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Effects of Moringa Oleifera Mouthwash on Plaque Index in Patients Having Fixed Orthodontic Appliances

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ABSTRACT

Background: With increasing trend towards improved facial esthetics, larger number of people are getting orthodontic treatments. Maintenance of adequate oral hygiene become difficult with fixed orthodontic appliances because tooth brushes can't reach all the surfaces due to hindrance provided by fixed appliances. Thus, in order to prevent deleterious effects of braces adjunctive oral care measures like flossing and use of an efficient mouthwash is advised by orthodontists. Commercially available chemical mouthwashes especially Chlorhexidine Gluconate is very effective in preventing plaque accumulation, gingivitis and other gum health problems, but it is associated with wide variety of side effects. So, the interest is being shifted to herbal alternatives that are equally effective but have minimal to zero side effects. In the recent history Moringa Oleifera has emerged as a miracle tree and studies have shown that it has numerous health benefits and medicinal advantages as well. It also has anti-inflammatory a property. **Objective:** The objective of this study was to find out the efficacy of Moringa Oleifera mouthwash in preventing plaque development over the period of five months in patients undergoing orthodontic treatment with fixed appliances. **Materials and Method:** This parallel arm, triple blinded, randomized controlled trial was done at Islamic International Dental Hospital. Total 60 participants who were starting orthodontic treatment with fixed appliances were included in the study; they were randomly allocated to two groups, Moringa Group and Control group. Plaque Index was recorded before the fixed appliances i.e. T0. One month after that i.e. at T1, PI was recorded again and then a one litter bottle of mouthwash was given to the participants with the detailed instructions on how to use that. Placebo mouthwash was given to control group and Moringa Oleifera mouthwash was given to Moringa group. PI was recorded again after one month i.e. at T2 and then on monthly intervals i.e. T3, T4 and T5. Jamovi and Phyton was used to analyze the results, the difference between PI scores at T0, T1, T2, T3 and T4 was assessed using the Wilcoxon Signed-rank test. P value was set at ≤ 0.05 and was considered significant. Mann Whitney U test was applied to compare the plaque index scores between control and Moringa group. **Results:** The results showed significant increase in plaque index score in both the groups over time from T0 to T4. But it increases more in the control group as compared to the Moringa group. At T4 there is significant difference in plaque index scores between the two groups, control groups have higher score than the Moringa group. **Conclusion:** The Moringa Oleifera mouthwash efficiently reduces the plaque accumulation in patients undergoing orthodontic treatment with fixed appliances. Thus, it can be used as an adjunctive oral care measure to prevent gingivitis and other deleterious effects of fixed orthodontic appliances instead of chemical mouthwashes.

INTRODUCTION

Recently there is an increase in desire of improved dental and facial aesthetics among different social classes, with the number of individuals getting their teeth aligned with fixed orthodontic appliances is also increasing. Majority of these patients are adolescents and young adults and are usually careless. It is also difficult to maintain adequate oral hygiene with the braces on as the appliances cause hindrance for the tooth brush to reach all the areas effortlessly(1), so the iatrogenic degenerative effects of orthodontic fixed appliances like; plaque accumulation, gingivitis, gingival hyperplasia, periodontitis, white spot lesions, caries and bad breath are somewhat inevitable(2). In the oral cavity, Gingivitis in the most common inflammatory disease that affects the supportive structures of teeth and tissues. Gingivitis is the plaque

induced inflammation of the gingiva that is localized and reversible but if remain uncontrolled it leads to periodontitis. Orthodontists advise the use of mouthwash as an adjunctive to proper brushing and flossing to prevent or lessen the severity of plaque accumulation, gingival hyperplasia, gingivitis, periodontitis, enamel demineralization, white spot lesions, discoloration of teeth etc(3, 4).

A good deal of mouthwashes is available, promising to maintain oral hygiene, improve oral health and reduce gingival inflammation. Majority of these mouthwashes contain different concentrations of different chemicals, particularly chlorhexidine Gluconate (CHX) has been widely used(5). Chlorhexidine gluconate is a cationic bisbiguanide and is popular as broad spectrum antimicrobial agent, and also as anti-inflammatory and anti-plaque agent in oral cavity(5). However, numerous reports detail the harmful effects of long-term use of these chemical based mouthwashes, including teeth surface staining/ discoloration, irritation, ulceration, loss of taste and burning sensation(6). Thus, finding alternative agents with less or no side effects would be really beneficial to overcome these adverse effects. Recently, the interest is being shifted to natural and organic elements to gain similar or better results with zero or at least minimum side effects. Mouthwashes containing the extracts of various herbal products such as Neem, Miswak, Fennel, Propolis, Green tea etc, have been developed and are now commercially available. Reports have shown significant benefits of herbal products over chemical ones. Herbal mouthwashes may offer similar anti-plaque properties with minimal side effects. In search of finding herbal remedies and staying closure to mother nature Moringa Oleifera was discovered in Indian subcontinent in 1785.

Moringa Oleifera is a nutraceutical agent and widely known for its anti-cancer, anti-microbial, anti-inflammatory and many other properties(7). Recently in dentistry a lot of work has been done on flavonoids (a key ingredient in MO extract), including its effect on reduction in plaque development, anti-cariogenic effects, anti-bacterial properties, as an adjunctive in treatment of surgical wounds, prevention and treatment of gingivitis and periodontitis because of its anti- oxidative and anti-inflammatory properties(8).

Previously a lot of work have been done to study the efficacy of variety of herbal mouthwashes but no study has been done on Moringa Oleifera mouthwash as an active ingredient in a mouthwash, especially to check its effect on oral health of the patients undergoing orthodontic treatment with fixed appliances(9). Considering the promising anti-inflammatory properties of Moringa Oleifera we are expecting that the mouthwash with MO leaf extract as active ingredient will show significant reduction in accumulation of dental plaque and thus will prevent the gingivitis, gingival hyperplasia, periodontitis, calculus development, bad breath etc in patients undergoing treatment with fixed orthodontic appliances. If it proves to be equally efficient in preventing plaque development as chlorhexidine mouthwash then that means we can use natural ingredient to gain the same advantage without any of the associated side effects.

Aim of this clinical trial is to check the effect of Moringa Oleifera mouthwash on plaque development in patients undergoing treatment with fixed orthodontic appliances.

Null Hypothesis

MO mouth wash has no effect on the plaque accumulation during the fixed orthodontic appliance treatment.

Alternative Hypothesis

MO mouth wash prevents the accumulation of plaque during the fixed orthodontic appliance treatment.

MATERIALS AND METHODS

It was a parallel arm, triple blinded, randomized controlled trial conducted at Department of Orthodontics, Islamic International Dental College, Islamabad. Moringa Oleifera and placebo mouthwashes were prepared with the help of the pharmaceutical team of Riphah International University Using WHO calculator Sample size was calculated to be 50, i.e., 25 participants in each group, but we included 60 participants in total to account for dropouts. Level of significance was set at 5%, power of test 90% , population standard deviation at 0.05, population variance 0.25, test value of population mean 1.21 and anticipated population mean of 0.88(10).

Permission was taken from the hospital ethical review committee and informed written consent was taken from the candidates participating in the study. For the Participants under the age of 18 consent was taken from their parents or legal Guardian. A total of 60 participants, males and females fulfilling the inclusion criteria i.e. (Young healthy subjects ranging between 13 years to 30 years of age undergoing orthodontic treatment, Patients with all permanent teeth present without any pathology, Healthy gingiva with no bleeding on probing before the start of orthodontic treatment and a normal pocket depth (1-3mm) on all teeth and no white spot lesions), were enrolled in this study. The participants of the study were divided into two groups Group A (N=30) and Group B (N=30) Each participant was identified using a code and randomly allocated to one of the two groups based on simple random sampling using computer-based randomization software.

A video graphic demonstration on how to brush teeth properly with braces and proper use of mouthwash was given to all the participants.

Plaque Index was checked for all the 60 Participants at five intervals i.e. T0, T1, T2, T3 and T4.

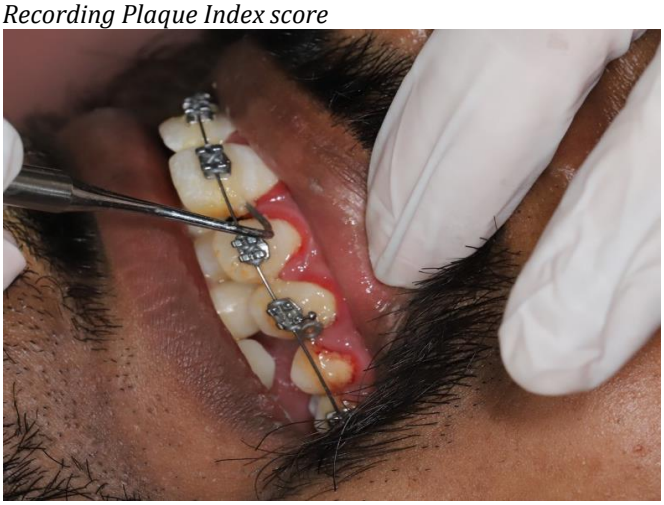
Plaque index (PI) was recorded before the placement of braces (T0). One month after the placement of braces (T1) PI was recorded again and after that 1000ml of MO mouthwash was given to the participants in group A and 1000ml of placebo mouthwash was given to the participants in control group i.e. group B. Participants in both groups was instructed to use 15ml (A Tablespoon) of the solution by swishing it vigorously in the mouth for 30 seconds twice daily and discarding it in the sink. Participants were instructed to use the mouthwash twice daily after brushing teeth according to the given instructions and then report on monthly basis to the department till 4 months.

Plaque index was recorded at five intervals, (T0) before treatment at (T1) 01 month, (T2) 02 months, (T3) 03 months, (T4) 04 months after the start of orthodontic

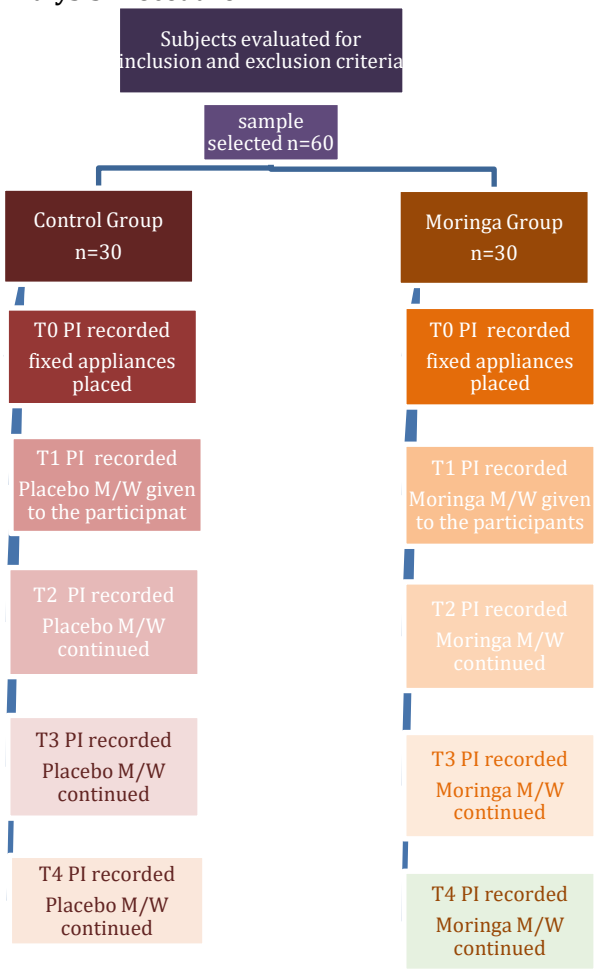
treatment. Plaque index (Loe and Sillness) was recorded by running a periodontal probe across the gingival margin, the amount of plaque on the probe determined the score ranging from 0-3(11) (fig 1). In this method labial, lingual gingival margins and interdental papilla of all erupted teeth except 3rd molars was examined and scored on a scale ranging from 0-4 according to severity.

Figure 1

Recording Plaque Index score



Data Analysis Procedure



The data set originally contained 59 entries with 59 columns, including information on participant groups (Control and Moringa), demographics (age and gender), Plaque Index Scores (Maxilla and Mandible from T0 to T4), the data set was first examined for inconsistencies and missing data. It was noted that columns "Plaque Index Maxilla T4" and "plaque Index Mandible T4" each had two missing values, For handling missing values, the missing values in "plaque Index Maxilla T4" and "Plaque Index Mandible T4" were replaced with their respective median values to ensure the integrity of these measurements without introducing bias.

Jamovi and Python used to analyze the data. Mean and standard deviation was calculated for the quantitative variable i.e. Plaque index. Mean or Median imputation and Imputation based on grouping was done to address the missing values and dropouts.

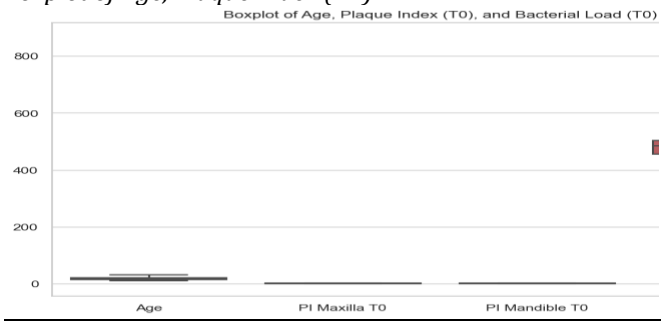
The difference between PI scores at T0, T1, T2, T3 and T4 was assessed using the Wilcoxon signed-rank test. P value was set at ≤ 0.05 and considered significant.

RESULTS

The baseline data analysis provides a comprehensive overview of the study participants. The mean age of participants is 18 years, with a range from 10 to 30 years, reflecting a young cohort undergoing fixed orthodontic appliance treatment. Plaque Index (PI) at T0, measured for both maxilla and mandible, shows relatively low initial values, with means of 0.46 for maxilla and 0.52 for mandible, indicating minimal plaque accumulation before treatment began (Fig 2).

Figure 2

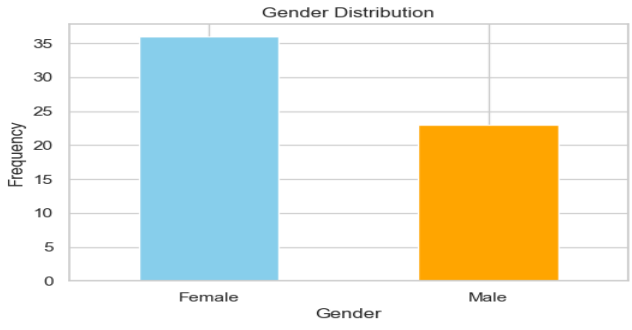
Boxplot of Age, Plaque Index (T0)



Gender distribution is slightly skewed, with 61. % female and 39% male participants. Overall, this baseline analysis sets a strong foundation for comparing changes in plaque index over time, particularly between control and Moringa mouthwash groups (fig 3).

Figure 3

Gender Distribution of the Participants



Mean plaque index score in maxilla at T1 was 0.81 and

mandible 0.86, at T2 it was 1.20 for maxilla and 1.28 for mandible. At T3 mean score was 1.54 for maxilla and 1.79 for mandible.
While at T4 it was 1.96 for maxilla and 2.15 for mandible. This shows that overall mean score increases over time, starting at around 0.46 (Maxilla T0) and 0.53 (Mandible T0), reaching approximately 1.97 (Maxilla T4) and 2.15 (Mandible T4).

Mean and Standard Deviation for Plaque Index

The table below provide the mean and standard deviation for the "Plaque Index (Maxilla and Mandible)" at the multiple time points (T0, T1, T2, T3, T4) comparing the Control and Moringa Groups.

Table 1

Plaque Index Maxilla and Mandible

Time point	Control (mean ± SD)	Moringa (mean ±SD)
PI Maxilla T0	0.57 ± 0.57	0.34 ± 0.55
PI Mandible T0	0.57 ± 0.57	0.48 ± 0.55
PI Maxilla T1	0.87 ± 0.63	0.76 ± 0.57
PI Mandible T1	1.00 ± 0.78	0.72 ± 0.87
PI Maxilla T2	1.50 ± 0.91	0.90 ± 0.80
PI Mandible T2	1.60 ± 0.73	0.97 ± 1.01
PI Maxilla T3	1.87 ± 0.67	1.21 ± 0.98
PI Mandible T3	2.30 ± 0.57	1.28 ± 0.90
PI Maxilla T4	2.47 ± 0.75	1.45 ± 1.19
PI Mandible T4	2.70 ± 0.88	1.59 ± 1.21

The Plaque Index score increases over time in both groups, but the Control group consistently shows higher values across all time points compared to the Moringa group, Suggesting the Potential benefit of Moringa Oleifera in Reducing Plaque accumulation.
Wilcoxon Signed- Rank Test was applied and it shows significant changes in Plaque index scores in Control group over all time points for both Maxilla and Mandible. The Moringa group shows significant changes in Plaque index scores in most comparisons. These Results suggest that plaque development is reduced in the Moringa group compared to the control Group.
Mann-Whitney U test was applied to compare the Plaque Index scores between control and Moringa group at each Interval.
There were *no significant differences* in Plaque Index scores between the Control and Moringa groups at the baseline (T0) and early time points (T1). From T2 onwards, there were significant differences between the two groups, with the Moringa group showing lower Plaque Index scores compared to the Control group (fig 4)
The differences become highly significant at T3 and T4, indicating a clear reduction in plaque development in the Moringa Group over time. These results suggest that Moringa Oleifera Mouthwash has a significant impact on reducing plaque index compared to the control group, especially from T2 onwards.
This Plot is showing the P-values over time for both Maxilla and Mandible, indicating where the differences between groups are statistically significant.

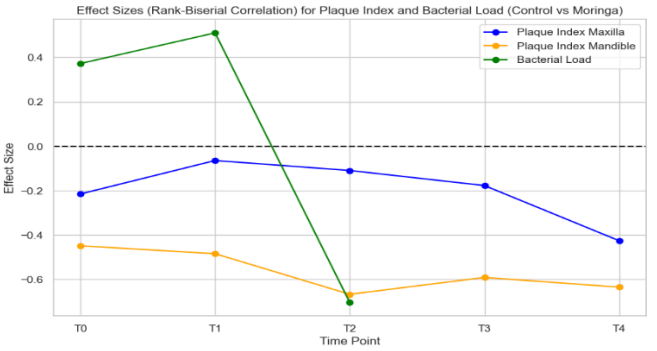
Figure 4

Mann-Whitney U Test p-values Over Time (Control v/s Moringa).

Rank-Biserial Correlation (Effect Size) for Mann-Whitney U test was calculated.
For Plaque Index score the effect size increases over time, with both Maxilla and Mandible showing large effects by T3 and T4. This indicates a substantial reduction in plaque accumulation in the Moringa group compared to the Control group (fig 5).

Figure 5

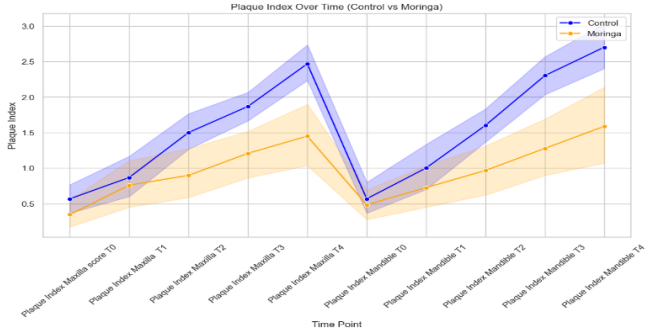
Effect Sizes (Rank-Biserial Correlation) for Plaque Index (Control v/s Moringa) against Time Point.



Friedman test was applied to analyze time series, to check the trend of Plaque Index over time within each group.
The Friedman test results indicate highly significant changes over time within both the Control and Moringa groups for Plaque Index scores. This Suggests that Plaque accumulation exhibit distinct trends over time with each group.
The Plaque Index increases over time in both groups, but the Moringa group showed a consistently lower Plaque Index compared to the Control Group, especially from T2 Onwards (fig 6).

Figure 6

Plaque Index over Time (Control v/s Moringa)

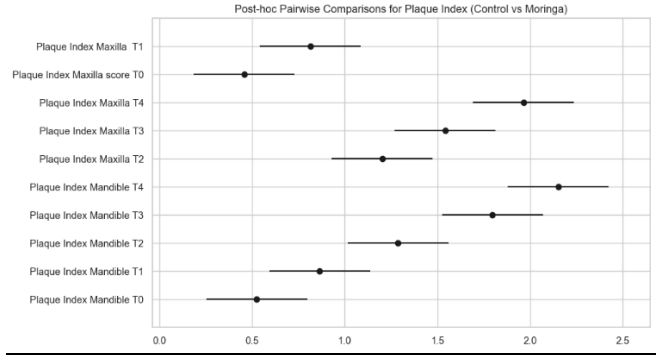


Post-hoc pairwise comparisons were done for Plaque Index (Control V/S Moringa).

Significant reductions in plaque index were observed between T0 and later time points in the Moringa group, suggesting that Moringa Oleifera Mouthwash is effective in reducing plaque development over time (fig 7).

Figure 7

Post-hoc Pairwise Comparisons for Plaque Index (Control v/s Moringa).



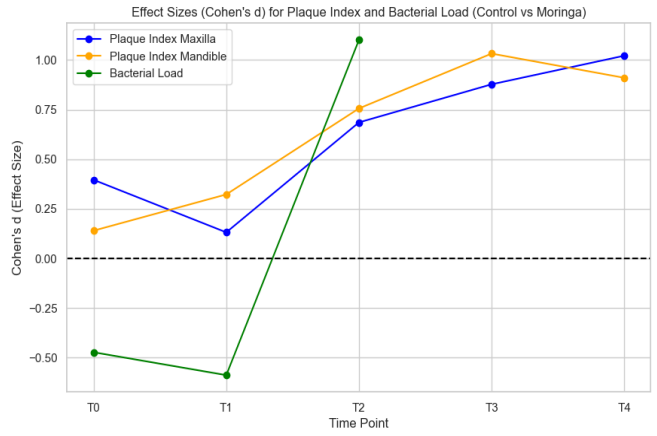
Cohen's d was calculated for each time point for plaque index effect sizes.

For Plaque Index the effect size grows over time, with small to moderate effects at earlier time points (T0 and T1), becoming large by T3 and T4. This indicates a substantial difference in plaque reduction between Moringa and Control at the later time points, with the Moringa Group showing much lower Plaque Index values (fig 8).

This analysis shows that the Moringa Oleifera mouthwash has a substantial and increasing effect over time.

Figure 8

Effect Sizes for Plaque Index (Control v/s Moringa)



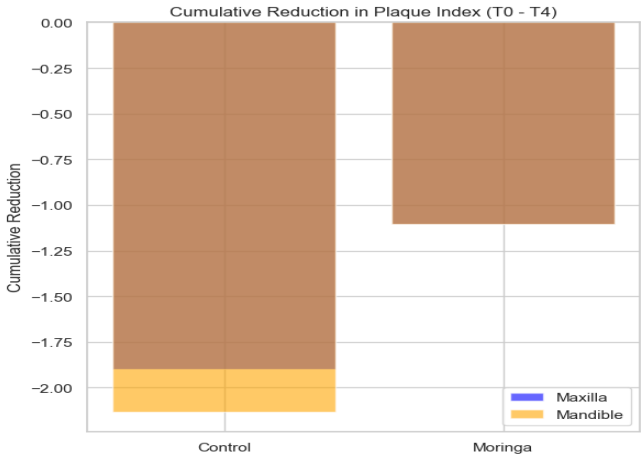
Cumulative reductions for Plaque Index (T0 to T4) were calculated.

The cumulative reduction in plaque index from T0 to T4 shows significant differences between the Moringa and Control groups for both Maxilla and Mandible, indicating that the Moringa group experienced much greater reductions in plaque accumulation.

Both maxilla and mandible show a greater cumulative reduction in plaque index for the Moringa group compared to the control group, highlighting the effectiveness of Moringa Oleifera Mouthwash in reducing plaque accumulation over time (fig 9).

Figure 9

Cumulative Reduction in Plaque Index Score (from T0 to T4).

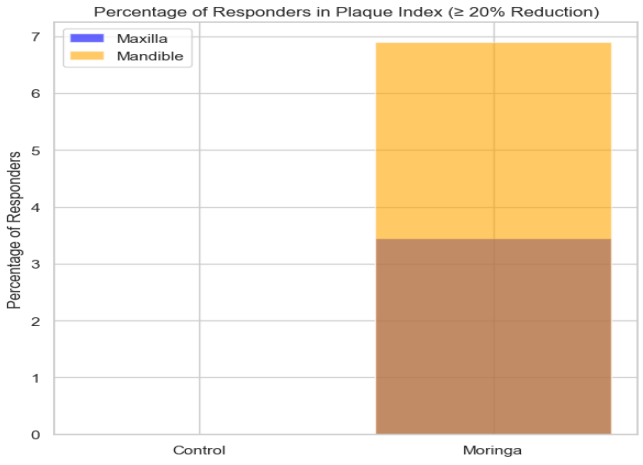


Responder analysis results show that in the Moringa group, a small but meaningful percentage of participants achieved a 20 % reduction in plaque index, while no participants in the control group reached this threshold. (Fig 10)

The analysis further confirms the clinical benefit of Moringa Oleifera mouthwash in reducing Plaque accumulation.

Figure 10

Percentage of Responders in Plaque Index.



DISCUSSION

The hypothesis of the present study was that the Moringa Oleifera Mouthwash prevents Plaque development in patients undergoing orthodontic treatment with fixed appliances. The results of our clinical trial revealed statistically significant difference in Plaque index score between control group and Moring group over period of time. This suggests that Moringa Oleifera Mouthwash is very effective in preventing plaque accumulation in patients undergoing orthodontic treatment with fixed appliances. These results are consistent with previous studies conducted to check the efficacy of Moringa Oleifera mouthwash as anti-plaque agent(10, 12).

In a study published in European Journal of Dentistry in 2022 where Moringa Oleifera was being used as an active ingredient in a dentifrice, plaque accumulation and gingival inflammation significantly reduced(13). Our study showed the similar results of decreased plaque

accumulation over time in patients undergoing treatment with fixed appliances in the Group who were using Moringa Oleifera Mouthwash as compared to increased plaque development in the control group.

As reported in previous studies this clinical trial showed that there was a significant increase in Plaque Index in patients undergoing Orthodontic treatment with fixed appliances over time,[14] proving that fixed orthodontic appliances causes machinal hindrance in effective tooth brushing and thus maintaining adequate oral hygiene is very difficult(13). Thus, in addition to proper brushing and flossing adjunctive oral hygiene maintenance measure are also necessary. Using proper mouthwash is very beneficial, a mouthwash that prevents dental plaque development.

Due to inadequate maintenance of oral hygiene some of the deleterious effects of fixed orthodontic appliances become more pronounced like gingivitis, bad breath, extrinsic staining of teeth, white spot lesions and periodontitis. In order to prevent all these side effects a good mouthwash that can prevent plaque development is very useful. According to our recent study Moringa Oleifera mouthwash can be used as an effective adjunctive measure to improve oral hygiene and prevent plaque accumulation in turn all other associated side effects of fixed orthodontic appliances.

This present clinical trial has shown that Moringa Oleifera mouthwash is very effective in preventing plaque development. When Moringa group was compared with the control group there was significant difference in plaque index score between Control and Moringa group at later intervals, T3 and T4 i.e. two and three months after the start of mouthwash use respectively. These results are in accordance with the previous studies(15). If Moringa Oleifera Mouthwash is effective in preventing plaque accumulation it will in turn prevent other harmful effects associated with long term use of fixed orthodontic appliances like, gingivitis, periodontitis, white spot lesions and calculus deposits.

This clinical trial has shown the efficacy of Moringa Oleifera mouthwash in preventing dental plaque development is as good as Chlorhexidine Gluconate. Thus, Moringa Oleifera Mouthwash can be a good and natural alternative to Chlorhexidine mouthwash to maintain adequate oral hygiene in patients undergoing orthodontic treatment with fixed appliances. Moringa Oleifera mouthwash can be used as an adjunctive oral care measure to improve oral hygiene and prevent gingival inflammation. This means that the patients can incorporate Moringa Oleifera mouthwash into their daily routines without any adverse effects associated with Chlorhexidine mouthwashes like burning sensation, extrinsic teeth staining, desquamation of oral mucosa etc.

CONCLUSION

In conclusion, this study highlighted the effectiveness of Moringa Oleifera mouthwash and the fact that it can be used in place of the chemical mouthwashes available. It showed that Moringa Oleifera mouthwash effectively reduced the plaque development in patients undergoing orthodontic treatment with fixed appliances. Thus, reduces the chances of gingival inflammation which in turn improves the gingival health and prevents gingivitis, periodontitis, white spot lesions and calculus development.

Further research work can be conducted to explore the long-term effects of Moringa Oleifera mouthwash on oral cavity and also on the fixed appliances. Studies can be conducted to check whether Moringa Oleifera Mouthwash has any deleterious effects on metallic wires and different types of fixed orthodontic appliances or not. Effects like discoloration of teeth with long term use of Moringa Oleifera Mouthwash and anti-cariogenic effects can be evaluated.

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