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COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF TWO COMMERCIALLY AVAILABLE HERBAL AND NON-HERBAL (FLUORIDATED) TOOTHPASTES ON SALIVARY MICROBIAL COUNT: AN *IN-VIVO* STUDY

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ABSTRACT: Dental caries is a complex disease which is expressed as an interaction between various factors including the host, agent, substrate and time. The interplay between the dental biofilm and oral microorganisms is an important feature for development of a cariogenic dental plaque. Formation of the biofilm needs to be controlled through regular brushing in order to prevent the development of caries and periodontal diseases. Hence a study was conducted to evaluate and compare *in-vivo* the effect of two commercially available herbal and non-herbal (fluoridated) toothpastes on salivary bacterial count in 5 - 10 years age children with 3 - 6 decayed teeth. 40 children were randomly allocated into two groups I and II. Group I were provided with Fluoridated (Kidodent) toothpaste and Group II with Herbal (Babool) toothpaste. Participants were scored for plaque index and saliva sample was collected as the baseline score (day 0). After demonstration of brushing technique participants were asked to brush twice daily. The plaque index score and saliva sample was once again collected at the end of Day 15. The baseline mean bacterial counts significantly reduced from 37.65 to 15.57 in Group I and from 38.08 to 16.88 in Group II. Whereas mean plaque index score were reduced from 1.75 to 0.71 in Group I and 1.87 to 0.79 in Group II. Both Kidodent and Babool significantly reduce bacterial and plaque scores at 15 days interval. But there was no statistical significant difference between two pastes.

INTRODUCTION: Dental caries is considered as a localized and transmissible pathological infectious process that results in the destruction of hard enamel tissue (Loesche 1986) [1]. It is a complex disease which is expressed as an interaction between various factors including the host, agent, substrate and time [2]. The main etiological agents of dental caries are *Streptococcus mutans* and *Lactobacillus species* [3].

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Their interplay within the dental biofilm is an important feature for the establishment and maintenance of the micro flora and it is also linked with the development of a cariogenic dental plaque [4]. Dental plaque is considered as the precursor of dental caries, gingivitis and periodontitis [6].

The oral bacterial species metabolize sucrose to lactic and other acids in dental plaque produced on the surface of the tooth and dissolve calcium phosphate in the enamel, consequently giving rise to dental caries [5]. Formation of the biofilm is a natural process in the oral environment, but it needs to be controlled through regular brushing in order to prevent the development of caries and periodontal diseases [7]. Both chemical and mechanical oral hygiene aids are used for removal

and prevention of plaque. Mechanical plaque control measures, such as toothbrushes, dental floss, toothpicks and interdental brushes are very popular and are mostly used in conjugation with chemical plaque control aids, *e.g.* mouth rinses and medicated toothpastes [7]. Fluoride therapy has been the cornerstone of caries preventive strategies ever since the introduction of water fluoridation schemes over five decades ago [1].

Antimicrobial mechanisms of toothpastes containing fluoride are through interfering the glucose transport, carbohydrate storage, extra-cellular polysaccharide formation and acid formation by oral streptococci [8]. Recently, a number of chemical agents have been advocated which are available in a toothpaste or dentifrices or in the form of a mouthwash [9, 11]. Many herbal and homeo-based dentifrices claim to have antimicrobial properties.

It is important to determine the efficiency of antibacterial effect of different toothpastes brands which have different ingredient to reduce bacterial load in human mouth and contribute to dental health [10]. As Herbal formulations can provide an option for safe and long term use as they do not require alcohol, artificial preservatives, flavours or colours for their activity [12], interest in alternative toothpaste based on plant extracts has increased recently [2].

Because the popularity of natural medicines and dentifrices continues to rise, dental professionals are in a position to provide information to patients about safety and efficacy of these products. This study therefore seeks to evaluate the efficacy of Fluoride and Babool containing toothpastes which are commercially available (over-the counter) on the salivary microbial count level as well as to compare if any difference exists in the antimicrobial action between the Fluoridated and Babool containing toothpastes or both have equal efficacy against salivary microorganisms.

MATERIALS AND METHOD: The study was conducted among children in the age group of 5 - 10 years. 40 children who fulfilled inclusion and exclusion criteria were selected from Department of Pedodontics and Preventive Dentistry of MGV Dental College and Hospital, Nashik.

Inclusion Criteria:

- 40 children in the age group of 5 - 10 years with 3 - 6 decayed teeth were included.

Exclusion Criteria:

- Marked intraoral soft tissue pathology.
- Subjects with history of taking antibiotics three months prior or during the course of study.
- Medically compromised patients.
- Children undergoing orthodontic therapy.
- Children with history of professionally applied topical fluoride.
- Children with previously restored teeth.

After obtaining ethical committee clearance and informed consent from parents, 40 children were selected randomly and were categorized into two groups.

Group I: Consist of 20 children to use Fluoridated (Kidodent) toothpaste.

Group II: Consist of 20 children to use Herbal (Babool) toothpaste.

A. Method of Data Collection: Prior to the commencement of the study, through oral prophylaxis of all the participants were done using ultrasonic scaler to render them plaque, stain and calculus free. They were then asked to refrain from normal tooth cleaning habits. All the participants from both the groups underwent a washout period of 2 1/2 days to rule out any possible carryover of the previously used oral hygiene products [2, 14]. The washout was done by brushing with water alone [2, 13, 14] and then followed by the treatment period of 15 days. At the end of the washout period, participants were scored for plaque index and saliva sample was collected as the baseline score (day 0). Following baseline data collection, the participants were given toothpastes of their respective groups and were asked to brush their teeth with pea size of toothpaste and toothbrush twice daily under their parents supervision. The tooth brushing technique (Fonne's technique) was demonstrated to participants and parents. There were no changes in their dietary habits. The plaque index score and saliva sample was once again collected at the end of Day 15.

B. Method of Recording Plaque Index: The plaque index was assessed according to Silness P. and Loe H. (1964).

C. Method of Saliva Collection: Saliva collection was done in the morning after routine oral hygiene procedure. Children were made to sit comfortably on the chair. After swallowing pre-existing saliva; saliva was allowed to accumulate in the floor of the mouth for approximately five min and then saliva was collected by asking children to spit into a 30 ml sterile container. The collected Sample was taken to laboratory within 1 h of collection [1, 15].

D. Laboratory Procedures: The laboratory procedures were done in the Department of Microbiology, MGV Dental College. 0.1 ml of collected saliva was diluted to 1:10,000 times using micropipette and 0.1 ml of diluted sample was inoculated on nutrient agar plate. Using L- shaped glass spreader the salivary sample was uniformly spread on nutrient agar. The inoculated plates of nutrient agar were then incubated for 48 h in 37 ºC in an incubator. The number of colony forming units (CFU) were then counted by manual method and recorded. Computation of the number of colonies was done by multiplying the obtained count with 1×10^5 as the sample was diluted ten thousand times. The baseline score (Day 0) recorded served as control. After completion of the experimental period *i.e.* after 15 days again saliva sample was collected. The samples were then processed as discussed previously. The counts obtained were then compared with the baseline counts.

Statistical Analysis: Statistical analysis was performed using SPSS statistical computer software and the data were analyzed using paired t-test and un-paired t test.

RESULTS: At the baseline (Day 0) Group I had a mean bacterial count of $37.65 \times 10^5 \pm 2.86$ cfu/ml of saliva. Group II had $38.08 \times 10^5 \pm 2.32$ cfu/ml of saliva. When an unpaired t-test was performed, p value was 0.60, which was not statistically significant. After 15 days of toothpaste therapy the mean bacterial count of Group I was $15.57 \times 10^5 \pm 2.72$ cfu/ml of saliva and Group II was $16.88 \times 10^5 \pm 1.98$ cfu/ml of saliva. When an unpaired t-test was performed, p value was 0.08. There is no statistically significant difference between the two toothpastes although there has been a significant reduction of bacterial count in two groups when compared to baseline as depicted in **Table 1**.

TABLE 1: INTER GROUP COMPARISON OF FLUORIDATED AND HERBAL TOOTHPASTES FOR BACTERIAL COUNT AT DAY 0 AND 15

		Fluoridated (Kidodent) toothpaste		Herbal (Babool) toothpaste		
Time	**N**	**Mean**	**SD**	**Mean**	**SD**	**P Value**
Day 0	20	37.65×10^5	2.8651	38.08×10^5	2.32	0.60
Day 15	20	15.57×10^5	2.7290	16.88×10^5	1.98	0.08

At the baseline (Day 0) Group I had a mean Plaque index score 1.75 ± 0.26. Group II had 1.87 ± 0.20. When an unpaired t-test was performed, p- value was 0.11, which was not statistically significant. After 15 days of toothpaste therapy the mean Plaque index score of Group I was 0.71 ± 0.19 and Group II was 0.79 ± 0.15. When an unpaired t-test was performed, p-value was 0.14. There is no statistically significant difference between the two toothpastes although there has been a significant reduction of plaque index scores in two groups when compared to baseline as depicted in **Table 2**.

TABLE 2: INTER GROUP COMPARISON OF FLUORIDATED AND HERBAL TOOTHPASTES FOR PLAQUE INDEX AT DAY 0 AND 15

		Fluoridated (Kidodent) toothpaste		Herbal (Babool) toothpaste		
	N	**Mean PI**	**SD**	**Mean PI**	**SD**	**P Value**
Day 0	20	1.7550	0.26170	1.8765	0.20145	0.11
Day 15	20	0.7135	0.19980	0.7910	0.15927	0.14

DISCUSSION: Fluoridated dentifrices are considered most effective caries control products [17] and most commonly employed technique for caries reduction [18, 15]. The caries preventive effect of low fluoride toothpastes may be slightly less as compared to that of adult strength alternatives but considering the fact that most children tend to swallow toothpaste while brushing and the adverse

effects associated with it, the use of low-fluoride toothpaste by children under 6 years of age seems justified. Herbal extracts have received special attention for being non-chemical and non-synthetic in nature [19]. Indian gum Arabic tree Acacia, belong to the family Leguminosae, and has been recognized worldwide as a multipurpose tree [20]. *Acacia arabica* bark has been used as demulcent, nutritive supplement, expectorant, styptic and tonic and have astringent, immunosuppressant, antibacterial, antitumor, antithrombotic, hypoglycemic and anti-helminthic activities [21]. Babool serves as a source of polyphenols. The phytochemicals contribute chemically to a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes [22]. The bark, root, gum, leaves and flowers have found use for skin diseases, diarrhea, dysentery, cough, diabetes, eczema, wound healing, burning sensation and as an astringent, demulcent, anti-asthmatic. The tender twigs are used as toothbrushes.

The finding of the present study is in accordance with the study conducted by Patil *et al*., [1] where in a steady decrease in Strep. Mutans was observed over a period of 5 months after using Fluoridated and Herbal toothpaste containing neem. Toothpaste containing neem and fluoride were equally effective in reducing counts of bacteria. Study done by Gibraiel *et al*., [23] 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.

J. Sunitha *et al*., [24] compared the antimicrobial effect of few herbal dentifrices against cariogenic organism such as *Streptococcus mutans* and *Lactobacillus acidophilus*. The study concluded that in case of *S. mutans*, the maximum antimicrobial effect among the six dentifrices (Neem, Vicco Vajradanti, Himalaya Herbal, Colgate Herbal, Dabur Red, and Dabur Babool) was shown by Babool followed by Colgate Herbal. For *L. acidophilus*, the antimicrobial zone exhibited by all the six dentifrices were similar to the positive control (colgate total).

Maria *et al*., [25] compared *in vitro* antimicrobial effect of herbal based and fluoride based toothpaste on *S. mutans*. Fluoride toothpaste showed superior antibacterial activity. Studies done by Williams *et al*., [26] and Dumas *et al*., [27] also reported colgate total as a better dentifrice as compared to herbal dentifrice. The results of the present study are not in accordance with these studies as they were performed *in-vitro* and the effect of dentifrice when used *in-vivo* is influenced by saliva and time of brushing.

Mohan Kumar *et al*., [28] and Sabiha *et al*., [19], conducted *in vitro* study comparing various herbal dentifrice with conventional dentifrice by measuring zones of inhibition created around the disc concluded that herbal toothpastes are equally efficient and can also be recommended like conventional formulations, as found in the present study. Even if patient swallows Babool toothpaste no harm is caused. On contrary it provides systemic benefits. This may be beneficial to disabled, bedridden patients, mentally or physically handi-capped children whom cannot perform daily oral hygiene care. Fluoridated toothpaste (Kidodent) used in the present study costs about Rs.88 for 75g, which might be too expensive for people from lower economic strata. The promising results shown by Babool suggests that further studies should be undertaken to explore the antibacterial efficacy of Babool against various other specific microorganisms and also with combination of other equally effective herbal ingredient.

CONCLUSION: Although the results showed by herbal toothpaste were not superior as compared to fluoridated toothpaste, it proved equally effective in reducing bacterial counts and plaque scores. Hence it can be recommended as a cost effective alternative to conventional fluoridated toothpaste, thus reducing the changes of fluoride toxicity.

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CONFLICT OF INTEREST: The authors hereby declare no conflict of interest in the publication of this paper.

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