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Efficacy of various mouth rinses on dental plaque, gingival inflammation and microbial count in children: An in –vivo study

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Abstract

Aim: The aim of this study is to evaluate and compare the efficacy of various mouth rinses on plaque accumulation, gingival inflammation and microbial counts in children.

Materials And Method: The study was conducted on 90 (Ninety) healthy children with permanent dentition in the age range of 13 to 15 years. The subjects were divided into 3 experimental groups namely Group A, Group B and Group C who rinsed with Chlorhexidine mouth rinse (CHX) 0.2% , Essential oil mouth rinse (EO) and Povidone- iodine mouth rinse (PVP-I) respectively. Plaque index and gingival index were recorded at baseline ,7th day and 14th day of intervention. Salivary samples were collected for evaluation of total microbial colony count at baseline ,7th day and 14th days of intervention . Results were tabulated and statistically analyzed.

Results: At final evaluation, the decrease in mean Plaque index (i.e. Mean change from baseline to day 14) of Group A was found to be the highest (45.7%) followed by Group B (28.8%) and Group C (26.7%) the least , gingival index (i.e. Mean change from baseline to day 14) of Group A was found to be the highest (67.8%) followed by Group B (58.1%) and Group C (48.4%) the least and microbial counts (i.e. Mean change from

baseline to day 14) was 100.0% in all three groups. **Conclusion**: There are some limitations of home oral hygiene practices in children like inadequacy in brushing, constant education, motivation and manual dexterity which makes it difficult in reducing plaque solely by mechanical means and bacteria are usually left behind. Therefore, the use of chemotherapeutic agents in the form of mouth rinses have a key role as adjuncts to daily home care.

Keywords: Mouthrinses, Plaque Index, Gingival Index, Microbial Count

Introduction

Plaque is the primary etiological factor in gingival inflammation. Oral hygiene failure results in the formation of pathogenic plaque.[1]Therefore, plaque control represents the cornerstone of good oral hygiene practice. The mechanical supragingival plaque control includes the toothbrush, floss, woodsticks, and interdental brushes. However, the degree of motivation and skill required for the effective use of these oral hygiene products may be beyond the ability of the majority of patients.[2] and this is a bit more difficult to achieve in young children as they require good manual dexterity. Therefore, there may be a need of chemical plaque control in the form of mouthrinses apart from routine teeth brushing. The chemical control of plaque includes organic or inorganic chemicals, which inhibit the accumulation, growth and survival of microbiota and debris. The main advantage of chemicals and their action depends on increased concentration in gingival cervicular fluid and saliva, which improves gingival health.[3] A variety of products are available for chemical plaque control, which are divided into first generation (e.g., phenols, quaternary ammonium compounds), second generation (e.g., bisbiguanides-chlorhexidinegluconate [CHXG]), third generation (e.g. Delmopinol).[4] CHXG is widely used with concentration of 0.12% and 0.2% as mouth rinse. Due to the antiplaque effect of CHXG and substantivity property is used as an adjunct to scaling when compared with other antiseptics it is cationic broad-spectrum antimicrobial agent, which mainly acts by preventing pellicle formation, and involves in destabilization of the outer bacterial membrane thereby preventing bacterial cell wall adsorption and binding of mature plaque[5]. CHX is available in various forms such as digluconate, acetate and hydrochloride salts which are sparingly soluble in water. Chlorhexidine is regarded as the “gold standard” anti-plaque agent.[6] However, it is not a “Magic Bullet” due to certain side effects like tooth staining, taste disturbance, etc. It has served the dental profession over three decades and has also been recognized by the pharmaceutical industry as the positive control, against which the efficacy of alternative anti-plaque agents should be measured[7]. Listerine is an essential oil containing mouthwash that is available over the counter. Listerine has been reported since the 1890s. Short-term and long-term clinical studies have indicated that the daily use of Listerine (Pfizer), a mouthwash that contains phenolics such as thymol, eucalyptol, menthol, and methyl salicylate, may retard plaque build-up and reduce gingivitis.[8,9] The effect of List on plaque was ascribed to its bactericidal properties. Povidone- iodine was discovered in 1955 at the Industrial Toxicology Laboratories in Philadelphia by H. A. Shelanski and M. V. Shelanski. Povidone-Iodine is a potent microbicidal agent with several advantages over other iodine solutions. Combining iodine with Polyvinyl pyrrolidone (PVP) increases its ability to dissolve in water and alcohol, reduces irritability and decreases the staining caused by pure iodine. PVP, the hydrophilic polymer that acts as a carrier in povidine- iodine, does not have

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any intrinsic antibacterial activity, but because of its affinity to the cell membrane, it delivers free iodine directly to the bacterial cell surface. Delivery of iodine to the sensitive elements of the cell membrane is a crucial event of antibacterial action. Iodine targets are in the bacterial cytoplasm and cytoplasmic membrane, and its killing action takes place in a matter of seconds[10]. Hence, this study was conducted to evaluate the efficacy of various mouth rinses on plaque accumulation gingival inflammation and microbial counts in children.

Materials and Method

The study was conducted on 90 (Ninety) healthy children with permanent dentition in the age range of 13 to 15 years attending the outpatient department of Pedodontics and Preventive Dentistry, Career Post Graduate Institute of Dental Sciences & Hospital, Lucknow. (CPGIDSH).

Inclusion criteria

Healthy children without any known systemic illness.

Children with permanent dentition and age range of 13 to 15 years.

Exclusion criteria

Children with known history of allergy to any commercial mouthwash.

Children using any other oral hygiene aid other than routine teeth brushing.

No recent history of use of antimicrobial agents or any other drugs (within 4 weeks)

Methodology

The subjects were divided into 3 experimental groups depending on the type of mouth rinse used having 30 samples each as shown in table1.

Table.1 showing distribution of samples.

Groups	Number of sample. N	Mouthrinse
Group A	n=30	Chlorhexidine mouth rinse0.2%
Group B	n=30	Essential oil mouth rinse (EO)
Group C	n=30	Povidone- iodine mouth rinse (PVP-I)

Examination of the study was done at baseline,7th and 14th day of intervention. Baseline scores of plaque index (Fig.5) using Turesky - Gilmore –Glickman method (modification of QUIEGLY HEIN index) and gingival index (Fig.4) using Loe and Silness method was taken for all the participants followed by full mouth prophylaxis. The designated mouthrinses were dispensed to the subjects of respective groups. Subjects of each groups were instructed to rinse once daily with 15 ml of allocated mouth wash for 30 seconds after 1\2 an hour of tooth brushing in the morning (for 14 days) .The parents/guardians were asked to supervise the children during the use of mouth wash and also instructed to refrain from using any other oral hygiene aid / mouth wash during the study period and advised to exercise routine self- performed oral hygiene practices (tooth brushing) and unstimulated saliva was collected by draining method (Fig. 3)**.** Patient sitting quietly, with the head bent down and mouth open to allow the saliva to drip from the lower lip and saliva was collected with help of new disposable syringes.

Microbiological analysis

The saliva samples were vortexed, to uniformly mix the saliva. Using a calibrated loop (4mm inner diameter) 10 µl of the vortexed samples were streaked on sheep blood agar selective for growth of all bacteria respectively. At baseline,7 and 14 days samples were streaked on sheep blood agar and cultured plates were incubated aerobically for 48 hours at 37°C in an incubator. After the completion of incubation period all plates were examined for presence of visible colonies of bacteria. A semi quantitative estimation of colony count was done and count was expressed as the number of colony forming units per mililiter (CFU/ML) of salivary samples.

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Fig.1: Shows Armamentarium used in study

Fig. 2: Shows sheep blood agar and Petri dish

Fig.3: Shows collection of saliva by draining method

Fig. 4: Shows evaluation of gingival index

Fig.5: Shows evaluation of plaque index

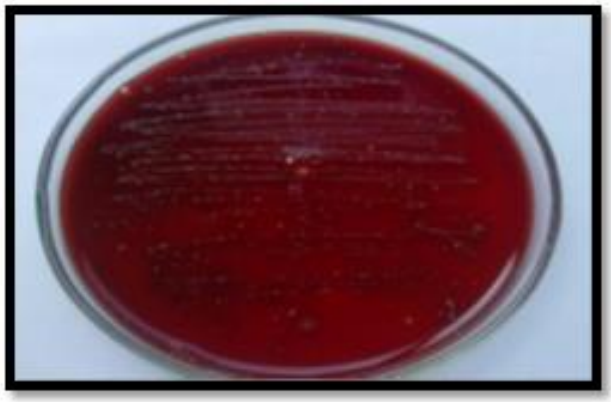

Fig.6: Shows microbial culture of saliva

Statistical analysis

The data collected for plaque and gingival index and microbial count for each group at baseline, 7th day ,14th day was tabulated and subjected to statistical analysis. Data were summarized as Mean ± SE (standard error of the mean). Groups were compared by repeated measures two factor analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance by Levene's test. A two-tailed p value less than 0.05 ($p<0.05$) was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0).

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Observations and results

The present in-vivo study evaluates the efficacy of various mouth rinses on dental plaque, gingival inflammation and microbial counts in children.

Plaque index -The pre (baseline) and post (day 7 and day 14) PI of three groups is summarized in Table 2 the mean of plaque index in all three groups decreased comparatively at day 7 whereas increase at day 14 but at both post periods it still remained lower as compared to respective baseline and the decrease was evident highest in CHX (Group A) followed by EO (Group B) and PVP I (Group C) the least.

Table 2: PI (Mean ± SE, n=30) of three groups over the periods

Time periods	Group A	Group B	Group C
Baseline	1.75 ± 0.06	1.74 ± 0.04	1.81 ± 0.03
Day 7	0.73 ± 0.03	0.92 ± 0.02	1.18 ± 0.02
Day 14	0.95 ± 0.04	1.24 ± 0.03	1.33 ± 0.02

For each group, comparing the mean of plaque index between the periods, Tukey test showed significant ($p<0.001$) decrease in plaque index at both day 7 and day 14 as compared to respective baseline in all groups (Fig. 7) In contrast, in all three groups, it increase significant ($p<0.05$ or $p<0.001$) at day 14 as compared to day 7. However, at final evaluation, the decrease in mean of plaque index (i.e. Mean change from baseline to day 14) Group A was found to be the highest (45.7%) followed by Group B (28.8%) and Group C (26.7%) the least.

Gingival index

The pre (baseline) and post (day 7 and day 14) gi of three groups is summarized in table 3 the mean of gingival index in all groups decrease linearly at day 7 and day 14 as compared to respective baseline and the decrease was evident highest in chx (group a) followed by eo (group b) and pvp i (group c) the least.

Table 3: GI (Mean ± SE, n=30) of three groups over the periods

Time periods	Group A	Group B	Group C
Baseline	1.52 ± 0.04	1.61 ± 0.04	1.54 ± 0.07
Day 7	0.87 ± 0.04	1.07 ± 0.03	1.14 ± 0.05
Day 14	0.49 ± 0.03	0.68 ± 0.03	0.79 ± 0.02

For each group, comparing the mean of gingival index between the periods, Tukey test showed significant ($p<0.001$) decrease in gingival index at both day 7 and day 14 as compared to respective baseline in all groups (Fig. 8) Moreover, in all three groups, it also decrease significant ($p<0.001$) at day 14 as compared to day 7. However, at final evaluation, the decrease in mean of gingival index (i.e. Mean change from baseline to day 14) Group A was found to be the highest (67.8%) followed by Group B (58.1%) and Group C (48.4%) the least.

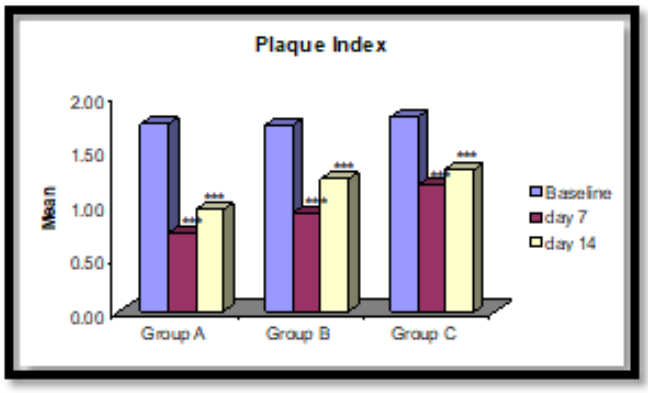

Fig.7: Bar graph showing comparisons of difference in mean PI between the periods among groups

Microbial count

The pre (baseline) and post (day 7 and day 14) microbial count (mbc) of three groups is summarized in table 4. After mouth rinse use, the mean of microbial counts in all groups decrease linearly at day 7 and day 14 as compared to respective baseline and the decrease was evident almost in all three groups.

Table 4: MBC (mean ± se, n=30) of three groups over the periods

Time periods	Group A	Group B	Group C
Baseline	229.90 ± 71.88	226.60 ± 72.16	231.70 ± 71.76
Day 7	0.42 ± 0.33	0.56 ± 0.33	1.99 ± 0.67
Day 14	0.009 ± 0.005	0.013 ± 0.004	0.030 ± 0.007

For each group, comparing the mean of microbial counts between the periods, Tukey test showed significant ($p<0.01$) decrease in microbial counts at both day 7 and day 14 as compared to respective baseline in all groups

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(Fig.9) However, in all three groups, it did not differ (p>0.05) between day 7 and day 14 i.e. found to be statistically the same. However, at final evaluation, the decrease in mean of microbial counts (i.e. mean change from baseline to day 14) was 100.0% in all three groups.

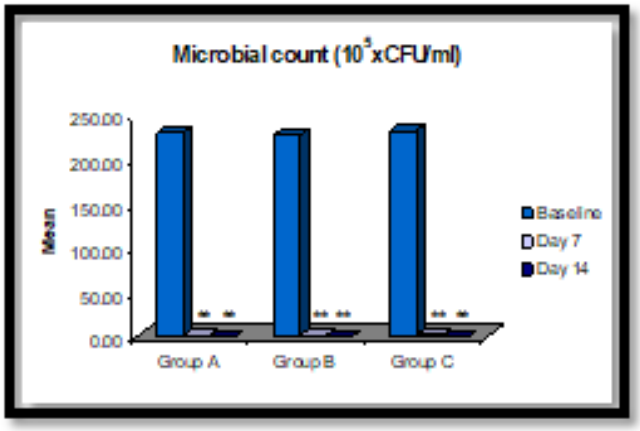

Fig.8: Bar graph comparisons of difference in mean MBC between the periods among groups

Discussion

In the present study was designed to be an *in-vivo* that aimed to evaluate and compare the efficacy of various mouth rinses on plaque accumulation, gingival inflammation and microbial counts in children. Chlorhexidine gluconate is the most effective antiseptic mouth rinse available today. Chlorhexidine tightly binds to tooth structure, oral tissues and dental plaque and releases slowly, resulting in 8-to-12-hour substantivity. Chlorhexidine (chx) is considered the most common and extensively studied chemical agent for plaque control till date. It has been used as an adjunct to mechanical plaque control such as tooth brushing and oral prophylaxis for the maintenance of proper oral hygiene. Inspite of potent antimicrobial and anti-plaque properties of chlorhexidine, the mechanisms of action for this mouth rinse are rupturing of the bacterial cell membrane resulting in cell death and inhibiting pellicle formation and plaque colonization[11]. Chlorhexidine has been shown to penetrate dental plaque biofilm killing pathogens. Due to the reduced effectiveness caused by positively charged dentifrice ingredients interacting with chlorhexidine, it is recommended to rinse 30 minutes after tooth brushing chlorhexidine gluconate provides the greatest anti–plaque and anti– gingivitis benefits available today the adverse effects of chlorhexidine include extrinsic staining of teeth, transient impairment of taste sensation and taste perturbation [12], enhanced supragingival calculus formation and less commonly, desquamation of the oral mucosa. Essential oil mouthwash that contains phenolics such as thymol, eucalyptol, menthol, and methyl salicylate, may retard plaque buildup and reduce gingivitis.[8,9] the effect of Listerine on plaque was ascribed to its bactericidal properties that were documented in-vitro and in-vivo.[13,14,15] phenolic compounds, however, are also known to interfere with the inflammatory process.[16,17] therefore, it was observed that when listerine was used as a mouthwash two times daily for 6 weeks, it had limited influence on plaque, but was effective in reducing gingival inflammation similar to chx. Other studies with a similar study design[18] showed that the regular use of listerine failed to retard plaque-associated gingivitis. Phenolic compounds[19],[20],[21]alter the cell membrane, resulting in leakage of the intracellular contents and eventual cell death and exhibit anti-inflammatory properties by inhibiting prostaglandin synthetase, anti-inflammatory effect of phenolic compounds occurs at concentrations lower than those needed for antibacterial activity and alter bacterial enzyme activity. Povidone-iodine came into commercial use in 1955. It is on the world health organization's list of essential medicines, the most effective and safe medicines needed in a health system. Povidone-iodine is available over the counter.[22]povidone-iodine is a chemical complex of povidone and the element iodine. It

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contains from 9% to 12% available iodine. Povidone-iodine is a potent antimicrobial agent that kills microorganism on contact.[22] moreover povidone- iodine has been found to significantly reduce the incidence of post-operative wound complications in surgery. However it is contraindicated in as a regular mouthwash in patients with thyroid disorders or on lithium therapy. Side effects related to povidone-iodine include mucosal irritation and hypersensitivity reaction.[23]the result of this experimental study showed that plaque index decreased in all three groups at 7th day whereas increased at 14th day but post period plaque index score still remained lower as compared to respective baseline and the decrease was evident highest in chx group followed by essential oil and povidone -iodine. Evaluating the effect of groups and periods on mean pi, anova showed significant effect of both groups (f=40.05, p<0.001) and periods (f=577.46, p<0.001) on pi. Further, the interaction effect of both groups and periods (group x period) on pi was also found to be significant (f=13.35, p<0.001).however ,the final evaluation of this in-vivo study showed that decreases in mean plaque index (mean change) from baseline to day 14 of chx group was found to be highest (45.7 %) followed by essential oil group (28.8%) and povidone- iodine group (26.7%) the least. The gingival index scores decreased in the present study in all three groups at day 7 and day 14 as compared to respective baseline and the decrease was evident highest in group a (chlorhexidine) followed by group b (essential oil) and group c (povidone- iodine) . Evaluating the effect of groups and periods on mean gingival index score, anova showed significant effect of both groups (f=13.22, p<0.001) and periods (f=481.94, p<0.001) on gingival index score . Further, the interaction effect of both groups and periods (group x period) on gingival index score was also found significant (F=4.87, p=0.001). However, at final evaluation, the decrease in mean Gingival index (i.e. Mean change from baseline to day 14) of Group A(CHX) was found to be the highest (67.8%) followed by EO (58.1%) and Povidone iodine (48.4%) the least.The result of the experimental study showed that microbial count decreased in all three groups at day 7 and day 14 as compared to respective baseline and the decrease was evident highest in Group A (CHX) followed by Group B(Essential oil) and Group C (povidone- iodine). Evaluating the effect of groups and periods on mean microbial count (MBC), ANOVA showed insignificant effect of groups (F=0.002, p=0.998) while significant effect of periods (F=30.412, p<0.001) on MBC. Further, the interaction effect of both groups and periods (Group x Period) on MBC it was found insignificant (F=0.001, p=1.000). However, at final evaluation, the decrease in mean microbial count (i.e. Mean change from baseline to day 14) was 100.0% in all three groups. **Prashant R Shetty et .al (2013)**[24] concluded that plaque index was significantly reduced at the end of 15 days in all groups (CHX, Listerine and Herbal) but plaque index was significantly increased at the end of 30 days of this study in all three groups. **Ahmed Jarrar et.al (2014)**[25] compared plaque index score for CHX, Essential oil, and sterile water and found that mean plaque index score was less for CHX when compared to Essential oil mouthrinse and sterile water. **Brecx M, et.al (1989)**[18] in a double blind study [21days] found that plaque indices remain lowest in CHX group while subject using Listerine and Meridol (amine fluoride and stannous fluoride)harboured similar indices but gingival index score were equal in all groups (Listerine and Meridol) and values amounted to half of these with CHX and concluded that CHX was superior to Listerine and Meridol and ability to maintain low plaque score and

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gingival health and found the decrease in vitality of bacteria was more pronounced in CHX group with minimum value of 41% after 7th days mouthrinsing and 56% at 21days mouthrinsing .This score was significantly lower than Listerine group and Placebo group at all examination period. **Shriparna Biswash et al (2015)**[26]compared the effect of commercially available Green tea mouthwash Listerine and CHX mouthwash in gingivitis patients. The result demonstrated that Green tea to be equally effective in reducing the periodontal indices as CHX. They concluded that use of Green tea mouthwash is an effective antiplaque agent that is comparable to CHX. **Bala Subramanya Goutham et al (2013)**[27]concluded that at the end of 28 weeks Chlorohexdine & Listerine significantly reduced plaque growth & gingivitis compared when to placebo (sterile saline solution).However chlorhexidine rinse was more effective against plaque regrowth than the phenolic rinse. **Ram kumar et al(2006)**[28] in an in-vivo study on 11-18 years children indicated that the 0.2% CHX showed maximum potential for the control of pathogenic organisms and controlling disease and plaque accumulation , whereas essential oil mouthwash found next to 0.2% CHX while Sodium fluoride and normal saline were observed to be least effective. **Shah HP et al (2017)**[29]compared Chlorhexidinedigluconate, Phenolic (Essential oil), Chitosan anti-adhesive mouthwash and placebo (distilled water as control group). Results of this study showed that there is highly statistically significant decrease in plaque and gingival index in chlorhexidine and Essential oil group, statistically significant decrease in chitosan and placebo group. They indicated chlorhexidine to be the most effective anti-plaque and anti-gingivitis agent, followed by Essential oil mouthwash with the benefit of reduced staining, thus can be used on long term basis, and whereas chitosan is not significantly effective compared to placebo group. **Hemant Gupta et al (2014)**[30] evaluated the effectiveness of Povidone- iodine 2% and CHX 0.2% in reducing the count of S.mutans in saliva and concluded that CHX and Povidone – iodine were effective in reducing S.mutans count. However Povidone-iodine was more effective in decreasing S. Mutans count as compared to CHX. **Takehiro Oyanagi et al (2012)**[31]compared Chlorhexidine gluconate, Benzethonium chloride (BTC), Essential oil (Listerine), and Povidone-iodine. CFU counts showed significant reduction after treatment with Listerine and PVP-I compared to other solutions. Listerine also displayed significant inhibition effects in preventing the progression of demineralization. Bactericidal potencies of the mouthwashes varied significantly, suggesting that mouthwashes like Listerine can be useful for the prevention of caries and secondary caries. In review of literature 67 a number of trials have demonstrated the long –term plaque and gingivitis reducing properties of both CHX and EO mouthwashes. These studies clearly demonstrate that these agents have lasting efficacy and can access hard to reach areas. The present study demonstrated that the use of the chemotherapeutic mouthwash resulted in a significant reduction of gingivitis, plaque and microbial count at the end of the mouthrinsing phase. PI in all three groups decreased comparatively at day 7 whereas increased at day 14 but at both post periods it still remained lower as compared to respective baseline. However Brecx et al[18] reported a continuing increase of the PI score during use of the CHX rinse. This may be due to the small quantity of plaque always present on the tooth surface when CHX is used. This amount of plaque might be too small to increase the PI score but large enough to influence the GI score with time. However gingival index scores in

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this study in all groups decreased linearly at day 7 and day 14 as compared to respective baseline. Chlorhexidine proved to be superior in the reduction of PI, GI and Microbial count as compared to Listerine and Povidone-iodine. In one of the studies, evaluated the efficacy of CHX , Listerine and Placebo in school children aged 10 to 12, plaque was reduced 16% and gingivitis 67% compared to placebo. In a second study, conducted on adults, plaque was reduced 61% and gingivitis 39% [32].Microbial counts in all groups decreased linearly at day 7 and day 14 as compared to respective baseline. No doubt Chlorhexidine is considered the gold standard and is also evident in this study because of its superior antiplaque effect, which is a result of its superior degree of persistence on the tooth surface. The high efficacy of Chlorhexidine could be due to its bactericidal action during the time of application followed by bacteriostatic action due to adsorption at the tooth surface. Chlorhexidine, Essential oil and Povidone-iodine were equally effective in reducing the microbial count and were statistically same. However, CHX was found to be significantly effective in reducing the PI score and gingival index score compared to EO and PVP-I. Thus it can be derived from the study that CHX, EO and PVP-I mouthrinses are effective in reducing the plaque accumulation, gingival inflammation and microbial counts as their efficacy reflects their previously proved physical and chemical properties.

Conclusion

There are some limitations of home oral hygiene practices in children like inadequacy in brushing, constant education, motivation, and manual dexterity which makes it difficult in reducing plaque solely by mechanical means and bacteria are usually left behind. Therefore, the use of chemotherapeutic agents in the form of mouthrinses have a key role as adjuncts to daily home care. For a definitive conclusion a larger sample size with clinical and in-vitro investigations with these mouth rinses are suggestive for future study.

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