrasive particles:

The paste was extruded about 15 to 20 cm length from the collapsible tube of each sample on a butter paper. Then all the samples were tested by pressing it along its entire length by a finger for the presence of hard and sharp edged abrasive particles[6].

Determination of spreadability:

About 1 gm of sample was weighed and placed at the centre of the glass plate (10X10 cm) and another glass plate was placed over it carefully. Above the glass plates 2 kg weight was placed at the centre of the plate avoid sliding of the plate. The diameter of the paste in centimeters was measured, after 30 min for all samples. The experiment was repeated three times and the averages were reported[6].

Determination of fineness:

A sample of 10 gm was accurately weighed and placed in a 100 ml beaker. To this 50 ml of water was added and allowed to stand for 30 min with occasional stirring until the toothpaste was completely dispersed. This solution was passed through 150 micron Standard sieve. Then the sieve was washed with running tap water. Washing should be continued until all the matters passed through the sieve. After washing the residue remained on sieves was collected and dried in an oven at 105 °C. After drying the sample was collected carefully and weighed[6].

Fineness was calculated by using the following formula,

$$\text{Percentage by mass} = \frac{M1}{M} \times 100$$

Where M1 - Mass in grams of residue retained on sieve

M - Mass in grams of material taken for the test

Determination of pH:

5 gm of sample was accurately weighed and placed in a 150 ml beaker. To this 45 ml of freshly boiled and cooled water was added at 27 °C. It was stirred well to make a thorough suspension. The pH was determined for all samples within 5 min by using digital pH meter[6].

Determination of foaming power:

5 gms of sample was weighed and placed in a 100 ml glass beaker. To this 10 ml of water was added and the beaker was covered with a watch glass and allowed to stand for 30 min, this operation was carried out to disperse the toothpaste in water. The contents of the beaker were stirred with a glass rod and the slurry was transferred to a 250 ml graduated measuring cylinder, during this transfer ensure that no foam was produced and no lump paste went into the measuring cylinder. The residue left in the beaker was transferred with further portion of 5-6 ml of water to the cylinder. The content of cylinder was adjusted to 50 ml by adding sufficient water and was maintained at 30 °C. The contents were stirred with a glass rod to ensure a uniform suspension. As soon as

the temperature of the content reached 30 °C, the cylinder was stoppered and 12 complete shakes were given to it. The cylinder was allowed to stand for 5 min and the volume of foam with water and water only was noted.

Foaming power is calculated by using the following formula

Foaming power = V1 – V2

Where V1 - Volume in ml of foam with water
V2 - Volume in ml of water only

Antimicrobial evaluation
Microorganisms:
Standard indicator strains were used for determining antimicrobial effect of toothpaste. The strains of *Streptococcus mutans*and *Candida albicans* were cultured in nutrient agar broth. The above mentioned organisms were subcultured on to their respective media *Streptococcus mutans* on Mutans Sanguis Agar*,* and *Candida albicans* on Sabroud Dextrose Agar.

Antimicrobial assay:
The antimicrobial activity of different toothpastes was determined by modified agar well diffusion technique. 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 were punched in each plates. The toothpastes were placed on predesignated area of 8 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. Diameters of the zones were measured with a Hi Antibiotic Zone Scale[7].

RESULTS AND DISCUSSION
The toothpastes were evaluated according to the standards specified by Bureau of Indian Standards.
The developed toothpastes were evaluated for hard and sharp edged abrasive particles and the formulations were free from hard and sharp edged abrasive particles.The results showed that the ingredients added to the toothpastes were ground properly and concluded that trituration is a good method for the preparation of toothpaste. The toothpastes are safe for the gums and enamel.
The maximum spreadability of toothpaste is 8.5 cm according to BIS standards. The prepared toothpastes

showed spreadability ranging from 5.8cm – 5.9 cm which concluded that all the formulations complied with the standard value. There was no difficulty in the filling of toothpastes and extrusion of toothpastes from the tubes.
According to BIS standards the maximum pH value of toothpaste is 10.5. The pH of the prepared formulations ranged from 9.4 – 9.5 and complied with the standard range of the toothpaste. So all toothpastes are considered safe for use.
The maximum fineness of the toothpaste is 0.5 % by mass according to the BIS standards. The fineness of the evaluated formulations ranged from 0.30- 0.32 % by mass which complied with the BIS standard of the toothpaste. It was concluded that there were no coarse particles in the toothpaste which cause scratching on enamel surface.
The minimum foam formation is 50 ml according to BIS standards. The foam formation of the evaluated toothpastes ranged from 55 - 56 ml showing that all the formulations complied with the standard values. The results showed that the foam formation of the evaluated toothpastes is sufficient for its cleansing action.
All the toothpastes which had been evaluated complied with the standards specified by BIS. Hence all the toothpastes were found to be of good quality.

ANTIMICROBIAL STUDIES
The toothpaste containing arginine (F1) was more effective against *Streptococcus mutans* with a zone of inhibition of 22.5 mm diameter and toothpaste containing proline (F2) showed a zone of 22mm diameter. Both toothpastes showed equal effectiveness against *Candida albicans* with a zone of inhibition of 17.75mm diameter. Antimicrobial study showed that all the formulations have significant activity against *Streptococcus mutans and Candida albicans*).

CONCLUSION
Thus the objectives of this investigation were realized with respect to development of toothpastes with arginine and proline and their evaluation and therefore the selected toothpastes can be consider as promising formulation for the treatment of dental caries caused by *Candida albicans* and *Streptococcus mutans*.

Table 1
Composition of Toothpastes containing Arginine 8%(F1) and Proline 8% (F2)

Ingredients	Quantity	
	F1	F2
Arginine (%)	8	-
Proline (%)	-	8
Calcium carbonate (%)	55	55
Glycerin (%)	30	30
Sodium carboxy methyl cellulose (%)	1.2	1.2
Sodium saccharine (%)	0.2	0.2
Sodium lauryl sulphate (%)	1.5	1.5
Menthol (%)	1	1
Titanium dioxide (%)	1	1
Methyl paraben (%)	0.1	0.1
Water up to (%)	100	100

Table 2
Evaluation of toothpastes as per Bureau of Indian Standards

Formulation	Hard and sharp edged abrasive particles	Spreadability (cm)	pH	Fineness (% by mass)	Foam formation (ml)
F1	Absent	5.9	9.4	0.3	55
F2	Absent	5.8	9.5	0.32	56

Table 3
Mean diameter of the zone of inhibition of toothpastes obtained after 18 hours of incubation.

Test organisms	Formulation	Mean Zone of inhibition (mm)
Candida albicans strain	F1	17.75
	F2	17.75
Streptococcus mutans strain	F1	22.5
	F2	22

Fig 1
Determination of hard and sharp edged abrasive particles

Fig 2
Toothpaste after spreadability test

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