Antibacterial activity of fluoride and nano-hydroxyapatite against Lactobacillus as an ingredient of toothpaste and mouthwash formulations: An In-vitro study.

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Scientific motivation

Nano-hydroxyapatite can bind to tooth and it is more effective in penetrating subsurface enamel, as well as fragments of plaque and bacteria when contained in toothpastes and mouth rinses. Fluoride has anticariogenic effect on biofilms it is known as anti-caries agent. For caries prevention fluoride and nano-hydroxyapatite are capable antibacterial agents.

Research indicates that nano-hydroxyapatite containing tooth paste and mouthwash are comparable to commonly used fluoride containing

toothpaste and mouthwash. However, the studies testing the antibacterial activity of nano-hydroxyapatite containing toothpaste and mouthwash against Lactobacillus are little known. Hence the study is to evaluate the antibacterial efficacy of nano-hydroxyapatite against Lactobacillus.

Tittle

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Abstract

Aim: The aim of the study is to evaluate the antibacterial efficacy of fluoride and nano-hydroxyapatite against Lactobacillus as an ingredient of toothpaste and mouthwash formulations.

Methods: The antimicrobial activity of fluoride containing toothpaste, mouthwash and nanohydroxyapatite containing toothpaste, mouthwash against Lactobacillus were determined by modified agar well diffusion method. Rogosa agar plates were used.

Experimental groups were made- Group I – control group (n=4 rogosa agar plates). Group II - Toothpaste containing fluoride (n=4). Group III - Toothpaste containing nanohydroxyapatite (n=4 rogosa agar plates). Group IV - Mouthwash containing fluoride (n=4 rogosa agar plates). Group V - Mouthwash containing nanohydroxyapatite (n=4 rogosa agar plates).

Plates were seeded with 0.5 mL of 24 h broth cultures of Lactobacillus acidophilus. The plates were allowed to dry for 1 h. One central and five wells at equidistance in each of the plates were cut. The selected toothpaste and mouthwash solutions were made. 0.2 ml of these prepared solutions were introduced into the wells. The plates were incubated at

37°C for 24 h. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition.

Statistical analysis: One-way Analysis of Variance (ANOVA) test was performed for intergroup comparison, followed by post hoc test.

Results: Amongst all the experimental groups, Group II (Fluoride containing toothpaste) had maximum zone of inhibition. {Mean=32.5 ± 1.47}. Amongst all the three group, Group I (Control group) had no zone of inhibition.

Conclusion: Group II (Fluoride containing toothpaste) showed maximum inhibition zone, i.e highest antimicrobial activity. Followed by Group IV (Fluoride containing mouthwash), Group III (Nano-hydroxyapatite containing toothpaste), Group V (Nano-hydroxyapatite containing mouthwash) and Group I (Control).

Key words: Nano-hydroxyapatite, Fluoride, lactobacillus, Rogosa agar plates, antimicrobial activity.

Introduction

In India, as in other developing countries, a very important proportion of dental problems are due to microbial infections. Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases.[1,2] Dental caries is a multifactorial disease that results from the interaction of specific bacteria and ingredients of the diet within plaque formed on the tooth surfaces.[3]

Cariogenic bacteria convert sugars into acids through a glycolytic process called fermentation. If left in contact with the tooth for a extended time period, these acids cause demineralization.[4] This process is dynamic; however, remineralization can also occur if the acid is neutralized and appropriate minerals are available in the mouth from saliva and also from preventive aids, such as toothpaste and mouthwash. If sufficient acid is

produced over a extended period of time in the favor of demineralization, caries will progress forming a cavity.[5]

Lactobacilli are Gram-positive bacteria that are transmitted to the oral cavity through the first few years of life. Lactobacilli are considered a major contributor to the advancement of caries.[6] Increased levels of Streptococcus mutans and Lactobacillus in the oral microbiota is related with the onset and advancement of tooth demineralization and caries.[7] Fluoride is one of effective agents for caries prevention. Fluoride ions make the tooth structure resilient against demineralization. In addition, they play a role in remineralization of demineralized tooth structure.[8] Some studies have shown that fluoride even inhibits colonization, metabolism, and growth of bacteria, prevents plaque maturation and reduces acid production by some species.[9] Hydroxyapatite is a naturally occurring and biocompatible mineral occurring in our teeth, bones and saliva. Nano Hydroxyapatite has a strong ability to bond with proteins, fragments of plaque and bacteria, when available in toothpastes. This is due to the size of the Nano particles. Nano Hydroxyapatite remineralizes teeth by refilling lost minerals like calcium and phosphate. It helps to repair teeth damaged by caries and also prevents caries progression.[10]

Experimental

Preparation of agar plates:

20 Rogosa agar plates were used.

Experimental groups were made:

Group I – control group. (n=4)

Group II - Toothpaste containing fluoride. (n=4)

Group III - Toothpaste containing nanohydroxyapatite. (n=4)

Group IV - Mouthwash containing fluoride. (n=4)

Group V - Mouthwash containing nanohydroxyapatite. (n=4)

Agar plates were seeded with 0.5 mL of 24 h broth cultures of each isolate of lactobacillus acidophilus. The plates were allowed to dry for 1 h. One central and five wells at equidistance in each of the plates were cut.

Preparation of toothpaste and mouthwash solutions:

The selected toothpaste i.e., fluoride and nano-hydroxyapatite containing toothpaste solutions were made by mixing the calculated amount of toothpastes (2.0 gm) in measured volume (2 ml) of sterile pyrogen-free distilled water to give 1:1 dilution. Similarly, each mouth rinse i.e., fluoride and nano-hydroxyapatite containing mouthwash (2 ml) were mixed with 2 ml of sterile distilled water.

0.2 ml of these prepared solutions were introduced into the wells. The same amount of sterile distilled water was introduced into the central well as a control.

The plates were incubated at 37°C for 72 h. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition.

Statistical analysis

The data obtained was coded and entered in Microsoft Excel 2007/2013. Descriptive and Frequency analysis was done by using Statistical Product and Service Solution (SPSS) (v.21.0) software. Normality of the data was checked by using the Shapiro-Wilk normality test. One way Analysis of Variance (ANOVA) test was performed for intergroup comparison, followed by post hoc test. p-value of < 0.05 was considered statistically significant at 95% confidence intervals.

Results

Table 1: Antibacterial properties of fluoride and nano-hydroxyapatite against Lactobacillus as an ingredient of toothpaste and mouthwash formulations of each sample in terms of zones of inhibition (in mm)

Sr no.	Group 1 (Control)	Group 2 (Fluoride toothpaste)	Group 3 (Nanohydroxyapatite toothpaste)	Group 4 (Fluoride mouthwash)	Group 5 (Nanohydroxyapatite Mouthwash)
1	0	33.5	25.5	27.8	24
2	0	31.5	28	28	28.2
3	0	31	25	29.5	24.5
4	0	34	26.8	30	23
AVERAGE	0	32.5	26.325	28.825	24.925

Table 2: Descriptive statistics of antibacterial properties of fluoride and nano-hydroxyapatite against Lactobacillus as an ingredient of toothpaste and mouthwash formulations of each sample in terms of zones of inhibition (in mm)

	Mean	SD	SE	Minimum	Maximum
Group 1 (Control)	0.0	0.0	0.0	0.0	0.0
Group 2 (Fluoride toothpaste)	32.5	1.47	0.73	31.0	34.0
Group 3 (Nanohydroxyapatite toothpaste)	26.32	1.35	0.67	25.0	28.0
Group 4 (Fluoride mouthwash)	28.82	1.09	0.54	27.8	30.0
Group 5 (Nanohydroxyapatite Mouthwash)	24.921	2.27	1.13	23.0	28.2

Table 3: Overall Comparative statistics of antibacterial properties of fluoride and nano-hydroxyapatite against Lactobacillus as an ingredient of toothpaste and mouthwash formulations of each sample in terms of zones of inhibition (in mm)

	Mean	SD	One way Anova F test	P value, Significance
Group 1 (Control)	0.0	0.0	F = 322.60	p < 0.001**
Group 2 (Fluoride toothpaste)	32.5	1.47		
Group 3 (Nanohydroxyapatite toothpaste)	26.32	1.35		
Group 4 (Fluoride mouthwash)	28.82	1.09		
Group 5 (Nanohydroxyapatite Mouthwash)	24.921	2.27		

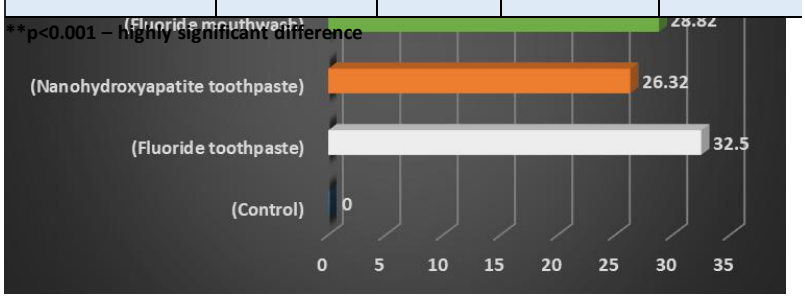

Table 4: Pairwise Comparative statistics of antibacterial properties of fluoride and nano-hydroxyapatite against Lactobacillus as an ingredient of toothpaste and mouthwash formulations of each sample in terms of zones of inhibition (in mm)

Tukey's post hoc test for pairwise comparison			
Group	**Comparison Group**	**Mean Difference**	**P value, Significance**
Group 1 (Control) vs	**Group 2 (Fluoride toothpaste)**	**32.5**	**p<0.001****
	Group 3 (Nanohydroxyapatite toothpaste)	**26.32**	**p<0.001****
	Group 4 (Fluoride mouthwash)	**28.82**	**p<0.001****
	Group 5 (Nanohydroxyapatite Mouthwash)	**24.92**	**P<0.001****
Group 2 (Fluoride toothpaste) vs	**Group 3 (Nanohydroxyapatite toothpaste)**	**6.17**	**P<0.001****
	Group 4 (Fluoride mouthwash)	**3.67**	**P =0.018***
	Group 5 (Nanohydroxyapatite Mouthwash)	**7.57**	**P<0.001****
Group 3 (Nanohydroxyapatite toothpaste) vs	**Group 4 (Fluoride mouthwash)**	**2.5**	**P =0.153 (NS**
	Group 5 (Nanohydroxyapatite Mouthwash)	**1.4**	**P =0.650 (NS**
Group 4 (Fluoride mouthwash) vs	**Group 5 (Nanohydroxyapatite Mouthwash)**	**3.9**	**P =0.012***

p>0.05 – not significant (NS) *p<0.05 – significant difference **p<0.001 – highly significant

Amongst all the experimental groups, Post hoc analysis showed that statistically significant results were obtained when the control group was compared with fluoride containing toothpaste, nano-hydroxyapatite containing toothpaste, fluoride containing mouthwash and nano-hydroxyapatite containing mouthwash.

- Group II (Toothpaste containing fluoride) had maximum zone of inhibition. {Mean=32.5 ± 1.47}. Group I (Control group) had no zone of inhibition.
- Group II (Toothpaste containing fluoride) had maximum inhibition zone {Mean=32.5 ± 1.47} followed by Group IV (Mouthwash containing fluoride) {Mean=28.82 ± 1.09}, Group III (Toothpaste containing nano-hydroxyapatite) {mean=26.32 ± 1.35}, Group V (Mouthwash containing nano-hydroxyapatite) {Mean= 24.921 ± 2.27}, Group I (Control).

DISCUSSION

The use of toothpaste as an adjunct to tooth brushing and the use of mouth rinse may assist to improve the effectiveness of oral hygiene practices in several ways.[11] It include inhibition of the bacterial adhesion to tooth surface, reduces the overall rate of accumulation of plaque and also decreases bacterial count of saliva.[12] In the current study, Antimicrobial activity was assessed by the agar diffusion method that is based on the measurements of the microbial inhibition zones against the tested microorganisms.

Among all the groups, Group II (Toothpaste containing fluoride) had maximum inhibition zone followed by Group IV (Mouthwash containing fluoride). The antibacterial effect of fluoride is well recognized and depends on the influx of hydrogen fluoride into bacterial cells and the dissociation to the H+ and F– ions in the cytoplasm. Fluoride also act as an inhibitor of bacterial enzymes, such as adenosine triphosphatase and enolase.[12,13] The metabolism of bacteria is inhibited by fluoride it also inhibit the energy and biosynthetic metabolism of bacteria.[14]

Group IV (Mouthwash containing fluoride) showed less zone of inhibition as compare to Group II. Fluoride toothpaste contains more

fluoride as compare to mouthwash which is not sufficient to fight bacteria. More resistance is seen with sodium fluoride containing toothpaste as it forms impervious layer rich in fluoride, which act against acid exposure.[15]

Group III (Nano-hydroxyapatite containing toothpaste) showed less zone of inhibition as compare to group II and group IV. This may be due to micro-cluster of hydroxyapatite reduce bacterial adhesion without killing the bacteria; this means biofilm management without effects on the ecology of the oral cavity. Bacterial receptors are blocked; coaggregation and bacterial adhesion to the pellicle are reduced.[16] There was no statistical difference seen between group III (Toothpaste containing nano-hydroxyapatite) and group IV (Mouthwash containing fluoride) Group III and group V (Mouthwash containing nano-hydroxyapatite).

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