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EVALUATION OF THE ANTIMICROBIAL ACTIVITIES OF THREE COMMONLY AVAILABLE TOOTHPASTES ON ORAL MICROBIAL FLORA.

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Abstract
The oral cavity provides a rich ecological niche within which microbes strive to survive, replicate and cause harmful effects. Toothpastes, with the aid of toothbrushes are used to clean the teeth and so improve on the integrity of the teeth. The efficacies of these toothpastes vary with some possessing higher activities than others. The activities of three brands of toothpastes (A, B, C) were evaluated for their antimicrobial efficacy employing the agar diffusion test method and also by determining the viability of the microbes in vitro and in vivo in the presence of the toothpaste. The results showed that brand A – a gel, had the greatest activity followed by B also a gel. Brand C, a paste, was the least active. There was a slight decrease in the viability of oral microbes after brushing compared to the control. Formulation type may also have some influence on activity.

Keywords: Antimicrobial activity; Toothpastes; Oral flora"

INTRODUCTION

The teeth and the oral mucosa are subject to disease conditions such as dental caries and non-specific chronic inflammation of the soft tissues immediately related to the teeth. The primary aetiological agents are oral bacteria and to a lesser extent, fungi. The bacteria in the mouth are present in the saliva, adherent to epithelium and deposits on tooth surfaces (Lee and Macdonald, 1984).

The oral cavity is also where food (carbohydrates, proteins, fats, vitamins, etc.) digestion starts. Bacteria break down carbohydrate or sucrose in the mouth to produce acids. Over every tooth, there is usually a layer of saliva and mucus in which bacteria thrive on food residues building up a coating known as plaques on the teeth. When left uncleaned, the plaques accumulate leading to the formation of a hard layer called tar-tar or calculus, on the tooth. This causes enamel decalcification and permits the influx of bacteria, weakening the structure and leading to cavity formation. As this continues, the dentine is softened and painful, pulp becomes inflamed and there is necrosis which is irreversible (Lee and Macdonald, 1984).

The bacteria implicated is the α-haemolytic streptococci of which *Streptococcus mutans* is the most important colonizer and causative agent of dental caries (Hennessy 1975; Hamada and Slave, 1980; Cohen et al, 1983; Hugo and Russell 1998).

The need and desirability for cleaning the teeth necessitated the formulation of safe and effective dentifrices. Toothpastes, which are used primarily for cleaning the teeth with the aid of toothbrushes are composed of

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medicaments compounded together to improve on dental integrity. This ranges from removal of plaque from the teeth surfaces, reduction of sensitivity of teeth to weather changes, increasing the whitening nature of teeth to prevention of halitosis (Odontocat®, 2003).

The study was undertaken to evaluate the effectiveness of three commonly available toothpastes found within the locality against oral microbes obtained from volunteers.

MATERIALS AND METHODS

Materials: Three (3) brands of commercial toothpaste: A, B (both gels) and C (a paste and imported) were obtained from local stores; sterile normal saline, Nutrient agar (NA) (Lab M International).

Sensitivity test: Mouth rinses from ten (10) volunteers were taken prior to brushing of their teeth with toothpaste in the morning using 50ml sterile normal saline. Aliquots of each were seeded into molten sterile nutrient agar such that the plates produced a dense but not confluent growth, poured into sterile Petri dishes and allowed to set. Cups were cut into the plates using a sterile cork-borer (no 5). Two concentrations of each toothpaste brand (undiluted and diluted) were filled into the cups such that 0.5g and 0.1g respectively of the original paste/gel filled each cup. A pre-diffusion time of 30mins was allowed before incubating at 37oC for 24hours. The zones of inhibition were then measured using a transparent plastic rule. The average of duplicate test results was taken.

Viability test: The ten volunteers were made to brush their teeth with sterile normal saline using new toothbrushes alone (saline wash of the new brushes showed a count of zero to <10 cfu). The base viable counts were obtained. Using the volunteer with the highest count, mouth rinses were obtained at timed intervals of 30minutes up to 180 minutes and aliquots were plated in NA. This served as the control. From the mouth rinse of the same volunteer at zero time, an aliquot of 8ml was mixed with 0.5g of toothpaste and at timed intervals of 30minutes up to 180 minutes 0.2ml were plated to determine survivors. The above procedure was repeated on another day but first the mouth was brushed with toothpaste and immediately rinses were obtained for baseline count and thereafter at timed intervals up to 180mins.

RESULTS AND DISCUSSION

The sensitivity test results showed toothpaste A as having the highest activity at both concentrations used followed by toothpastes B and C respectively as shown by the group performance (Table I). The variation in the sensitivity may be attributed to the formulation characteristics, active ingredients and release patterns of the active ingredients in the various toothpastes. Brands A and B, both gels, exist as flocculated systems which have different consistency, density and cohesiveness compared to C, a paste. Gels have faster release properties of their active medicaments independent of its water solubility compared to pastes which exist as disperse system containing of as much as 50% colloidal solute (Naim, 1995). This may explain why B showed a higher activity than C even though the active ingredients are the same (sodiumfluorophosphate). In addition to the fluoride salt, A also contains triclosan whose action has been shown to persist for 14 hours and may be synergistic with fluoride (Odontocat® 2003).

Brand C showed no activity when not diluted (Table 1) but in the diluted sample, activity could have been due increased release of the medicament due to increased degree of freedom of its colloidal solutes.

The in vivo viability test of A showed an initial drop, probably due to the mechanical effect of brushing, followed by a gradual increase in viable count (Table II). This may be attributed to interfering effect of organic

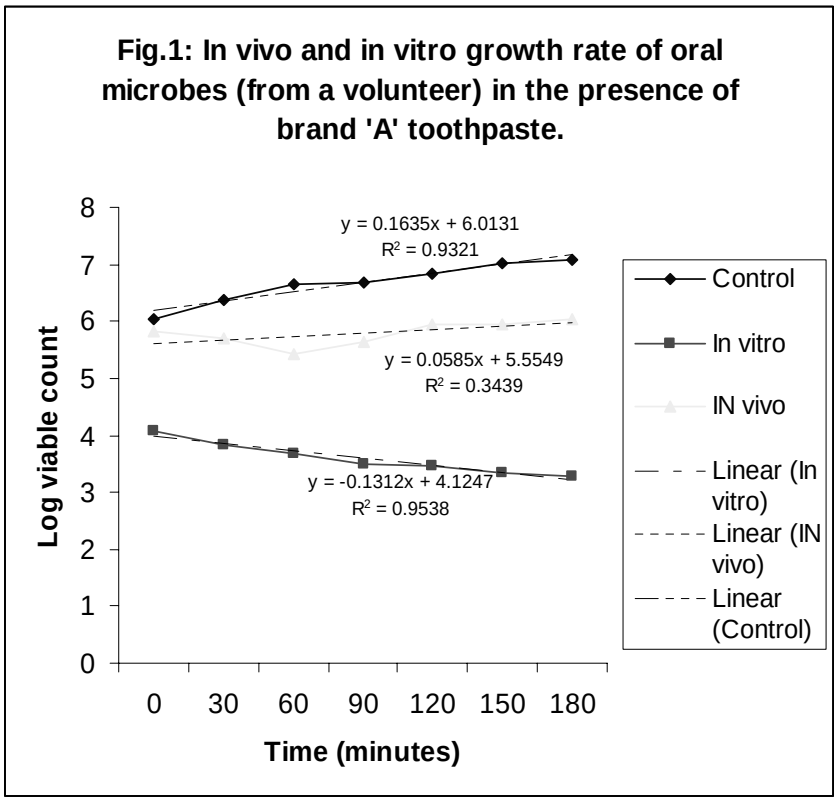
matter, ions, adhering plaques and mucous substances.

Table I: In-vitro sensitivity of oral microbes from ten (10) volunteers to three brands of toothpaste (A, B, and C)

Volunteers	Inhibition zone diameters (mm)					
	A		B		C	
	Diluted	Undiluted	Diluted	Undiluted	Diluted	Undiluted
I	13.25±0.25	28.25±2.25	11.25±0.25	22.75±0.25	12.50±1.50	-
II	14.00±0.00	24.00±5.00	16.50±2.50	26.50±0.50	14.00±1.00	-
III	13.00±0.50	25.75±0.75	12.25±1.75	25.25±2.25	11.50±1.50	-
IV	13.75±0.75	28.00±2.00	12.75±0.75	24.50±0.50	10.00±0.00	-
V	14.50±0.50	23.50±0.50	12.75±0.25	23.75±0.25	13.25±0.75	-
VI	14.50±1.50	25.25±0.75	14.00±1.00	23.50±1.00	11.25±1.75	-
VII	15.00±0.50	29.25±1.25	12.25±1.25	19.25±1.25	12.50±1.50	-
VIII	19.25±4.75	30.00±1.00	20.00±1.00	24.00±0.00	14.50±4.50	-
IX	13.25±0.25	21.75±1.75	12.50±1.00	22.00±0.50	-	-
X	14.75±0.75	24.25±1.75	13.50±0.50	24.75±0.75	-	-
Average	14.53±2.36	26.00±3.34	13.78±2.75	23.63±2.11	12.43±2.43	-

Table II: Effect of brand A toothpaste on in vivo and in-vitro growth rate of oral microbes.

Time (min)	*In vivo* (cfu/ml)	Log viable count	*In vitro* (cfu/ml)	Log viable count	Control (cfu/ml)	Log viable count
0	6.78×10^5	5.8312	1.21×10^4	4.0828	1.06×10^6	6.0269
30	4.96×10^5	5.6955	7.01×10^3	3.8457	2.45×10^6	6.3892
60	2.65×10^5	5.4232	4.85×10^3	3.6857	4.53×10^6	6.6561
90	4.34×10^5	5.6375	3.19×10^3	3.5038	5.13×10^6	6.6710
120	8.97×10^5	5.9528	3.01×10^3	3.4786	6.87×10^6	6.8370
150	8.96×10^5	5.9523	2.17×10^3	3.3365	1.02×10^7	7.0086
180	1.07×10^6	6.0294	1.85×10^3	3.2672	1.20×10^7	7.0792



However, the rate of increase in count was lower than that of the control (Fig 1). The consistent decrease shown in the in-vitro analysis may be ascribed to the continuous contact without interfering effects of the factors mentioned above.

CONCLUSION

The findings from this study indicate some value in the use of toothpaste as a way of maintaining oral hygiene with respect to oral bacteria. It may be suggested, however, that more than one application of brushing per day should be encouraged for greater efficacy as indicated by the results of the viability test. The formulation type, whether gel or paste, seem to have some influence on the efficacy of the product.

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