



**Research Article**

## **EVALUATION OF ANTIMICROBIAL EFFICACY OF THREE COMMERCIALY AVAILABLE TOOTHPASTES ON SALIVARY MICROBIAL COUNT**

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### **ABSTRACT**

**Aim:** To assess the antimicrobial efficacy of three commercially available toothpastes on salivary microbial count.

**Materials and Methods:** Thirty children were randomly allocated into three groups. Group I provided with Cheerio Oral Gel, Group II provided with Himalaya complete care toothpaste, Group III provided with Vicco Vajradanti toothpaste. Saliva samples were then processed at the baseline score (Day 0) and after completion of the experimental period i.e. after 15 days for antimicrobial efficacy.

**Results:** Data collected was statistically analysed using SPSS version 21.0 and ANOVA test.

**Conclusion:** Cheerio oral gel was superior as compared to Himalaya and Vicco Vajradanti and all the three pastes proved equally effective in reducing bacterial counts.

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### **INTRODUCTION**

Dental caries is the most prevalent dental disease affecting human race which are globally rendered as one of the most frequently occurring oral diseases affecting 60-90% of schoolchildren and almost 100% of adults worldwide. (Shadin. A.S. *et al*, 2018) The development of caries is dependent on several ingredients, which include; elevated bacterial counts of mutant streptococci (MS) and lactobacillus (LB) in dental plaque, consumption of sugar-filled and carbohydrate-based foods, along with vulnerable/susceptible teeth. (Soham.B *et al*, 2015)

Saliva is a complex fluid consisting of 99% of the water and remaining 1% of organic and inorganic molecules. It plays a major role in the demineralization and remineralization processes of the teeth. Human saliva is 99.5% water, while the other 0.5% consists of electrolytes, mucus, glycoproteins, enzymes, and antimicrobial agents such as secretory immunoglobulin and lysozyme. The normal pH of saliva is 6.7–7.4, but as bacteria break down carbohydrates, they release lactic acid, butyric acid, and aspartic acid which bring down the pH of saliva. (Mary.D *et al*, 2018)

When the pH in the oral cavity decreases, the thermodynamic conditions become unfavorable, and there is a driving force for hydroxyapatite dissolution or demineralization. (Van Zyl A.W. *et al*, 2010)

Dentifrices are considered agents with antibacterial potential which could have a beneficial effect on plaque control and disease prevention. (Pires.J.R *et al*, 2007) The attributes of the dentifrices which may affect their cosmetic or therapeutic effect are their physical form, chemical composition, their pH, their solubility. (Kalyan CP, 2013) Therefore the aim of this article was to assess the antimicrobial efficacy of three commercially available toothpastes on salivary microbial count which includes Cheerio oral gel, Himalaya Complete Care and Vicco Vajradanti.

### **MATERIALS AND METHODS**

**Study Design**

The study was conducted in Mathrusya Child Home, an orphanage, in Tirupati, Chittoor district. A total of 30 children of age group of 5–10 years who fulfilled the inclusion and the exclusion criteria were selected to participate in the study.

**Inclusion and exclusion criteria**

Inclusion criteria included children who had DMF/def score zero. Children free from any systemic illness or using any medication. Parents who were able to understand the purposes, risks and benefits of this study. Parents who were able to give written consents if needed.

The exclusion criteria for selection included presence of any marked intraoral soft tissue pathology, subjects with history of taking antibiotics 3 months prior to or during the course of study, medically compromised patients, children undergoing

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orthodontic therapy, and children with history of professionally applied topical fluoride.

**Division of Samples**

Consent was taken from the orphanage authority for conducting the study on the participants selected 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, whitening formulations, etc. Participants were instructed not to visit any dental surgeon during the study period. Not participating in other studies was agreed upon by the participants.

The study subjects were provided with same toothbrushes (oral B kid) and were demonstrated same tooth brushing technique (Fones technique). The time (2 min), duration (twice daily), and amount of dentifrice were also kept same for all children to maintain standardization, which was personally monitored.

The selected children were divided into three groups, each consisting of 10 subjects as follows: Group I: Cheerio oral gel (Dr.Reddy's Laboratories Ltd., Hyderabad, India), Group II: Himalaya Complete Care (The Himalaya Drug Company, Bengaluru, India), Group III: Vicco Vajradanti (Vicco Laboratories, Nagpur, India). The study took place over a period of 15 days.

**Collection of salivary samples**

Saliva collection was done in the morning after routine oral hygiene procedure. Standardization of the saliva collection technique was done by asking the subjects not to perform any physical exercise and not allowing them to eat or drink (except water) 1–2 h before salivary sample collection. The saliva was collected over a period of 4 mins. The procedure was repeated on 1st day, at the end of 15<sup>th</sup> day.

Samples were transported for microbiological evaluation in dry ice (at -70°C) within 4–6 h.

**Laboratory procedure**

The samples were vortexed to uniformly mix the saliva. 1 ml of collected saliva was diluted to 1:10,000 times. Using an inoculation loop (standard loop with 4 mm diameter), 10 µl of the vortexed sample was streaked on nutrient agar plate. Inoculated plates were incubated for 48 hours at 37 °C in an incubator. The bacterial colonies on the plate were counted using a colony counter machine and were expressed as number of colony forming units per milliliter (CFU/ml) of saliva.

The baseline score (Day 0) recorded served as control. The counts obtained after 15 days were then compared with the baseline counts.

**Statistical analysis**

The data obtained were statistically analyzed using SPSS software, version 21 using ANOVA at 95% confidence level (p≤ 0.05, significant).

**RESULTS**

The results of this investigation showed that Cheerio oral gel tooth paste had maximum reduction in microbial colony count compared to all other tooth paste formulations. Table 1 shows the mean bacterial counts at base line and day 15.

**Table 1** Mean percentage of colony forming units per milliliter (CFU/ml) in Groups I, II, and III

GROUPS		n	Mean ± S.D	f - value	p - value
Base Line	Group A	10	77.80 ± 6.5	3.7	0.005
	Group B	10	83.20 ± 5.7		
	Group C	10	86.60 ± 8.9		
	Total	30	82.53 ± 7.87		
Day 15	Group A	10	40.10 ± 5.1	9.2	0.001
	Group B	10	49.70 ± 9.7		
	Group C	10	55.30 ± 8.3		
	Total	30	48.37 ± 10.0		

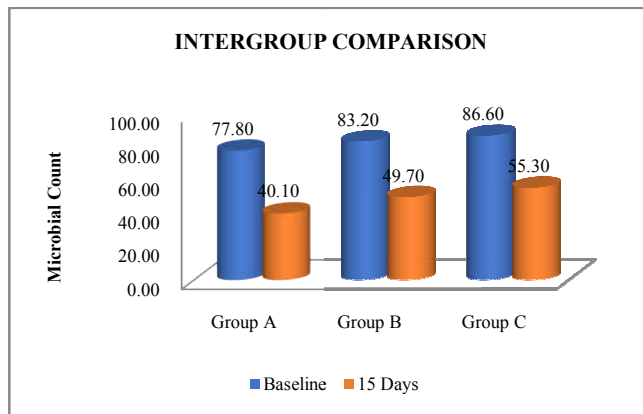
Table 2 shows the intergroup comparison of all the three commercially available toothpastes where the Cheerio oral gel showed the maximum decrease in colony count at the end of 15 days.

**Table 2** Intergroup Comparison of Mean percentage of colony forming units per milliliter (CFU/ml)

GROUPS	Period	n	Mean ± S.D	f-value	p - value
GROUP A Cheerio Oral gel	Base Line	10	77.80 ± 6.5	14.3	<0.001
	Day 15	10	40.10 ± 5.1		SS
GROUP B Himalaya Complete Care	Base Line	10	83.20 ± 5.7	9.3	<0.001
	Day 15	10	49.70 ± 9.7		SS
GROUP C Vicco Vajradanti	Base Line	10	86.60 ± 8.9	8.1	<0.001
	Day 15	10	55.30 ± 8.3		SS

SS – Statistically Significant

Graph 1. shows the intergroup comparison between the three toothpastes at the baseline and after 15 days. The mean colony count statistically reduced in Cheerio oral gel group than the Vicco Vajradanti and Himalaya Complete Care toothpaste. The figures 3, 4 and 5 represent the bacterial colonies in each group after 15 days which was statistically significant and greater reduction of colonies were seen in Cheerio oral gel Group.



**Graph 1** Intergroup Comparison of three tooth pastes



**Fig 1** Microbial colony count of Cheerio



Fig 2 Microbial colony count of Himalaya



Fig 3 Microbial colony count of Vicco Vajradanti

## DISCUSSION

Microorganisms play a vital role in causation of dental caries. Various studies have provided evidence of bacterial specificity in caries etiology. Complete removal of microorganisms from the oral cavity is impossible, but their count can be reduced so that it becomes less cariogenic with the help of various preventive measures, for example, probiotics, antibiotics, fluorides, and oral hygiene aids.(Mc Donald, 2004)

Various oral hygiene measures are available, such as tooth brushing, dental flossing, mouthwashes, dentifrices, etc., among which tooth brushing with dentifrice is the most commonly used. These mechanical measures are feasible, cost-effective, and can easily be used by children. Dentifrices are therapeutic mechanical aids which are available as tooth powder or toothpaste and aid in removal of plaque.(Bhati.N *et al*,2015)

Several clinical studies have demonstrated the inhibitory effects of antimicrobial dentifrice on oral bacteria and gingiva. The findings of the present study is in accordance with the study conducted by Patil *et al.*, where in a steady decrease in *S mutans* was observed over a period of 5 months after using Fluoridated and Herbal toothpaste containing neem.(Patil.S *et al*, 2010)

Fluorides are abundantly used in many oral health products including toothpastes and mouthrinses as they help in caries prevention.(Grant.D.A *et al*, 1979) When formulated correctly

and used as directed, fluoride toothpaste will help to safely and effectively prevent tooth decay. It is well documented the ability of fluoride to inhibit or even reverse the initiation and progression of dental caries.(Marinho.V.C.C, 2009) Thus in the present study the fluoridated tooth paste Cheerio oral gel shows the superior activity.

Fluoride retention in saliva and plaque increases significantly with the frequency of application.(Nordstrom A *et al*, 2012) Fluoridated dentifrices are considered most effective caries control products (Hirose.M *et al*, 2015) and most commonly employed technique for caries reduction.(Gamboa F *et al*, 2004; Saravana KK *et al*, 2015) Herbal extracts have received special attention for being non-chemical and non-synthetic in nature.(Shaheen SS *et al*, 2015)

Formulations II and III are herbal and ayurvedic based products and exhibited least effectiveness compared to the other test formulations. This may be due to the ingredients present. Using natural medicines to cure various diseases has become an increasing trend. Herbal medicine has made significant contribution to modern medical practice.(Almas.K *et al*, 2001)

Though studies in animals and invitro have shown the antimicrobial properties of several of these herbs, there is no other way of knowing their real clinical effects without a randomized clinical trial. In the present study, the herbal formulations studies appeared to be equally effective as the fluoride formulations, but not superior to them.(Amruthesh.S *et al*, 2010)

Study done by Gibraiel *et al.*,<sup>19</sup> also inferred that the toothpaste formulation containing natural anti-microbial agents were more effective in controlling the oral microflora compared to toothpastes containing synthetic antimicrobial agents. Maria *et al.*,<sup>20</sup> compared *in vitro* antimicrobial effect of herbal based and fluoride based toothpaste on *S. mutans*. Fluoride toothpaste showed superior antibacterial activity.

When intercomparison of mean bacterial counts was done between groups where dentifrices were used, the result was found to be significant at the interval of 15 days of the study [Table 2]. This indicates that the fluoridated dentifrices showed maximum reduction in bacterial colony counts compared to the other two formulations.

The promising results shown by Cheerio oral gel suggests that further studies should be undertaken to explore the antibacterial efficacy of Cheerio against various other specific microorganisms and also with combination of other equally effective herbal ingredient.

## CONCLUSION

The present study was conducted to compare three commercially available toothpastes and their effect on salivary microbial count level. Results from this study have shown that Cheerio oral gel was superior as compared to Himalaya and Vicco Vajradanti and proved equally effective in reducing bacterial counts.

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