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Evaluation of Gingival Status in Orthodontic Patients by Three Different Mouthrinses – Anti-Oxidant, Essential Oil and Chlorhexidine - A Randomised Control Trial

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KEYWORDS

Antioxidant, Chlorhexidine, Essential oils, Gingivitis, Orthodontic, Treatment

ABSTRACT:

Objective: To evaluate the clinical efficacy of an antioxidant mouth wash on fixed appliance orthodontic patients with generalized gingivitis. The mouthrinse contains the antioxidants ferulic acid and phloretin.

Materials and Methods: A total of eighty one patients undergoing fixed orthodontic appliance therapy participated in the study were divided into three active interventional groups of n=27 each. Group I were given antioxidant mouthwash, group II with essential oil mouthwash and group III with chlorhexinde mouth wash were given as a interventional measure immediately after bonding of the brackets. Each patient was evaluated at four orthodontic treatment visits (T0, T1, T2, T3)for 3 months. Between T2 and T3, intervention was with withdrawn. A periodontal examination, including Gingival index (GI), Bleeding on probing (BOP), and Orthodontic plaque index (OPI) was performed at each visit. The severity of index scores was assessed by Repeated measure ANOVA followed by **Post-hoc** Bonferroni test and Tukey test.

Results: Gingival index:Mean differences across the three time points T1, T2 and T3 for GI in all the three groups (T2-T1: 1.03±0.35, 0.47±0.2, 1.17±0.37 and T3-T2: 1.2±0.35, 0.45±0.18, 1.65±0.24 respectively) showed a statistically significant difference ($p<0.001$). No statistical difference is observed for Bleeding on probing between the groups across different time periods.

Conclusion: Antioxidant-mouthwash equally effective as essential and Chlorhexidine mouthwashes in reducing severity of gingivitis in early stages of fixed orthodontic appliance therapy.

1. Introduction

Orthodontic treatment significantly improves the dental and skeletal condition, but also causes some side effects such as gingivitis due to the change in oral hygiene habits. The prime reason for the occurrence of gingivitis in patients treated with fixed orthodontic appliances is the presence of brackets and other components which provide an increased retention area for biofilm accumulation. This increase in the retention areas of plaque by adherence around bands and brackets leads to changes in the oral environment and a shift in microbial ecology, causing reactions in gingiva and periodontal breakdown. Even while it holds fact that the inflammatory alterations, such as elevated gingival index

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and deeper pockets, are mostly present during the course of treatment, it's still true that chronic inflammation can harm the periodontium in ways that are long-lasting.**1**

Control of the plaque that causes gingivitis can be achieved by interrupting, reducing, or eliminating the microorganisms. Mechanical removal of microbial biofilm using toothbrushes with dentifrices is the most common way of controlling gingivitis. Nevertheless, patients find it difficult to maintain an appropriate level of oral hygiene, which is difficult to attain by the brushing alone.

Because of the inefficiency of mechanical methods in maintaining proper oral hygiene after brushing, mouthwash solutions and dentifrices containing antimicrobial agents were included.**2**

Chlorhexidine is the most potent anti-microbial agent available today. It is regarded as the gold standard agent against which other anti-plaque agents are compared. **3**

Various mouthrinses containing chemotherapeutic agents are introduced, among which essential oil mouthrinse is termed most effective compared to chlorhexidine4 (Ulkur F, 2013), as chlorhexidine mouthrinses have various side effects like disturbance of the tase perception,discoloration of the enamel, erosion of the mucosa, burning sensation of mouth, dryness of mouth,carcinogenic effects, and the deterioration of compositeadhesive limit their use to around 5-6 weeks. **5**

Recent systematic reviews concluded that chlorhexidine mouthwash, essential oil, and herbal mouthwashes successfully combat gingivitis and plaque accumulation, increase the effectiveness of oral hygiene measures, and limit plaque accumulation in orthodontic patients.6,7,8

Recent studies have implicated oxidative stress and cytotoxic effects of various metallic and nonmetallic materials used in fixed appliances as factors in gingival inflammation.9,10 In response to the colonization of bacteria in the plaque, phagocytes are activated to produce cytokines and Reactive Oxygen Species (ROS) which are presumably harmful to tissues. Free oxygen radicals and derivatives of radical oxygen such as hypochlorous acid (HOCl) and hydrogen peroxide (H2O2) are some of these reactive oxygen species.When there is an imbalance between the presence of reactive oxygen species and the presence of antioxidants in the body, it results in oxidative stress. Oxidative stress is a major factor in the development of a wide range of human diseases and one of the most common oral conditions is the gingivitis.**10**

This idea of oxygen species-induced damage has prompted researchers to look for a suitable complementary antioxidant therapy to treat inflammatory periodontal conditions.

There is also growing evidence that antioxidant mouthwash has similar antiplaque anti-gingivitis, and antibacterial benefits to chlorhexidine mouthwash.**11**

Phloretin is a natural anti-oxidant that is extracted from apple tree leaves and ferulic acid is extracted from the cell walls of the plants. The use of this antioxidant as a topical gel for treating gingivitis has been evaluated and found to decrease the severity of gingivitis and decreased bleeding on probing in orthodontic patients.**12** (Benjamin J. Martina, 2016)

A closer look at the literature reveals that no previous research has investigated the efficacy of antioxidant mouthrinses in direct comparison with the essential oils and chlorhexidine mouthrinses in the treatment of orthodontic gingivitis. The aim of the present study was to test the null hypothesis that is there is no difference in the treatment of orthodontic gingivitis between antioxidant, essential oil, and chlorhexidine mouthrinses in the early stages of fixed orthodontic therapy.

2. Materials and Methods

This three-arm parallel randomized clinical trial was unicentered carried out in the Narayana Dental College, Nellore, Andhra Pradesh,India. The study was approved by Institutional Review Board registered with the Control Trial Registry of India (CTRI/2021/01/030264). A minimum of 24 subjects per each of the three comparative groups were needed assuming an effective size of "1" between any two scores of Orthodontic Plaque Index and Gingival Index. This sample size will provide a minimum power 80% with an alpha value of 0.05.

The study comprised of orthodontic patients due for bracket bonding start up of up of fixed appliance. A final sample of eighty one (81) subjects were selected after inclusion and exclusion criteria and had expressed their willingness to participate in the study. The patients with

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good physical and mental health in the age group between 18 to 25 years were selected. Patients with caries-free, normal gingival health with Irregularity of less than 4-6 mm mm as assessed by Little's Index in the lower arch were selected. Patients with bleeding on probing of atleast 30% at appropriate probing sites, including all teeth bonded mesial to the first molars and not adjacent to a band tooth. Subjects with associated systemic diseases and on prolonged drug therapy were excluded. It was confirmed with the participant if the information sheet had been read and understood after the proceduresbeing explained in English and their known native language. Standard safety procedures and verified established protocols were followed in this study.

The randomization and allocation was done using the Fish-bowl method and the patients were assigned to all three active interventional groups equally (n=27) without any control group..

GroupI(n=27);Anti–Oxidant Mouthrinse(*Periosciences AO ProRinse Hydrating Mouthrinse, Periosciences LLC, Dallas, USA*)

Group II (n=27); Essential – Oil Mouthrinse (*LISTERINE® Cool Mint Mouthrinse, Johnson & Johnson Pvt Ltd, Bangalore, India*)

Group III (n=27); Chlorhexidine Mouthrinse (*CLOHEX Mouthrinse, Dr. Reddy's Laboratories Ltd., Hyderabad, India*)

The randomization and allocation was performed by one of the staff nurse not involved in the study. Patients were assigned to one of the groups in sequential and alternative manner. Assessors who performed the outcome measurement were also blinded to treatment allocation. This , in essence it was a double-blind study.

Outcome exposure Measured: The Primary outcome measured is the **gingivitis status**. on a quantitative scale **by** Sillness and Loe gingival index (GI)**[13]** and bleeding on **probing index (BOPI).**[14] Orthodontic Plaque Index (OPI)[15]was also measured as secondary outcometo assess the amount of plaque accumulation around the orthodontic bracket

Gingival index**[13]** scoring-.The buccal surface and the palatal surface of the tooth were measured. Four surfaces on each tooth were considered. The distal and mesial axial sides of buccal surface,buccal surface and the palatal surface were recorded.The average of the scores for each individual teeth was obtained. The cumulative score for each participant is obtained by adding up the scores of individual teeth divided by the by the number of teeth examined.

Bleeding on probing- Bleeding on probing index 14 is measured in percentage, calculated by the total teeth that bleed during probing by total teeth examined.If bleeding occurs within ten seconds, it is recorded as positive, if not, recorded as negative. Negative scoring is equivalent to the gingival index score of 0 and 1. A positive recording is equivalent to the gingival index score of 2 and 3.

For scoring of orthodontic plaque index, the same procedure as described for GI was followed

Clinical procedure- (Figure-1- Flow chart of the study)

All participants had undergone professional teeth cleaning with polishing paste free of fluoride content four weeks prior to bonding procedure. Participants were given thorough oral hygiene instructions based on a uniform preventative strategy.This procedure involved manual teeth brushing by using fluoride toothpaste (Colgate max fresh cool mint fluoride toothpaste) twice a day for 2 min followed by cleaning of the interdental spaces with an interdental brush or dental floss. There were strictly advised not to use other oral hygiene methods. This was followed by bonding procedure where etching of the tooth surface was done using 37% phosphoric acid for about 15-20 seconds. In the next step bonding agent and primer were applied and cured for about 20 seconds with high power LED curing light. It is followed by placement of 0.22 slot MBT metal bracket. Excess composite around the bracket was removed using Bracket placer sickle and then curing is done using LED curing light for 45 seconds. An initial Nickel-Titanium archwire of .014 inch is placed and ligated with clear elastic modules. Initial periodontal examination and Indices were recorded as **T0 (Day 1**) on the day of bonding immediately after the bonding procedure. The patient is disposed of after bonding after giving instructions regarding oral hygiene procedures.

Follow up & Recall visits: All the patients were recalled for regular orthodontic follow up visits on 28th day or at

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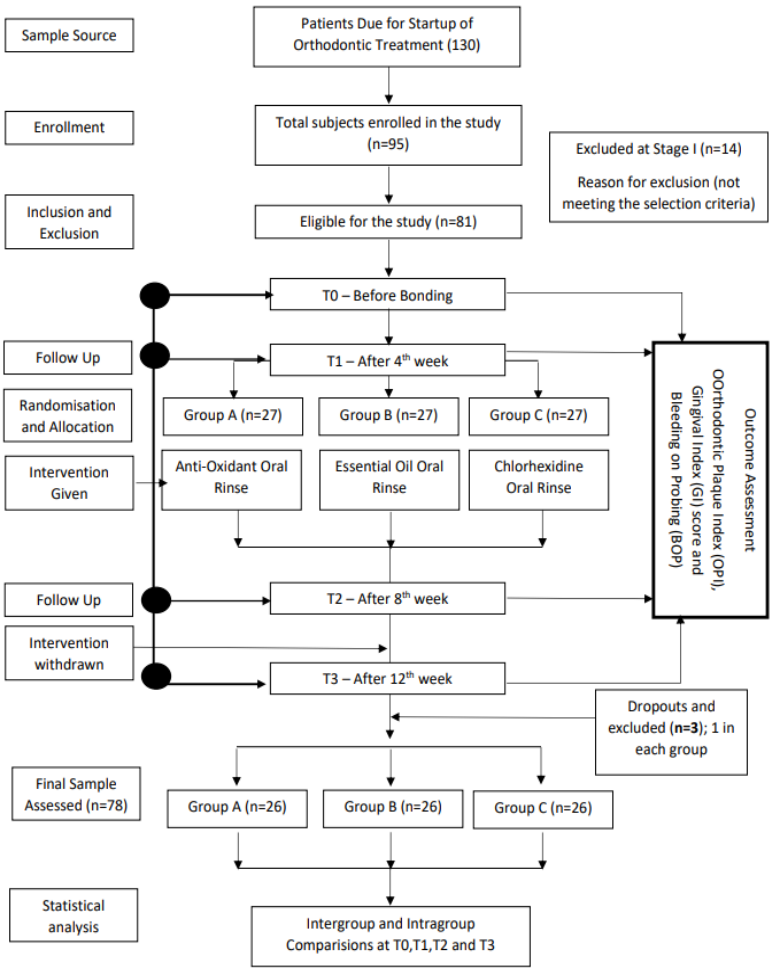
the end of 4th week . At the end of 4th week(**T1),** measurement of Orthodontic Plaque Index and Gingivitis was repeated. This was followed by groups allocation and the patient is asked to start using the mouth wash. At the end of 8th week(T2), intervention was withdrawn. As a part of treatment procedures, all the subjects were regularly followed up every month and the Gingival status was assessed at the end of 4th week(T1), 8th week(T2), and 12th week (T3),

Mode of intervention: Group I received anti-oxidant mouthrinse as a treatment procedure. Patients in Group are instructed to rinse their mouth using anti-oxidant dental rinse once daily for 1 month. After brushing the teeth, the patient is advised to vigorously swish their mouth with 1 teaspoonful (5ml) of the solution for 1 min and spit it out. Then the patients are instructed not to eat or drink for 30 min after rinsing. Same procedures were repeated **for Group II with** essential oil mouthrinse.

The group **Group III** were instructed the similar method of rinsing as in the other two groups using Chlorhexidine mouthrinse.The patients were daily reminded of the regimen by automated message system once on the previous night and early morning of the same day on using the mouthrinses through messages on mobile.

At the end of the 8th week, status of Orthodontic Plaque Index and Gingivitis was reassessed (T2) using the standard protocol and patients were asked to stop the usage of mouthrinse. The indices were again recorded after one month of withdrawal at the end of the 12th week(T3). Among these participants, one each from group 1 and 2 did not show up for the follow up appointments regularly and 1 participant of group 3 discontinued the treatment in the initial stages itself. The final sample consists of 78 participants with 26 in each group analysed for the trial.



Flowchart . 1: Consort Flowchart of the Study

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3. Statistical Analysis

The data after entering Microsoft excel sheet was analyzed by *IBM SPSS* version 26 for Windows. The age distribution among the three groups was calculated by ANOVA and gender distribution was calculated among the three groups by *Chi-square* test. The GI and OPI are calculated as ordinal data by examining the four surfaces of each tooth (mesiofacial, facial, distofacial, lingual) and it was converted into continuous data by taking the mean of the teeth examined. Bleeding on Probing is measured as the percentage of bleeding sites to the number of teeth examined, it is a discrete data and it is converted into continuous data by taking the mean of all subjects of individual groups. The normality of the GI, BOP and OPI at T1 are verified by *Kolmogorov-Smirnov* Test. Mean differences across the three time points T1, T2 and T3 for all the indices GI, BOP and OPI in all the three groups were analyzed by *Repeated measures ANOVA* test and followed by *Post-hoc* analysis for intrapair comparisons within each group between three different time periods for each index recorded. The intergroup comparisons for each of the index at a given time period is done using *One way ANOVA* test and followed by pairwise comparisons between the groups at time points using Post Hoc *Tukey test.* The level of significance was set at <0.05 for all tests.

4. Results

The final analysis evaluated consists of 78 participants with n=26 in each group). There was no varaiation between the three groups regarding age distribution and gender**(Table- 1)**. The descriptive data for the three groups with minimum and maximum values at different periods of time is given in table parametric tests were applied for analysis as the individual values were centered over the mean **(Table 2). (Figure 2)**

Gingival index:Mean differences across the three time points T1, T2 and T3 for GI in all the three groups (T2-T1: 1.03±0.35, 0.47±0.2, 1.17±0.37 and T3-T2: 1.2±0.35, 0.45±0.18, 1.65±0.24 respectively) showed a statistically significant difference (*p<0.001)* Bleeding on probing**:** Mean differences across the three time points T1, T2 and T3 for BOP in all the three groups (T2-T1: 45.54±9.93, 48.19±10.24, 44.77±10.66 and T3-T2:50.88±9.36, 54.96±8.76, 56.19±9.75 respectively) showed no statistically significant difference (*p=0.707).* Orthodontic Plaque index:Mean differences across the three time points T1, T2 and T3 for OPI in all the three groups (T2-T1:1.04±0.3, 1.1±0.3, 0.98±0.41 and T3-T2:1.23±0.32, 1.24±0.32, 1.27±0.38 respectively) showed statistically significant difference (*p=0.915)* (**Table 2**) . **(Figure 2)**

Mean differences across the three time points T1, T2 and T3 for all the indices GI, BOP and OPI in all the three groups were analysed by Repeated measures ANOVA test and intrapair comparisons within each group between different time periods is given(**Table 3**).The intergroup comparisons for each of the index at a given time period is done using One way ANOVA test.followed by pairwise comparisons between the groups at the any given time point is done by using Tukey test. (**Table 4**) Pairwise comparisons within the group showed a statistically significant difference between T2 and T3 in Gingival Index and Orthodontic Plaque Index in anti-oxidant mouthrinse group. Gingival Index in the Chlorhexidine mouthrinse group also displayed statistical significant difference between T1 and T3. There is significant difference (*p=0.001)* in GI at T1 between Anti-oxidant and Essential oil groups and Essential oil and Chlorhexidine groups. The results also showed a statistically significant difference (*p=0.001)* in GI at T2 between Essential oil and Chlorhexidine groups. Comparisons between the change of scores between two time periods (T2 - T3) between the three different groups has showed a statistically significant difference (*p=0.001)* in all the three groups for GI, BOP and OPI **(Table -5)**

5. Discussion

The various components of the fixed appliance make it difficult for the individual undergoing fixed orthodontic therapy to maintain proper oral hygiene by mechanical means like brushing. It facilitates the accumulation of oral microbial flora onto the tooth surface which further leads to gingival inflammation.

One of the important reasons for the development of orthodontic gingivitis is an increase in biofilm or dental plaque in fixed orthodontic treatment patients. The biofilm accumulates acidogenic bacteria causing white spot lesions and carious lesions. Fixed orthodontic appliances cause hindrance to proper tooth brushing, making oral hygiene methods difficult and provides sites for increased adhesion of bacteria and plaque formation.16

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The increase in microbial flora activates the defence mechanisms of the body which in turn destroys the host tissues also. Activated phagocytes produce cytokines and . Reactive oxygen species (ROS).The imbalance between antioxidants and ROS leads to oxidative stress significantly increasing gingivitis17 (Wei PF, 2004). The metals used in orthodontics also interacts with various agents such as physical-chemical and biological agents leading to oxidation10.18

Most commonly used method of controlling gingivitis among these methods in day to day life is brushing, but it cannot act on the micro level(microbes) hence additional support of a chemical agent is necessary. **Sharma N et al (2004)[19]** proposed that use of chemical agents as an adjunctive to brushing proved much more effective in controlling gingivitis in a better way, as brushing helps in reducing the biofilm while it cannot act on microflora affecting the gingival tissue which can be overcome by chemical agents. Among the chemical agents, the chlorhexidine is termed as the golden standard. The different means of plaque control and gingivitis include chemical (chlorhexidine, Essential Oil Mouthwash and the latest antioxidants) and mechanical (toothbrushes, interdental aids, water irrigation devices, etc) methods.

The main ingredient of chlorhexidine mouth wash is cetylperidium chloride(CPC) which has antiplaque and anti-gingivitis properties. For maximum antimicrobial activity of CPC, rinse should not be done immediately after brushing. [20] **(Sheen S, 2003).** It was also confirmed by earlier studies that the CPC-containing mouthrinses result objectionable in extrinsic tooth stains **21 (Ciancio SG, 1978).**Hence nowadays use of Essesntial Oil Mouth Wash (EOMW) is more preferred as an alternate chemical agent to chlorhexidine mouth wash.

Essential oils are the organic compounds that are made up of various by-products obtained from different kinds of plants for specific purposes. Van Leeuwen M.P.C**22** suggested essential oil mouthrinses, though doesn't have added benefits in controlling microbes, considering the lesser side effects of them compared to CHX they are termed to be a better alternative.

The most recent agents used in controlling the gingivitis are antioxidants. As it is already known that disturbance in the antioxidants and ROS, causes oxidative stress accentuating the progress of biofilm driven gingivitis and oxidative stress increased by the metals used in fixed orthodontic treatment.

The vital chemical agents known as antioxidants have the ability to neutralize free radicals before they damage human cells. Highly sophisticated antioxidant systems, either enzymatic or non enzymatic, have been developed in humans. These systems function in concert with one another to protect organs—especially cells—from free radical damage. Both endogenous and exogenous antioxidants are available. Hazardous chain reactions can be halted or completely destroyed by utilizing the antioxidants. According to their kind, antioxidants can be categorized into subcategories like ascorbic acids and polyphenols. Antioxidants can also work by becoming oxidized themselves. **23** (Aksakalli S, 2013).

In this study the Ferulic acid and phloretin ingredients present in antioxidant mouthrinse are known for their strong anti-oxidant, anti-inflammatory, and anti-microbial properties which acts against gram +ve, gram -ve, viruses and fungi. **24**, (Srinivasan M 2007).Phloretin is known for its strong antioxidant that prevents lipid peroxidation and peroxynitrite. The 2,6-dihydroxyacetophenome is the antioxidant pharmacophore of phloretin.This molecule's strong activity might result from tautomerization stabilizing its radical. **25,26** (Rezk BM, 2002; San Miguel, 2011).

Ferulic acid is a polyphenol present in a wide variety of foods, such as sweet corn, wheat, oranges, tomatoes, and carrots and phenoletin, a flavonoid obtained from tomatoes, apples, and strawberries as well as xylitol, thymol, and essential oils.

The present study evaluated the effect of newly introduced Phloretin and Ferulic Acid antioxidant mouthrinse with that of essential oil mouthrinse and chlorhexidine mouthrinse in reducing gingivitis in in the early stages of fixed orthodontic treatment. The reduction in gingival inflammation is statistically significant (*p=0.001)* in all the three groups during intervention period from T1 to T2.

The decrease in severity of gingivitis during this period of treatment may be attributed to the decrease in mediators of inflammation such as TNF-α and breakdown of Reactive Oxygen Species by anti-oxidants in Group I. Essential Oils (Group II) are also shown to improve Bleeding on probing and Gingival Index from the previous studies. **4,27**

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The treatment effects might have further added to the subsequent increase in gingival index and bleeding on probing that occurred followed by cessation of treatment intervention from T2 to T3. There is increase of gingival index scores in all the three groups except in essential oil (Group II) indicating a prolonged effect of the essential oils even after the withholding of the intervention.

The plaque index scores from T1 to T2 of the three groups did not show any statistically significant difference (p=0.915). All the three groups showed a significant increase in the orthodontic plaque index scores even after cessation of treatment intervention from T2 to T3. The fact that the orthodontic equipment were designed to retain plaque could potentially be the reason for the lack of plaque reduction.28. Gunsolley (2006)**29,** in a systematic review, explained the antigingivitis properties of essential oil containing mouth washes which decreased orthodontic gingivitis considerably thereby decreasing bleeding on probing. Studies carried out by *Sendamangalam V et al* (2011)**30** have shown the effect of anti-oxidants on plaque bacteria in-vitro and *Van Leeuwen MP et al* (2011) shown the essential oils are effective in reducing plaque bacteria in-vivo. According to study done by *Rosenbloom RG et al* (1991)**31**, these effects may be zeroed during fixed orthodontic therapy. *Chen et al* (2013)[26] has showed increase in plaque accumulation over 6 months in orthodontic patients using essential oil mouthrinses.

The bleeding on probing *(p=0.707*) and orthodontic plaque index (*p=0.915*) **(Table-3)** scores in the three groups across time periods shoed no statistical difference . When the mean differences at T3-T2 of the three groups were compared (**Table-5**), it showed negative values suggesting the importance of the intervention. Oral hygiene can be managed for sometime even after discontinuation of the active agent if the proper oral hygiene methods are followed regularly.

Phytogenic mouthwashes with antioxidant-rich ingredients have been found to be effective in reducing plaque and gingival inflammation. Thus they can be recommended as an alternative or adjunct to chemical based mouthwashes. However, there is more need to conduct further research in making direct comparisons with mouthrinses containing different anti-oxidants that are available for day to day usage. The formulation used in this study contains two types of anti-oxidants in combination making it difficult to decide whether the treatment outcome was due to which one of them both. Null hypothesis stands rejected as there is significant difference in treatment outcomes of antioxidant, essential oil and chlorhexidine mouthrinse groups. Essential oils have shown a marked significance in reduction of gingivitis in patients undergoing orthodontic therapy.

Every study comes with some limitations. Because of ethical considerations surrounding patient care during orthodontic treatment, there is a lack of a control group with no intervention to compare the outcomes of interventional groups. Only patients with a low to moderate risk of gingival inflammation were included in the current investigation. As a result, we were unable to assess any potential preventive benefits of the essential oil and antioxidant mouthrinses in those who had poor dental hygiene or a high risk of gingival inflammation.

6. Conclusion

Clinically significant reduction in gingival inflammation was observed in essential oil mouthrinse during the intervention period when compared with anti-oxidant and chlorhexidine mouthrinses. There was a statistically significant reduction of gingival inflammation observed in antioxidant, essential oil and chlorhexidine mouthrinse groups at three different time points (T1, T2, T3). To conclude Phloretin and Ferulic acid anti-oxidants were equally effective when compared with essential oils and chlorhexidine.

Table 1. Age and gender distribution among various groups

Sample characteristics	Group I AO Rinse		Group-II EO Rinse		Group-II CHX Rinse		ANOVA F value	p-value
N	26		26		26		2.343	0.103
Age* (years and months)	20.27 ± 3.47		21.96 ± 3.71		22.35 ± 3.85			
Gender**	Male	Female	Male	Female	Male	Female	-	0.504
	11	15	13	13	15	11		

*p≤0.05 statistically significant; *Age distribution is calculated using ANOVA **Gender distribution is calculated using Chi-Square test; AO: Anti-Oxidant (Group 1); EO: Essential Oil (Group 2); CHX: Chlorhexidine (Group 3)*

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Table 2: Descriptive Parameters under the study in three groups

Index	Time	Group	Mean	SD	95% Confidence Interval for Mean		Min	Max
		(n=26)			Lower Bound	Upper Bound		
GI	T1	AO	1.45	0.36	1.3	1.59	0.8	1.84
		EO	1.01	0.29	0.89	1.13	0.5	1.66
		CHX	1.55	0.28	1.44	1.66	0.98	1.98
	T2	AO	1.03	0.35	0.89	1.17	0.3	1.58
		EO	0.47	0.2	0.38	0.55	0.16	0.83
		CHX	1.17	0.37	1.02	1.32	0.5	1.72
	T3	AO	1.2	0.35	1.06	1.34	0.5	1.83
		EO	0.45	0.18	0.37	0.52	0.16	0.83
		CHX	1.65	0.24	1.55	1.75	1.2	1.98
BOP	T1	AO	59.42	11.58	54.74	64.1	38	76
		EO	61.85	11.48	57.21	66.48	33	76
		CHX	59.5	12.58	54.42	64.58	33	76
	T2	AO	45.54	9.93	41.53	49.55	25	60
		EO	48.19	10.24	44.06	52.33	24	60
		CHX	44.77	10.66	40.46	49.07	25	60
	T3	AO	50.88	9.36	47.1	54.67	33	62
		EO	54.96	8.76	51.42	58.5	30	68
		CHX	56.19	9.75	52.25	60.13	33	75
OPI	T1	AO	1.44	0.3	1.32	1.57	0.8	1.83
		EO	1.44	0.34	1.3	1.58	0.8	1.98
		CHX	1.41	0.39	1.25	1.56	0.8	1.98
	T2	AO	1.04	0.35	0.9	1.18	0.3	1.56
		EO	1.1	0.32	0.97	1.23	0.3	1.62
		CHX	0.98	0.41	0.81	1.14	0.3	1.62
	T3	AO	1.23	0.32	1.1	1.37	0.5	1.67
		EO	1.24	0.32	1.12	1.37	0.5	1.71
		CHX	1.27	0.38	1.12	1.43	0.5	1.8

**. The mean difference is significant at the .05 level. $p \leq 0.05$ statistically significant*

GI: Gingival Index; BOP: Bleeding on Probing; OPI: Orthodontic Plaque Index; T1: At the end of 4^{th} week after bonding of orthodontic brackets; T2: At the end of 8^{th} week after bonding of orthodontic brackets;T3: At the end of 12^{th} week after bonding of orthodontic brackets; AO: Anti-Oxidant (Group 1); EO: Essential Oil (Group 2); CHX: Chlorhexidine (Group 3)

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Table 3- Comparision of the parameters within the Individual Groups measured at three different points of time (Intragroup measurements)-Repeated measures ANOVA). Pairwise comparison-*Post-hoc* Bonferroni test.

Index	Group	Time points				
		T1	T2	T3	F value	P value
GI	Group I (AO)	1.45[a]	1.03[b]	1.2[c]	45.127	0.001*
	Group II (E O)	1.01 [a]	0.47 [bc]	0.45 [bc]	184.182	<0.001*
	Group III (CHX)	1.55 [a]	1.17 [b]	1.65 [c]	27.483	<0.001
BOP	Group I (AO)	59.42 [a]	45.54 [b]	50.88 [c]	118.437	<0.001*
	Group II (E O)	61.85 [a]	48.19 [b]	54.96 [c]	115.107	<0.001*
	Group III (CHX)	59.5 [ac]	44.77 [b]	56.19 [ac]	35.38	<0.001
OPI	Group I (AO)	1.44 [abc]	1.04 [ab]	1.23 [c]	24.255	<0.001*
	Group II (E O)	1.44 [a]	1.1 [b]	1.24 [c]	106.246	<0.001*
	Group III (CHX)	1.41[a]	0.98 [bc]	1.27 [bc]	32.28	<0.001

**. The mean difference is significant at the .05 level. p≤0.05 statistically significant*

*[abc] Different superscript letters **within a row , without a common superscript alphabet** indicate significant difference (P,0.05) compared with the other time periods across the columns -Pairwise comparison (Post-hoc Bonferroni test)*

Table 4- Comparison of the the parameters among different groups (Intergroup Comparisons) at individual time points T1, T2, and T3.(ANOVA): Pairwise comparison – Tukey test

Index	Time points	GROUPS				
		Group I (AO)	Group II (EO)	Group III (CHX)	F value	P value
GI	T1	1.45[ac]	1.01[b]	1.55 [ac]	22.289	0.001*
	T2	1.03 [ac]	0.47 [b]	1.17 [ac]	36.435	0.001*
	T3	1.2[a]	0.45 [b]	1.65 [c]	136.449	0.001*
BOP	T1	59.42	61.85	59.5	0.349	0.707
	T2	45.54	48.19	44.77	0.793	0.456
	T3	50.88	54.96	56.19	2.321	0.105
OPI	T1	1.44	1.44	1.41	0.089	0.915
	T2	1.04	1.1	0.98	0.789	0.458
	T3	1.23	1.24[c]	1.27	0.098	0.907

*. The mean difference is significant at the .05 level. p≤0.05 statistically significant [abc] Different superscript letters **within a row , without a common superscript alphabet** indicate significant difference (P,0.05) compared with the other Groups across the columns -Pairwise comparison (Tukey test)

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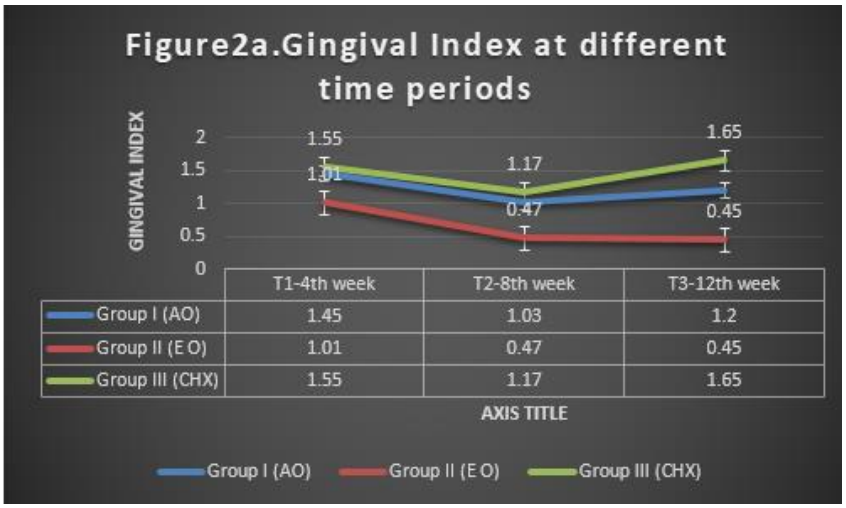
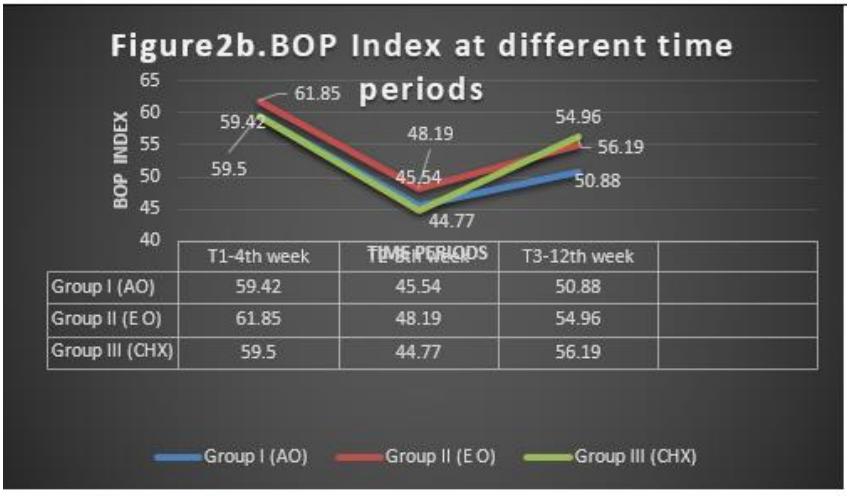
Table 5– Comparisons of the Individual parameters in three different groups between two time periods T2 and T3

Parameters	Time	Groups	Mean	Std. Deviation	F value	P value
GI	T3-T2	AO Rinse	0.17	0.20	21.133	0.001*
		EO Rinse	-0.03	0.13		
		CHX	0.47	0.42		
BOP	T3-T2	AO Rinse	5.34	2.79	9.559	0.001*
		EO Rinse	6.76	4.13		
		CHX	11.42	7.58		
OPI	T3-T2	AO Rinse	0.19	0.21	3.857	0.025*
		EO Rinse	0.14	0.07		
		CHX	0.29	0.27		

$p \leq 0.05$ statistically significant





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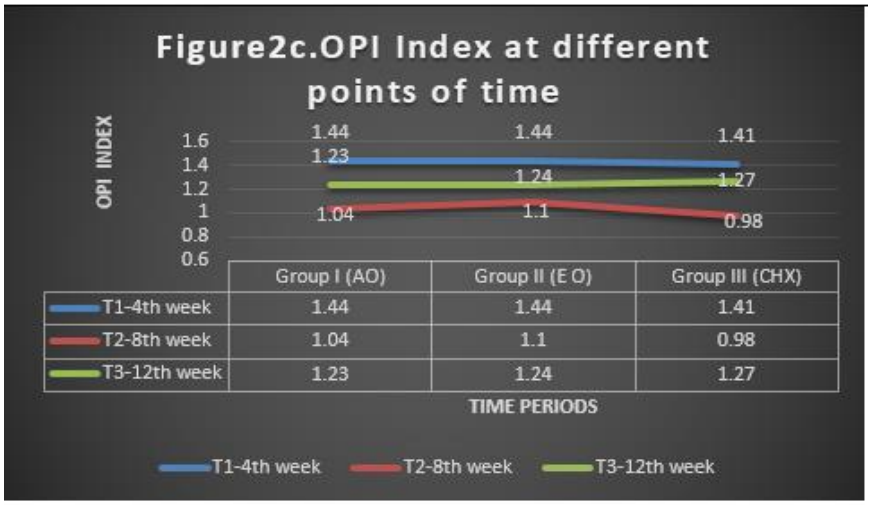

Figure 2. The graph showing the change of trend in Indices over given period of time.

References

1 Boke F, Gazioglu C, Akkaya S, Akkaya M. Relationship between orthodontic treatment and gingival health: A retrospective study. Eur J Dent 2014;8(3):373-380.

2 Shilpa M, Jain J, Shahid F, Gufran K, Sam G, Khan MS. Efficacy of three types of plaque control methods during fixed orthodontic treatment: a randomized controlled trial. J Pharm Bioallied Sci 2019;11(suppl 2):S246–S251

3 Löe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. J Periodontal Res 1970;5:79–83) .

4 Ulkur F, Arun T, Ozdemir F. The effects of three different mouthrinses in a 4-day supragingival plaque regrowth study. Eur J Dent 2013;7:352–358.

5 Tufekci E, Casagrande ZA, Lindauer SJ, Fowler CE,Williams KT. Effectiveness of an essential oil mouthrinse in improving oral health in orthodontic patients. Angle Orthod 2008;78:294–298.

6 Karamani I, Kalimeri E, Seremidi K, Gkourtsogianni S, Kloukos D. Chlorhexidine Mouthwash for Gingivitis Control in Orthodontic Patients: A Systematic Review and Meta-Analysis. *Oral Health Prev Dent*. 2022;20(1):279-294. doi:10.3290/j.ohpd.b3170043

7 Ren, X., Zhang, Y., Xiang, Y. *et al.* The efficacy of mouthwashes on oral microorganisms and gingivitis in patients undergoing orthodontic treatment: a systematic review and meta-analysis. *BMC Oral Health* **23**, 204 (2023). https://doi.org/10.1186/s12903-023-02920-4.

8 Panagiotou A, Rossouw PE, Michelogiannakis D, Javed F. Role of Essential Oil-Based Mouthwashes in Controlling Gingivitis in Patients Undergoing Fixed Orthodontic Treatment. A Review of Clinical Trials. Int J Environ Res Public Health. 2021 Oct 15;18(20):10825. doi: 10.3390/ijerph182010825. PMID: 34682572; PMCID: PMC8535870

9 Grimsdottir MR, Hensten-Pettersen A, Kullmann A. Cytotoxic effect of orthodontic appliances. Eur J Orthod 1992;14:47–53.

10 Spalj S, MlacovicZrinski M, Tudor Spalj V, IvankovicBuljan Z. In-vitro assessment of oxidative stress generated by orthodontic arch wires. Am J Orthod Dentofacial Orthop 2012;141:583–589.

11 Kumar KM, Varghese SS. Views on antioxidant mouthwashes as adjunct in periodontal therapy. Bioinformation. 2020 Dec 31;16(12):1069-1079. doi: 10.6026/973206300161069. PMID: 34938007; PMCID: PMC8600207.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1198-1210 | ISSN:2251-6727

12 Benjamin J. Martin, Phillip M, Campbell, Terry D. Rees, and Peter H. Buschang. A randomized controlled trial evaluating antioxidant–essential oil gel as a treatment for gingivitis in orthodontic patients. Angle Orthod 2016;86:407-412.

13 Löe H. The gingival index, the plaque index and the retention index systems. The Journal of Periodontology. 1967 Nov;38(6):610-6.

14 Jones CG. Chlorhexidine: is it still the gold standard?. Periodontology 2000. 1997 Oct 1;15:55-62.

15 Beberhold K, Sachse-Kulp A, Schwestka-Polly R, Hornecker E, Ziebolz D. The Orthodontic Plaque Index: An oral hygiene index for patients with multibracket appliances. ORTHODONTICS: The Art & Practice of Dentofacial Enhancement. 2012 Mar 1;13(1).

16 Sari E, Birinci I. Microbiological evaluation of 0.2% chlorhexidine gluconate mouthrinse in orthodontic patients. The Angle Orthodontist. 2007 Sep;77(5):881-4.

17 Wei PF, Ho KY, Ho YP, Wu YM, Yang YH, Tsai CC.The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1beta in gingival crevicular fluid: implications for oxidative stress in human periodontal diseases.J Periodontal 2004;39:287–293.

18 Nassar PO, Bombardelli CG, Walker CS, Neves KV, Tonet K, Nishi RN, Bombonatti R, Nassar CA. Periodontal evaluation of different toothbrushing techniques in patients with fixed orthodontic appliances. Dental press journal of orthodontics. 2013 Feb;18(1):76-80.

19 Sharma N, Charles CH, Lynch MC, et al. Adjunctive benefit of an essential oil-containing mouthrinse in reducing plaque and gingivitis in patients who brush and floss regularly: a six-month study. J Am Dent Assoc 2004;135: 496-504

20 Sheen S, Eisenburger M, Addy M. Effect of toothpaste on the plaque inhibitory properties of a cetylpyridinium chloride mouthrinse. Journal of clinical periodontology. 2003 Mar;30(3):255-60

21 Ciancio SG, Mather ML, Bunnell HL. The effect of a quaternary ammonium-containing mouthwash on formed plaque. Pharmacology and therapeutics in dentistry. 1978;3(1):1-6.

22 Van Leeuwen MP, Slot DE, Van der Weijden GA. Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: a systematic review. Journal of periodontology. 2011 Feb;82(2):174-94.

23 Aksakalli S. Antioxidants in dentistry: Review of literature. Dentistry. 2013;4(1):2161-1122

24 Srinivasan M, Sudheer AR, Menon VP. Ferulic acid: therapeutic potential through its antioxidant property. Journal of clinical biochemistry and nutrition. 2007;40(2):92-100.

25 Rezk BM, Haenen GR, van der Vijgh WJ, Bast A. The antioxidant activity of phloretin: the disclosure of a new antioxidant pharmacophore in flavonoids. Biochemical and biophysical research communications. 2002 Jul 5;295(1):9-13.

26 San Miguel SM, Opperman LA, Allen EP, Svoboda KK. Use of antioxidants in oral healthcare. Compend. Contin. Educ. Dent. 2011 Mar;32:E156-9.

27 Santamaria M Jr, Petermann KD, Vedovello SAS, DeganV,Lucato A, Franzini CM. Antimicrobial effect of Melaleuca alternifolia dental gel in orthodontic patients. Am J Orthod Dentofacial Orthop 2014;145:198–202

28 Naranjo AA, Triviño ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. American Journal of Orthodontics and Dentofacial Orthopedics. 2006 Sep 1;130(3):275-e17.

29 Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. J Am Dent Assoc 2006;137:1649–1657.

30 Sendamangalam V, Choi OK, Kim D, Seo Y. The anti-biofouling effect of polyphenols against Streptococcus mutans. Biofouling. 2011;27(1):9-13.

31 Rosenbloom RG, Tinanoff N. Salivary Streptococcus mutans levels in patients before,

during, and after orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics. 1991 Jul 1;100(1):35-7.

32 Chen Y, Wong RW, Seneviratne CJ, Hagg U, McGrath C, Samaranayake LP. The effects of natural compounds-containing mouthrinses on patients with fixed orthodontic appliance treatment: clinical and microbiological outcomes. International Journal of Paediatric Dentistry. 2013 Nov;23(6):452-9.