Original Article

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COMPARATIVE EVALUATION OF VARIOUS MOUTHWASHES FOR THEIR EFFECT ON ORAL HEALTH: AN IN-VIVO STUDY

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ABSTRACT

AIM: To evaluate the efficacy of various mouthwashes for their effect on plaque accumulation, gingival inflammation and quantitatively with oral microflora. **MATERIALS AND METHOD:** Sample size selected for the study was 40 healthy children of 7-14 years with DMFT/deft= 0. Samples were randomly divided into four groups namely Group A, Group B, Group C and Group D who rinsed with water, chlorhexidine mouthwash, xylitol mouthwash and herbal mouthwash respectively. Plaque index (PI) and gingival index (GI) were recorded at baseline and after 30 days. Salivary samples were collected for evaluation of total microbial colony count at baseline and after 30 days. Results were tabulated and were statistically analyzed. **RESULTS:** It was found that after 30 days maximum reduction was shown by Group D (Herbal mouthwash) in plaque scores (from 0.431 to 0.268) and gingival scores (from 0.208 to 0.075) while minimum reduction was shown by Group B (Chlorhexidine mouthwash) in plaque scores (from 0.458 to 0.278) and gingival scores (from 0.189 to 0.078). After 30 days maximum reduction in total microbial colony count was seen in Group D (Herbal mouthwash) (from 146.1 to 34.2 CFU/ml) and minimum was seen in Group B (Chlorhexidine mouthwash) (from 141.6 to 77.2 CFU/ml). **CONCLUSION:** All the mouthwashes displayed anti-plaque, anti-gingivitis and anti-microbial activity, the maximum was seen in herbal mouthwash followed by xylitol mouthwash and least in chlorhexidine mouthwash.

KEYWORDS: Mouthwashes; plaque index; gingival index; microbial count

INTRODUCTION

Oral health is very important to the appearance and sense of well being. Emerging evidence has shown a strong link between the effects of oral health on the general health. According to WHO oral health is defined as a state of well being free from chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal diseases, tooth loss, other diseases and disorders that affect the oral cavity.[1] Oral health can be maintained on a regular basis by using different plaque control methods which include mechanical and chemical methods.[2] Mechanical methods include tooth brushing, interproximal cleaning using dental floss or interproximal brushes. Chemical methods involve the use of a dentifrice, mouthwash etc.[3] Regular tooth brushing is a very important step in preventing tooth decay and gum diseases. Brushing with a dentifrice removes bacteria that promote dental decay and plaque that can cause gum diseases.[4] Flossing removes plaque and bacteria that can't be reached with a toothbrush like the interproximal and cervical areas. If flossing is not done, more than one-third of tooth surface is missed of cleaning.[5] All the mechanical oral hygiene practices are a bit

Table 1: Mean values of Plaque Index (PI) and Gingival Index (GI) in Group A, Group B, Group C and Group D at baseline and after 30 days

Groups	Group A		Group B		Group C		Group D	
	PI	GI	PI	GI	PI	GI	PI	GI
Baseline	0.414 ± .02415	0.203 ± .03162	0.458 ± .06563	0.189 ± .04581	0.426 ± .10035	0.212 ± .04452	0.431 ± .06293	0.208 ± .05116
After 30 days	0.451 ± .03659	0.254 ± .02908	0.278 ± .04423	0.078 ± .04427	0.296 ± .08020	0.087 ± .03802	0.268 ± .03659	0.075 ± .02214

Table 2: Inter-comparison of mean percentage (%) increase or decrease of Plaque Index (PI) and Gingival Index (GI) in Group A, Group B, Group C and Group D after 30 days of study

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p – value	95% Confidence Interval	
						Lower Bound	Upper Bound
PI after 30 days	Water	Chlorhexidine	.14800	.02355	.000*	.0811	.2149
		Xylitol	.17600	.02355	.000*	.1091	.2429
		Herbal	.22000	.02355	.000*	.1531	.2869
	Chlorhexidine	Xylitol	.02800	.02355	.758	-.0389	.0949
		Herbal	.07200	.02355	.229	.0051	.1389
	Xylitol	Herbal	.04400	.02355	.349	-.0229	.1109
GI after 30 days	Water	Chlorhexidine	.17100	.01585	.000*	.1259	.2161
		Xylitol	.18400	.01585	.000*	.1389	.2291
		Herbal	.19400	.01585	.000*	.1489	.2391
	Chlorhexidine	Xylitol	.01300	.01585	.923	-.0321	.0581
		Herbal	.02300	.01585	.599	-.0221	.0681
	Xylitol	Herbal	.01000	.01585	.969	-.0351	.0551

Table 3: Mean values of total bacterial colony count in Group A, Group B, Group C and Group D at different time intervals

S. No.	Groups of Mouthwashes	At baseline	After 30 days
1	Group A	127.6±79.75	136.2±70.47
2	Group B	141.6±57.129	77.2±52.74
3	Group C	151.3±64.10	69.4±54.31
4	Group D	146.1±55.29	34.2±39.80

difficult to be accomplished in young children as they require good manual dexterity. So especially in children the maintenance of oral health can be supplemented with regular use of mouthwashes as they are commonly used chemical methods of plaque control.[4] Mouthwashes are antibacterial in nature and help in preventing carious bacteria to flourish in the mouth. They can be broadly classified as chemical mouthwashes and herbal mouthwashes. Chemical mouthwashes containing: chlorhexidine, a bis-biguanide which is the most commonly used and is gold standard in antimicrobial efficacy. [6]Inspite of them being used since ages, they still have certain disadvantage like discoloration of teeth, dryness of mouth, erosion of enamel etc.[2] Herbal mouthwash provides a viable alternative as they are alcohol-free, chemical free and contains time tested herbal oils and extracts like- neem oil, clove and peelu that actually promote oral health.[3]Nowadays a newer chemical xlitol, which is a sugar substitute, is introduced into mouthwashes. It has an advantage of being not easily degenerated by micro-organisms against

Table 4: Comparison of mean percentage (%) increase or decrease of total bacterial colony count of Group B, Group C and Group D with Group A after 30 days

S. No.	Comparison between	Mean ± S.D.	p value
1	Group A & B	1.648±.824	0.004*
2	Group A & C	2.572±2.867	0.0003*
3	Group A & D	3.759±6.338	0.0000*

Table 5: Inter-comparison of mean percentage (%) increase or decrease of total bacterial colony count in Group B, Group C and Group D at 30 days of study

Groups		N	Mean	Std. Deviation	Std. Error Mean	p value
Dimension 1	B	10	-84.2923	5.86728	2.03132	0.042*
	D	10	-80.4368	4.23689	1.86450	
Dimension 1	B	10	-84.2923	5.86728	2.03132	0.542
	C	10	-82.1581	4.66329	2.23685	
Dimension 1	C	10	-82.1581	4.66329	2.23685	0.448
	D	10	-80.4368	4.23689	1.86450	

easily degenerated by micro-organisms against other sugar polyols.[7] Efficacy of the use of mouthwashes on oral health are evaluated with the help of indices: Plaque Index (Silness and Low, 1964) and Gingival Index (Low and Silness, 1963)[8] and for antimicrobial efficacy of mouthwashes, microbiological colony count of the oral micro-flora is done.[9] Thus, if a twofold effect on bacterial count as well as plaque accumulation and gingival inflammation could be found with mouthwashes, practicing daily self oral care would be very much benefitted especially for children. Thus, keeping this in mind the following study was undertaken to evaluate the efficacy of various mouthwashes on oral health status and micro-flora.

MATERIALS & METHODS

SAMPLE SIZE SELECTION

A sample size of 40 healthy children under the age group of 7-14 years from an orphanage with DMFT/deft =0 and satisfying all the inclusion and exclusion criterion of the study were selected.

INCLUSION CRITERIA

1. Caries free children with DMFT/deft = 0.
2. Children without any known systemic illness.
3. Children >7yrs and <14yrs.
4. Children should be staying in same area or conditions for >2 yrs.
5. With no recent history of use of antimicrobial agents or any other drugs (1 week).

EXCLUSION CRITERIA

1. Children using any other oral hygiene aids other than routine brushing with a dentifrice.
2. Children undergoing orthodontic treatment.
3. Children with history of professionally applied topical Fluoride.
4. DMFT/deft >0.

Consent was taken from the orphanage authority for the conduction of study on selected participants and an agreement was made, not to use any other oral hygiene products than those assigned during the study, including mouthrinses, dentifrices, whitening or therapeutic chewing gums or whitening formulations etc. Participants were instructed not to visit any dental surgeon during the study period and no participation in other studies was agreed upon. The 40 participants were divided into 4 different groups with equal number of children in each group: Group A - Control group (water), Group B - Chlorhexidine group (Hexidine), Group C - Xylitol group (Spry), Group D -Herbal group (Hiora) (Fig. 1). The subjects in the study were demonstrated toothbrushing technique (Fonne's technique) and instructed to take same amount of toothpaste (pea size). They were also instructed to brush teeth twice daily for 2 minutes, thus standardizing the duration, technique and time for brushing teeth.

RECORDING OF PLAQUE INDEX AND GINGIVAL INDEX

Plaque Index (PI) (Silness and Loe, 1964) and Gingival Index (GI) (Loe and Silness, 1963) were recorded for all the participants at baseline and after 30 days (Fig. 2). Before collection of the saliva, subjects were instructed not to eat or drink (except water) for 30 min. and also not to perform any physical exercise for at least an hour. Stimulated saliva was collected for bacterial

Fig. 1: Materials used in the study

Fig. 2: Recording of Plaque Index and Gingival Index

Fig. 3: Collection of salivary samples

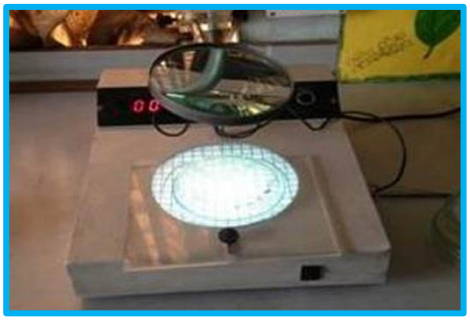


Fig. 4: Sample in colony counter machine for evaluation

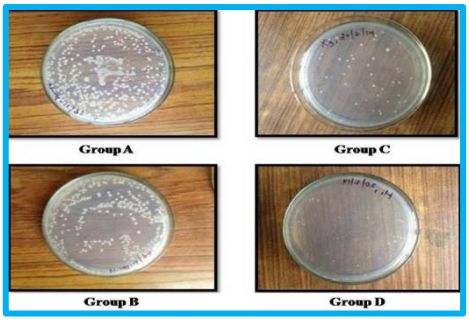
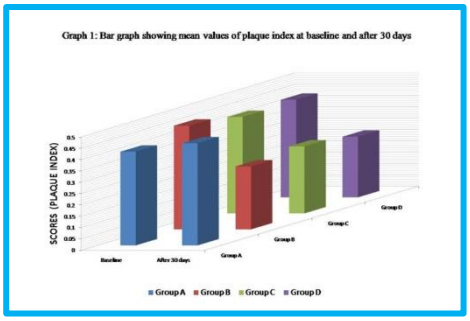
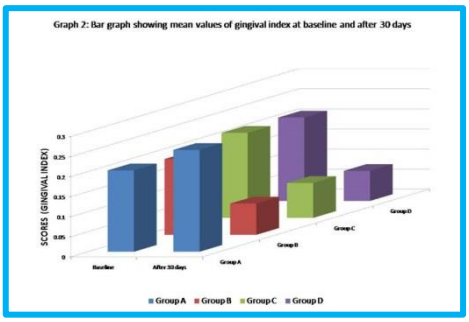


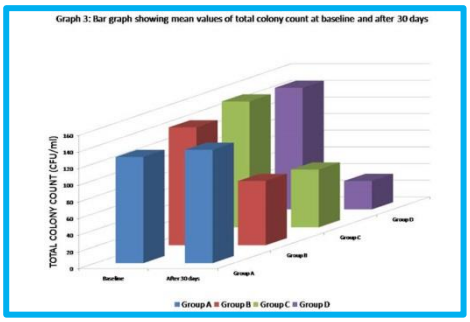
Fig. 5: Microbial Colony Morphology



Graph 1



Graph 2



Graph 3

examination. Subjects were given paraffin wax to chew to stimulate salivary flow which was then collected by expectorating in a sterile disposable measuring cup over the next 2 minutes and stored in the thermocol jar containing dry ice. The salivary samples were collected at baseline and after 30 days and were transported for the microbiological evaluation within an hour (Fig. 4 & Fig. 5).

Dispensing of Mouthrinses (According to the Manufacturer's Guidelines)

i. Group A was instructed to rinse twice daily with 10 ml of water for about 30 minute after toothbrushing for 1 min.
ii. Group B was instructed to rinse twice daily with 10 ml of chlorhexidine mouthwash with 1:1 dilution in water for about 30 min. after toothbrushing for 1 minute.

iii. Group C was instructed to rinse twice daily with 10 ml of xylitol mouthwash for about 30 min. after toothbrushing for 30 secs.
iv. Group D was instructed to rinse twice daily with 15 ml of herbal mouthwash for about 30 min. after toothbrushing for 30 secs.

STATISTICAL ANALYSIS

As the baseline values were variable thus percentage increase or decrease in the mean values has been taken to statistically evaluate plaque index, gingival index and microbial colony count. The data was statistically analyzed using Independent "t" test, and the intercomparison among various groups was done using Dunnett test. Statistical significance was taken at $p < 0.05$.

RESULTS

After 30 days maximum reduction was shown by Group D in both plaque scores(from 0.431 to 0.268) and gingival scores (from 0.208 to 0.075) while minimum reduction was shown by Group B in both plaque scores (from 0.458 to 0.278) and gingival scores (from 0.189 to 0.078) (Table 1) (Graph 1 & Graph 2). Total microbial colony count after 30 days, the maximum reduction was seen in Group D (from 146.1 to 34.2 CFU/ml) and minimum was seen in Group B (from 141.6 to 77.2 CFU/ml) (Table 3) (Graph 3). Group A (where no mouthwash was used) had shown an increase in the value of plaque scores (from 0.414 to 0.451), gingival scores (from 0.203 to 0.254) and total bacterial count (from 127.6 to 136.2 CFU/ml) (Table 1 & Table 3). The mean value of Plaque index and Gingival index was found to be significant at ($p < 0.05$) when comparison of Control group (Group A) was done with the Experimental groups (Group B, Group C and Group D) after 30 days. The p value was found to be non-significant (p-value > 0.05) when inter-comparison was done in the Experimental group i.e. between Group B, Group C and Group D after 30 days (Table 2). The mean value of total microbial count was found to be significant ($p < 0.05$) when inter-comparison of Control group (Group A) was done with the Experimental groups i.e. Group B, Group C and Group D after 30 days (Table 4). The p value was found to be non-significant (p-value > 0.05) in all the Experimental group inter-comparisons, except when Group B was compared with Group D (Table 5).

DISCUSSION

In the present study it was found that the mean value of Plaque Index (PI), Gingival Index (GI) and micro-biological evaluation decreased in all the experimental groups where mouthwashes has been used, from baseline till the cessation of the study (i.e. at 30 days). The maximum percentage decrease was seen in group D (Herbal mouthwash) followed by group C (Xylitol mouthwash) while minimum reduction was seen in group B (Chlorhexidine mouthwash). Thus, herbal mouthwash showed the maximum efficacy against plaque, gingivitis and micro-organisms. Its component Salvadora perisca has a bactericidal effect and Terminalia bellirica was found to help in tissue healing and repair. The astringent effect of tannins may help to reduce clinically detectable gingivitis. Tannins were found to inhibit the action of glucosyltransferase, thereby reducing plaque and gingivitis. The mildly bitter taste of the essential oils in herbal mouthwash stimulates the flow of saliva, which increases its buffering capacity.[10] High concentrations of chloride inhibit the formation of calculus and aid in removing stains from tooth surfaces. Peppermint has astringent and anti-inflammatory activity which is due to presence of ursane and glycosides.[11] Similar results were shown by studies done by several authors.[12-14] Xylitol mouthwash showed better efficacy than chlorhexidine mouthwash as xylitol is a naturally occurring non-cariogenic sugar substitute that cannot be metabolized by oral bacteria. It inhibits transfer of cariogenic bacteria from person to person and reduces bacterial recolonization over time. Xylitol reduces the total counts of micro-organisms and synthesis of adherent extracellular polysaccharides, leading to a change in the plaque-saliva distribution of plaque forming bacteria. The loosely bound plaque and micro-organisms are suggested to be easily shed to the saliva during mechanical saliva stimulation.[15] Similar results were shown by studies done by Arunakul M *et al*.,[7] and Ghiraldini B *et al*.,[16] Chlorhexidine is found to be effective against *S. mutans* but not much against other microbes in-vitro, but when used in-vivo it is much less effective as the micro-organisms are enclosed in a biofilm and are present deeper in the gingival crevices or mucosal folds and cannot be effectively reached. That is why chlorhexidine

reduces micro-organisms but does not usually eliminate it except with intensive use, with high-concentration and after repeated applications.[17] Current chlorhexidine products require patient compliance with a rinse that tastes bad and has the potential to stain.[18] Thus, least efficacy against micro-organisms was found in chlorhexidine mouthwash. When intercomparison of mean plaque & gingival index was done between experimental groups where mouthwashes were used, the result was found to be non-significant (at p-value > 0.05). Indicating that all the mouthwashes taken in the study had comparable efficacy in terms of oral and gingival health. Biswas G *et al*., did an in-vivo study to evaluate the anti-plaque and anti-gingivitis activity of chlorhexidine and herbal mouthrinse and found both of them to be equally effective.[19] When intercomparison of mean total colony count was done between experimental groups, the result was found to be non-significant at p-value > 0.05 in all the comparisons, except when herbal mouthwash was compared with chlorhexidine mouthwash. Reinforcing again that herbal mouthwash was found to display significantly more antimicrobial activity than chlorhexidine mouthwash though it is comparable to xylitol mouthwash. Balappanavar AY *et al*., did a study and found herbal mouthwash has more efficacy than 0.2% chlorhexidine for maintaining the oral health.[12] Well within the limitations of the study, it can be concluded that anti-plaque and anti-gingivitis and antimicrobial efficacy of xylitol and herbal mouthwash were equivalent and herbal mouthwash was found to have more antibacterial property as compared to chlorhexidine mouthwash. We recommend that further studies should be conducted to test various mouthwashes in a prospective randomized clinical trial.

CONCLUSION

1. All the mouthwashes displayed antiplaque, antigingivitis and antibacterial activity
2. The maximum antiplaque, antigingivitis and antibacterial activity was displayed by herbal mouthwash followed by xylitol mouthwash and minimum was shown by chlorhexidine mouthwash

CONFLICT OF INTEREST & SOURCE OF FUNDING

The author declares that there is no source of funding and there is no conflict of interest among all authors.

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