THE EFFECT OF COMMERCIALLY AVAILABLE LOCAL BRAND OF TOOTHPASTES AGAINST ORAL BACTERIA

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ABSTRACT

The objective of the study was to find the antimicrobial effect of local brands of toothpastes containing different active ingredients, against aerobic and anaerobic oral flora and Streptococcus mutans; the causative agent of dental caries and dental plaque.

The efficacy of eight locally formulated dentifrices was checked against aerobic and anaerobic oral bacteria of 30 subjects. Sterilized swabs were applied on the subject's teeth and inhibitory zones against these bacteria were measured by agar diffusion method. The magnitude and duration of Streptococcus mutans percentage reduction was evaluated against 1:100 and 1:1000 w / v dilutions of six dentifrices containing different active ingredients. The Streptococcus mutans were treated with selected dentifrices for 0,5,10,15,30,60 and 120 minutes. The CFU was done using spread plate method.

Triclosan containing toothpastes showed maximum inhibitory zones against aerobic and anaerobic oral bacteria followed by fluoridated, whitening and herbal dentifrices. The maximum percentage reduction of S. mutans was shown by triclosan containing toothpastes followed by fluoridated and herbal dentifrices.

All dentifrices showed antibacterial activity against aerobic and anaerobic oral bacteria, maximum being that of triclosan containing toothpastes. The longest and largest reduction of S. mutans was shown by triclosan containing dentifrices.

INTRODUCTION

The oral cavity is colonized by more than 400 species of aerobic and anaerobic bacteria. Anaerobic bacteria outnumber their aerobic counterparts by a ratio of 10:1 to 100:1. These organisms inhabit the teeth, the gingival crevice, the mucous membranes, the dorsum of the tongue and saliva(1). At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the mother in the first feeding. The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis.* These bacteria require non-desquamating (nonepithelial) surfaces in order to colonize[(2)].

The periodontal diseases are infections caused by /bacteria in the biofilm (dental plaque) that forms on oral surfaces. Two major divisions of periodontal diseases are gingivitis, which affects the gums and periodontitis, which may involve all of the soft tissue and bone supporting the teeth. Among the bacteria most commonly implicated in periodontal disease and bone loss are *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Veillonella parvula and Fusobacterium nucleatum*[(3)].

Dental caries is a disease, in which microbial fermentation of dietary carbohydrates forms lactic and other acids that leads to localized demineralization and destruction of the teeth. A cariogenic biofilm at a single tooth site may contain one-half-billion bacteria, of which species of mutans streptococci are critical components[(4)]. The characteristics contributing their

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cariogenicity are; ability to transport sugar, produces acid, tolerates acid's and produce extracellular and intracellular polysaccharides [5]

Various measures proposed for the prevention of dental diseases include antibacterial measures, dietary measures, modification in plaque metabolism and increasing resistance to tooth attack. The primary purpose of brushing the teeth with a dentifrice *(dens* - tooth, *fricare* - to rub) is to clean the accessible tooth surfaces of dental plaque, stains and food debris. Tooth cleaning with dentifrices dates back over 2000 years, while cleaning with toothpicks and brushes is an even older practice. Dentifrices have been prepared in several forms such as powders, pastes and gels. The most popular forms are the pastes and gels with over 5 billion tubes used worldwide each year. Several studies have shown that toothpaste has a key role in helping to remove dental plaque - the major cause of dental caries and periodontal diseases. Modern developments in toothpaste formulation have led to the addition of agents to provide therapeutic, as well as cosmetic, benefits. Frequently used, modern toothpastes can help prevent dental caries and limit the regrowth of dental plaque [6].

The exact composition of particular toothpaste varies with each manufacturer, but a typical formulation is abrasive 10-40%, humectants 20-70%, water 5-30%, binder 1-2%, detergent 1-3%, flavor 1-2%, preservative 0.05-0.5% and therapeutic agent 0.1-0.5%[6].

The therapeutic agents in toothpastes include fluoride and Triclosan. Use offluoride dentifrices is beneficial in the prevention of dental caries. Most dentifrices today contain fluoride, usually in the form of sodium monofluorophosphate (MFP). Triclosan, a broad spectruman antimicrobial agent used extensively in deodorants, soaps and other dermatological preparations, is the active agent in these new dentifrices[6]

The aims of the study are to obtain different isolates of *S. mutans* from subjects in Islamabad/ Rawalpindi city, to check the antibacterial activity of eight local brands of toothpastes against oral aerobic and anaerobic oral bacteria and to determine their anti-cariogenic activity against *S. mutans* for various durations at 1:100 w/v and 1:1000 w/v concentrations.

MATERIALS AND METHODS

Isolation of *S. mutans*

Collection of Sample

The paraffin stimulated saliva samples of 40 subjects were collected in sterilized glass tubes. The samples were carried in Microbiology Research Laboratory of Quaid-i-Azam University, Islamabad and processed immediately for isolation of *Streptococcus mutans* on GSTB agar media[7].

Identification of S. mutans isolates

The isolates were identified using morphological and cultural characteristics like size of colony, pigmentation, form, margin and elevation etc. The isolates were further identified by applying biochemical tests like Gram staining, MRVP test, Sugar fermentation tests, Hemolysis test, Bacitracin resistance test and Catalase test.

Inoculum preparation

A loop full of *S. mutans* isolates were inoculated in a test tube containing 10m1 of Jordan's Broth containing 5% sucrose and incubated at 37°C for 24 hrs.

Antimicrobial effect of locally formulated dentifrices

The anti-cariogenic activity of selected dentifrices containing different active ingredients was evaluated at various concentrations for various durations i.e. 0, 5, 10, 15, 30, 60 and 120 min.

Selection of dentifrices

Six locally formulated dentifrices, two triclosan containing toothpastes, two fluoridated and two herbal toothpastes were selected for observing the anti- bacterial activity against *S. mutans.*

Preparation of Toothpastes slurry

The toothpastes slurry was prepared by diluting them at 1:100 w/v and 1:100 w/v concentrations in distill water.

Experimental protocols

In sterilized eppendrofs, equal volume of S. *mutans* suspension and toothpaste slurry were dispensed and allowed to incubate for 0, 5, 10, 15, 30, 60 and 120 min.

at 37°C. No toothpaste was added to the *S. mutans* suspension in saline solution as a control. The bacteria were separated by centrifuging at 10,000 rpm for 15 min. The pelleted bacteria were washed with saline and were serially diluted. Using spread plated technique; the viable bacterial count was obtained on GSTB agar.

Agar Diffusion Assay

The sterilized swabs were applied on subject's teeth and lawn was made on duplicate nutrient agar plates. Toothpastes were diluted to 1:100 w/v concentrations and dispensed in two wells in agar plates. One plate was incubated aerobically for 24 hrs at 37°C and other anaerobically in candle extinction jar for 24 hrs at 37°C. Zones of inhibition were noted after 24 hrs.

RESULTS

The *S. mutans* isolates obtained from salivary samples of 40 subjects on GSTB agar media were identified on the basis of morphological, cultural characteristics and biochemical tests (Tables 1 and 2)[7][8]

Among Triclosan containing dentifrices (A and B), toothpaste A showed increase in percentage reduction of *S. mutans* at 1:100w/v concentration, from 0 min. (94.68%) to 15 min. (94.84%) Toothpaste B showed increase in reduction 0 min. (91.56%) to 15 min. (94.84%) (Fig 1). At 1:1000 w/v concentrations, toothpaste A showed increase in inhibitory growth from 0 min. (92.16%) to 30 min (94.29%) (Fig 3), toothpaste B from 0 min. (92.29%) to 120 min. (92.39%) (Fig 2).

TABLE 1: MORPHOLOGICAL AND CULTURAL TESTS RESULTS

Morphological identification on GSTB Agar	***Streptococcus mutans***
Size	0.02 to 0.7mm
Pigmentation	Beige to brown, dark gray, slate.
Form	Rough, frosted, circular and irregular.
Margin	Entire to undulate.
Elevation	Pulvinate to umbunate.

TABLE 2: DIFFERENTIAL CHARACTERISTICS OF *S. MUTANS* ISOLATES

Characteristics	***Streptococcus mutans***
Grams staining	**+**
Voges proskaur Test	**+**
Sugar fermentation test:	
Sucrose	+
Mannitol	+
Raffinose	+
Sorbitol	+
Salicin	+
Hemolysis Test	a
Bacitracin resistance	**+**
Catalase Test	**-**

a = No clearing of blood agar
+ = positive reaction - = negative reaction

At 1:100 w/v, two fluoridated toothpastes C and D exhibited increase in inhibitory activity. Toothpaste C showed increase in reduction from 0 min. (89.91%) to 15 min. (89.96%). Toothpaste D showed decrease in activity from 0 min. (86.32%) to 120 min. (78.87%) (Fig 3). Toothpaste C at 1:1000 w/v concentration, showed inhibition 0 min. (88.24%) to 60 min. (89.63%) (Fig 7) and toothpaste D 5 min. (85.91%) to 120 min. (81.76%) (Fig 4).

Herbal toothpastes (E and F) diluted to 1:100 w/v, toothpaste E showed increase in *S. mutans* reduction activity from 0 min. (87.21%) to 120 min. (91.54%) and toothpaste F showed 0 min. (89.31%) to 30 min. (89.89%) (Fig 5). At 1:1000 w/v, toothpastes E exhibited increase in percentage reduction 0 min. (83.00%) to 120 min. (89.27%) and dentifrice F showed increase in *S. mutans* reduction from 0 min. (87.56%) to 120 min. (89.48%) (Fig 6).

The inhibitory zones of two triclosan containing toothpastes (A and B) were checked against two fluoridated toothpastes (C and D), two whitening toothpastes (E and F) and two herbal toothpastes (G and **H).** Both triclosan containing toothpastes (Fig 7 and 8) produced maximum inhibitory zones against both aerobic and anaerobic oral bacteria followed by whitening, fluoridated and herbal dentifrices (Fig 9 and 10).

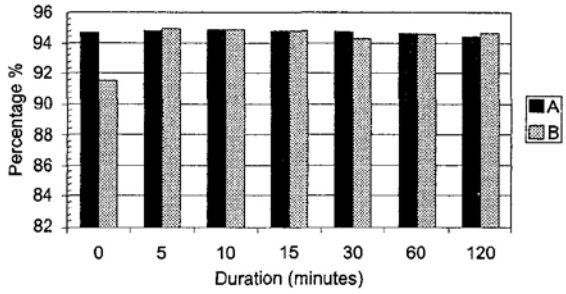

Fig 1: Percentage Reduction of *S. mutans* (2.5X10^5 CFU/ml) against two different dentifrices (1:100 w/v) containing Triclosan

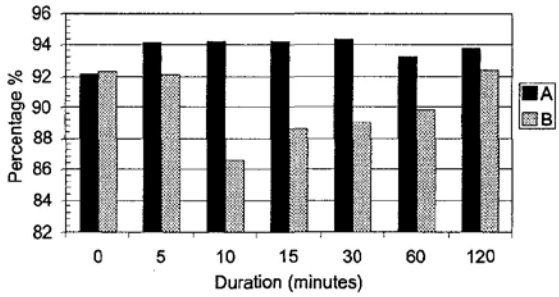

Fig 2: Percentage Reduction of *S. mutans* (2.5X10^5

against two different dentifrices
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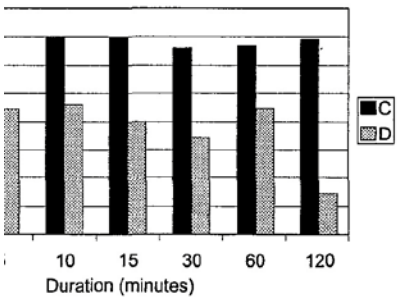

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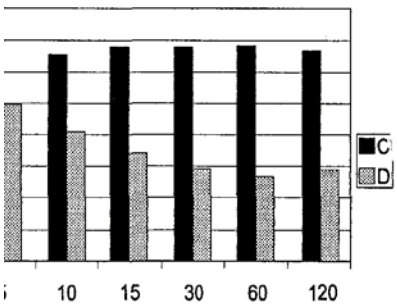


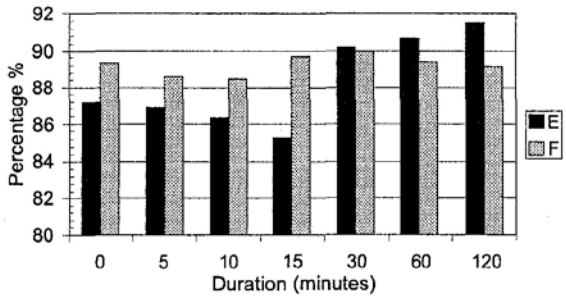
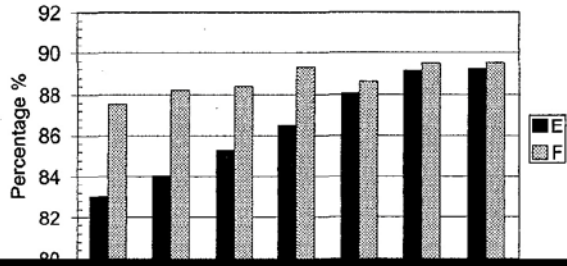


Fig 5: Percentage Reduction of *S. mutans* (2.5X10^5 CFU/ml) against two Herbal Dentifrices (1:100 w/v)



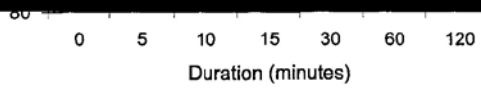
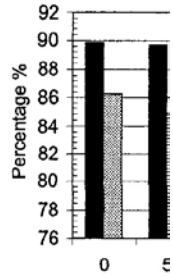


Fig 6: Percentage Reduction of *S. mutans* (2.5X10^5 CFU/ml) against two Herbal Dentifrices (1:1000 w/v)

CFU/ml)
(1:1000 w



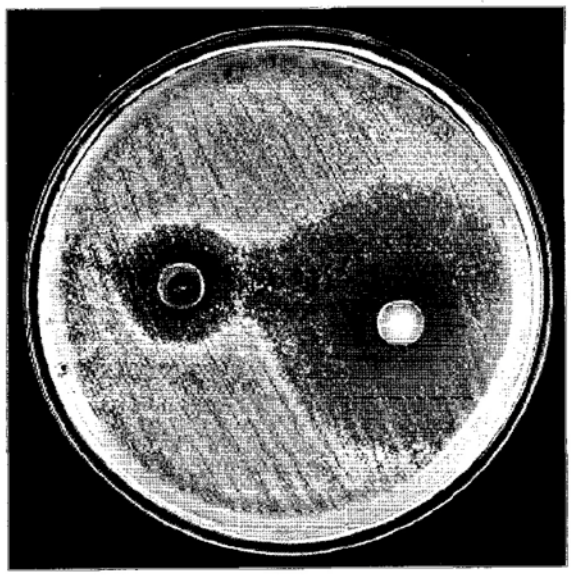
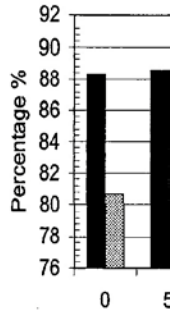


Fig 3: Percenta
CFU/ml)
w/v) cont



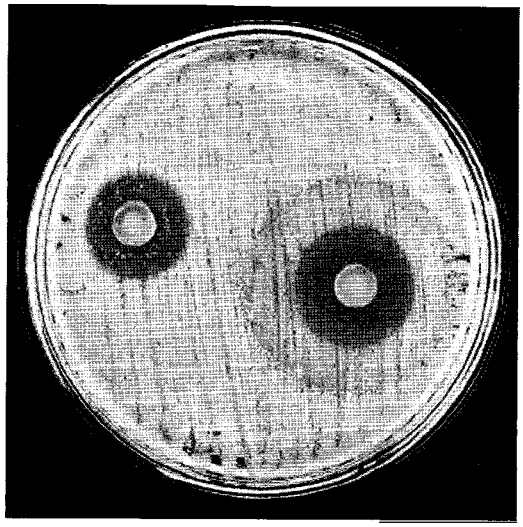


Fig 8: Inhibition Zones (cm) to triclosan containing dentifrices A and B against oral anaerobic bacteria.

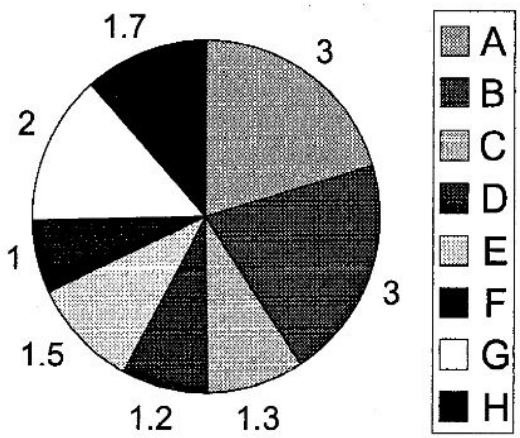


Fig 9: Inhibition Zone (cm) to different dentifrices against Oral Aerobic Bacteria

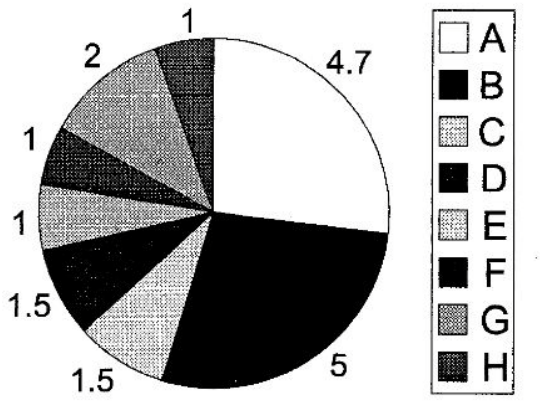


Fig 10: Inhibition Zone (cm) to different dentifrices against Oral Anaerobic Bacteria

DISCUSSION

Various preventive measures have been proposed for the prevention of dental diseases. The most popular measure to control the dental diseases is to alter the microbial flora by elimination of the pathogenic micro-organisms and by the enhancement of tooth resistance against the carious attack. The persistence of action of an antimicrobial agent relates to the plaque inhibitory action of that compound.

In present study, two triclosan containing tooth-pastes A and B exhibited considerable reduction in *S. mutans* as compared to other dentifrices. This study is in accordance with the finding in which consider-able reduction in salivary bacterial count was observed after treatment with triclosan containing dentifrice [9].

Triclosan dentifrices have also shown maximum inhibitory zones against oral aerobic and anaerobic bacteria. In various clinical studies, brushing with a 0.3% triclosan containing dentifrices when compared with a control paste resulted in significant reductions in dental plaque formation and gingival inflammation (10).

Fluoridated toothpastes also showed antibacterial activity against *S. mutans* and both aerobic and anaero-bic oral flora. Fluoride inhibits streptococci selectively an (12). Fluoride prevents caries by interfering with biofilm formation by the streptococci[13] However these effects are manifested only at high concentration of fluoride.

Herbal dentifrices antibacterial activity against oral bacteria was in consistent with the study of thirty-one Chinese medicinal toothpastes. The ability to inhibit growth, in vitro plaque formation and glucosyltransferase activity of *S. mutans* were observed by eighty-seven percent of the tested toothpastes [14]. The secondary metabolites in plants such as tannins, terpenoids, alkaloids and flavonides have been found to have antimicrobial properties [15].

Whitening toothpastes containing bicarbonate as active ingredient also exhibited antimicrobial activity against oral bacteria. The study evaluating the efficacy of bicarbonate toothpastes against placebo showed significant reduction of *S. mutans* as compared to placebo [16].

CONCLUSION

- The antibacterial activities against oral bacteria were exhibited by all dentifrices.
- The inhibition ofS. *mutans* was greater at 1:100 w/v dilutions than at 1:1000 w/v of toothpastes slurry.
- Triclosan containing dentifrices showed greater percentage reduction of *S. mutans* as compared to fluoridated and herbal toothpastes.
- Among all eight dentifrices, triclosan containing toothpastes exhibited maximum zone of inhibition.

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