



Effect of Moringa Oleifera Mouthwash on Bacterial Load in Plaque in Patients having Fixed Orthodontic Appliances

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Authors' Contribution

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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 and anti-bacterial properties. **Objective:** The objective of this study was to find out the efficacy of Moringa Oleifera mouthwash in reducing bacterial load in dental plaque in patients undergoing orthodontic treatment with fixed appliances. **Materials and Method:** A parallel arm, triple blinded, randomized controlled trial was conducted at Islamic International Dental Hospital. Total 30 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. Bacterial load in plaque was calculated by counting the colony forming units (CFU) on blood agar medium before the fixed appliances i.e. T0. One month after that i.e. at T1, BL was recorded again and then a one liter 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. BL was recorded again after one month i.e. at T2. Jamovi and Phyton was used to analyze the results, P value was set at ≤ 0.05 and was considered significant. Mann Whitney U test was applied to compare bacterial load in plaque between control and Moringa group. **Results:** Bacterial load in plaque increases from T0 to T1 but significantly decreases from T1 to T2 in Moringa group and increases from T1 to T2 in control group. **Conclusion:** The Moringa Oleifera mouthwash efficiently reduces the bacterial load in plaque 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). Inappropriate oral hygiene maintenance practices such as inadequate brushing time, substandard brushing technique, lack of use of dental floss or interdental brushes and use of antimicrobial or anti-inflammatory dental care products affect the level of oral hygiene and eventually cause inflammation. In the oral cavity, Gingivitis is 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-bacterial and 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). Moringa Oleifera leaves are also rich in proteins, vitamins, calcium and potassium. Substances like flavonoids, phenolics and carotenoids that are natural anti-oxidants and anti-inflammatory agents are also present in its leaves. Flavonoids act as an anti-oxidant because it protects cells from oxidative stress and quench free radicals(8). Recently in dentistry a lot of work has been done on flavonoids, 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(9).

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 and anti-microbial properties of Moringa Oleifera we are expecting that the mouthwash with MO leaf extract as active ingredient will show significant reduction in accumulation and bacterial load in dental plaque and thus will prevent the gingivitis, gingival hyperplasia, periodontitis, calculus development,

bad breath, teeth discoloration and white spot lesions in patients undergoing treatment with fixed orthodontic appliances. If it proves to be equally efficient in reducing pathogenic bacteria in plaque biofilm as chlorhexidine mouthwash then that means we can use natural ingredient to gain the same advantage without any of the side effects associated with chemical mouthwashes especially chlorhexidine gluconate ones.

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

HYPOTHESIS

Null Hypothesis

MO mouth wash has no effect on the bacterial load in dental plaque during the fixed orthodontic appliance treatment.

Alternative Hypothesis

MO mouth wash reduces the bacterial load in dental 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 32, i.e. 16 participants in each group. 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 32 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=16) and Group B (N=16) 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.

For analyzing bacterial load in plaque, we took help from microbiology department of Quaid-e-Azam International University Islamabad. We took a small amount of Plaque from patient's gingival sulcus using a dental probe, placed it on the sterile swab stick and dip it in sterile saline in the container. Name, serial number of the participant along with interval at which the sample was

taken was mentioned on the tube. Time and date at which the sample was taken was also mentioned, as it was duration sensitive. The plaque sample needed to be analyzed within 3-4 hours. The sample was then transported to Quaid-e-Azam university immediately. There in the microbiology department they placed the plaque sample on the blood agar medium and then after certain amount of time they counted the colony forming units (CFU)/ml and shared the results with us.

Bacterial load in the plaque was checked by microbial analysis by counting colony forming units (CFU) of *S. Mutans* on blood agar for both groups. Bacterial load in the plaque was checked right before the placement of braces (T0). One month after the placement of braces (T1) this parameter 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 was instructed to use the mouthwash twice daily after brushing teeth according to the given instructions and then report next month to the department.

Bacterial load in the plaque was checked again one month after the start of M/W i.e. two months after the start of braces treatment (T2).

Figure 1

Collection of Plaque sample for analyzing Bacterial Load in Plaque



Figure 2

Sterile swab for collection of plaque sample



Data Analysis Procedure

The data set originally contained 32 entries including information on participant groups (Control and Moringa), demographics (age and gender), we had three dropouts.

The data was carefully analyzed. Initial histogram and skewness measures indicated that bacterial load data was symmetrically distributed, with slight skewness, making median imputation a robust choice. Furthermore, group-based differences in bacterial load between the Control and Moringa groups were evaluated using both mean and median comparisons, revealing substantial differences between groups. Based on this, group-specific median imputation was selected to handle the missing bacterial load values.

Jamovi and Python used to analyze the data. Mean and standard deviation was calculated for the quantitative variable i.e., bacterial load in plaque. Frequency and percentage were calculated for qualitative variables like age, gender and CFUs. P value was set at ≤ 0.05 and considered significant.

In order to compare the mean number of CFUs between the two groups, independent sample T test was applied. For comparing the PI between the two groups, Mann Whitney U test was applied.

RESULTS

The baseline data analysis provides a comprehensive overview of the study participants. The bacterial load at T0 shows a mean value of 482.85 CFU, with a wide range from 7 to 874, suggesting varying levels of bacterial presence among participants at baseline.

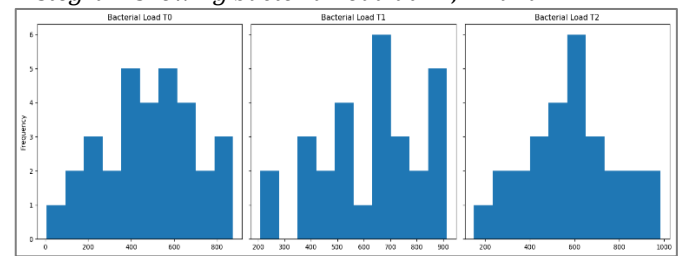
Mean score for Bacterial load in plaque was 482.85 at T0, 633.64 at T1 and 567.36 at T2. This shows that bacterial load increases from T0 to T1 but then decreases from T1 to T2.

The histogram of bacterial load for T0, T1 and T2 show a relatively symmetric distribution, without any significant skewness (fig 4.1).

Bacterial load in plaque gradually increases from T0 to T2 in both the groups.

Figure 4.1

Histogram showing bacterial load at T0, T1 and T2



Mean and Standard Deviation for Bacterial Load:

The table below provide the mean and standard deviation for the "Bacterial load" at the multiple time points (T0, T1 and T2) comparing the **Control** and **Moringa** Groups.

Table 4. 1

Bacterial Load in Plaque

Time Point	Control (Mean ± SD)	Moringa (Mean ± SD)
Bacterial Load T0	445.30 ± 176.94	521.69 ± 143.24
Bacterial Load T1	595.37 ± 133.17	673.24 ± 131.18
Bacterial Load T2	635.47 ± 124.04	496.91 ± 127.60

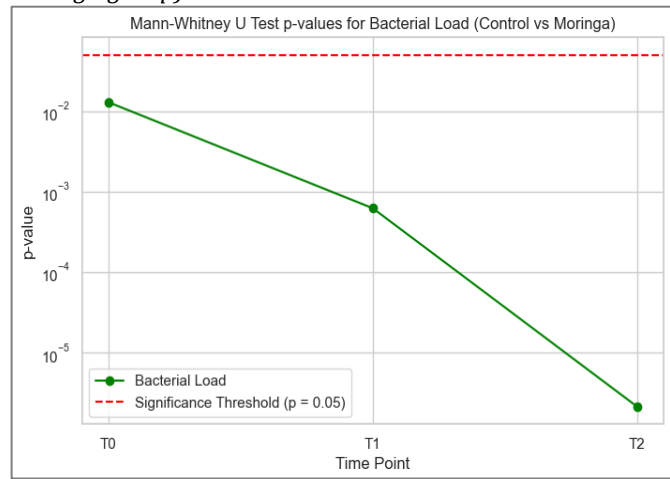
The Bacterial Load tends to increase from T0 to T1 in both groups. However, by T2 the Moringa group shows a notable decrease in Bacterial Load, while the control group continues to increase, indicating a possible reduction in bacterial load due to Moringa Oleifera mouthwash.

Mann-Whitney U test was applied to compare the Bacterial load in plaque between the two groups. At T0 there was a difference in Bacterial load in Plaque in Control and Moringa group but the differences became highly significant at T1 and T2, indicating a clear reduction in bacterial load in plaque in the Moringa group compared to the control group over time.

The plotted difference in the figure show a clear decrease over time, with T1 and T2 being highly significant (fig 4.2).

These results show that the Moringa Oleifera mouthwash significantly reduces bacterial load over time compared to the control group.

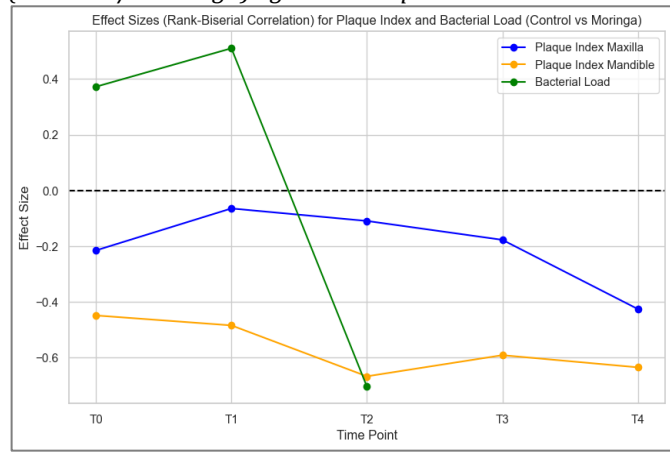
Figure 4.2
P-values for bacterial load against time point (Control v/s Moringa group).



Rank-Biserial Correlation (Effect Size) for Mann-Whitney U test was calculated.

For bacterial load, there is a moderate effect at T0, increasing to a large effect by T2 suggesting a significant reduction in bacterial load in the Moringa group (fig 4.3).

Figure 4.3
Effect Sizes (Rank-Biserial correlation) for Bacterial Load (Control v/s Moringa) against time point.

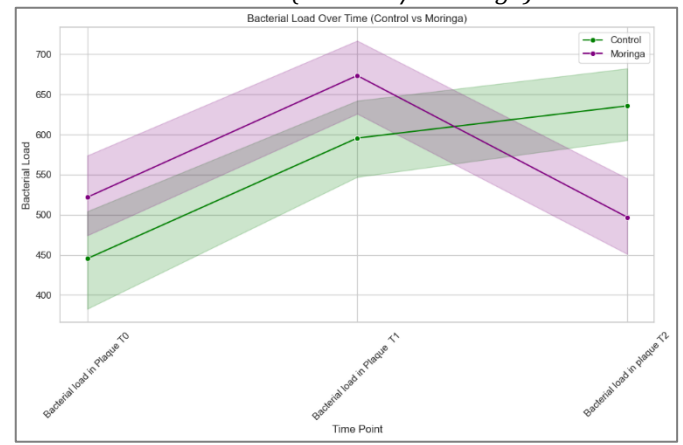


Friedman test was applied to analyze time series, to check the trend of Bacterial Load in plaque over time within each group.

The Friedman test results indicate highly significant changes over time within both the Control and Moringa groups for Bacterial Load in plaque. This Suggests that bacterial load exhibit distinct trends over time with each group.

Bacterial Load in plaque also increases over time, but the Moringa Group demonstrates a more substantial reduction compared to the Control group, particularly at T2 (Fig 4.4).

Figure 4.4
Bacterial Load over time (Control v/s Moringa)

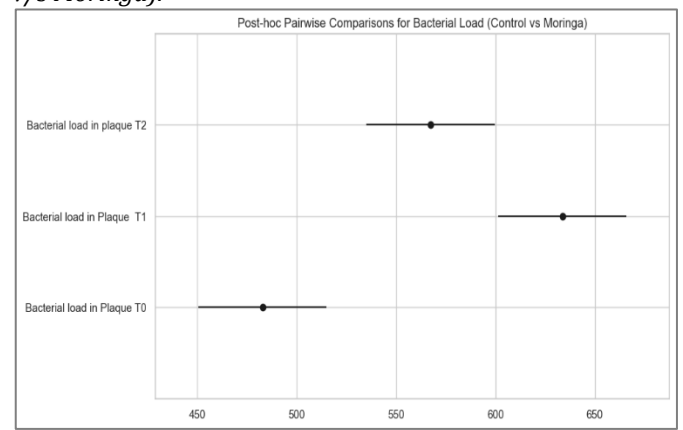


Post-hoc pairwise comparisons were done for Bacterial Load (Control V/S Moringa).

The Moringa group showed significant reductions in bacterial load, particularly between T0, T1 and T2, highlighting the effectiveness of the treatment (fig 4.5).

These Analysis confirm the Hypothesis that Moringa Oleifera Mouthwash significantly reduces bacterial load in plaque in patients undergoing treatment with fixed Orthodontic appliances over time.

Figure 4.5
Post-hoc Pairwise Comparisons for Bacterial Load (Control v/s Moringa).



Cohen's d was calculated for each time point for plaque index and Bacterial load in plaque effect sizes.

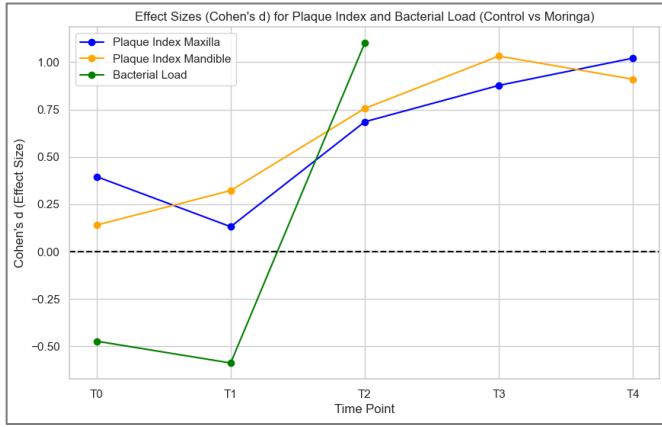
For Bacterial Load in plaque the effect sizes at T0 and T1 suggest that the bacterial load was initially higher in the control group but at T2, the effect size shifts dramatically

in favor of the Moringa group, indicating a large reduction in Bacterial load in plaque for those using Moringa mouthwash (fig 4.6).

The Plot confirms that the effect sizes for bacterial load increases over time, highlighting the growing impact of Moringa Oleifera Mouthwash on reducing Bacterial load in plaque in patients undergoing orthodontic treatment with fixed appliances (fig4.6).

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

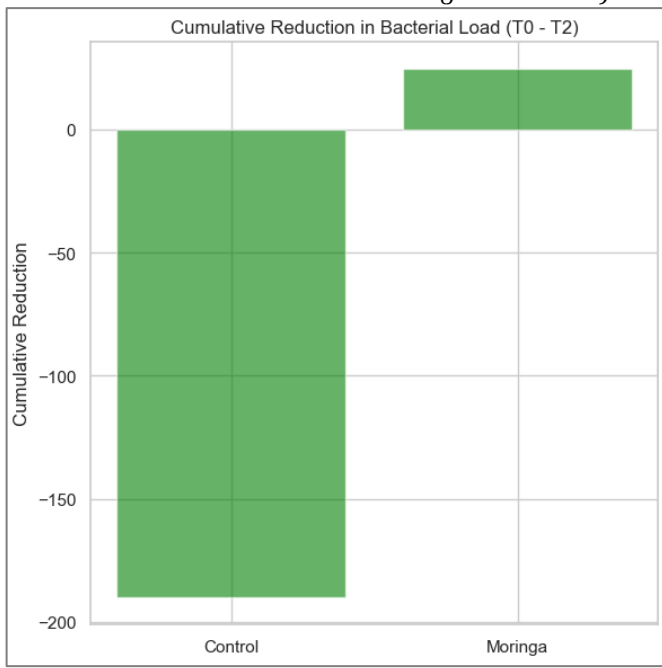
Figure 4.6
Effect Sizes for Bacterial Load (Control v/s Moringa)



Cumulative reductions Bacterial load (T0 to T2) was calculated.

The cumulative reduction in bacterial load from T0 to T2 is highly significant, suggesting that Moringa Oleifera Mouthwash effectively reduces bacterial load in plaque over time.

Figure 4.7
Cumulative Reduction in bacterial load (from T0 to T2).

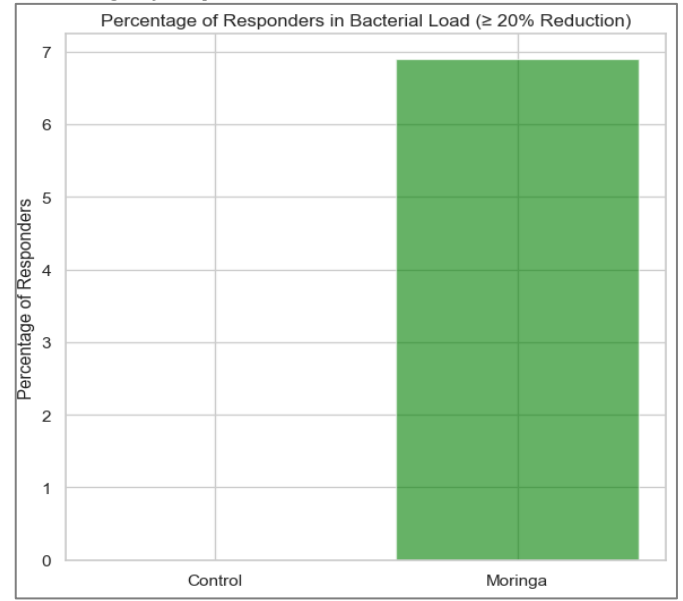


Responder analysis results show that in the Moringa group, a small but meaningful percentage of participants achieved a 20 % reduction in bacterial load in plaque,

while no participants in the control group reached this threshold.

The analysis further confirms the clinical benefit of Moringa Oleifera mouthwash in reducing Bacterial load in plaque.

Figure 4.8
Percentage of responders in Bacterial load.



DISCUSSION

The hypothesis of the present study was that the Moringa Oleifera Mouthwash reduces Bacterial load in plaque in patients undergoing orthodontic treatment with fixed appliances. The results of our clinical trial revealed statistically significant difference in Bacterial load in plaque in control group and Moringa Group especially at T2 interval that is one month after the use of Moringa Oleifera Mouthwash. These results are consistent with previous studies conducted to check the efficacy of Moringa Oleifera mouthwash as anti-bacterial agent(10, 11).

A study done in 2021 on herbal mouthwashes suggested a decrease in the percentages of candida spp. And staphylococcus spp. in the group where mouthwash containing extracts of Moringa Oleifera in combination with Citrus Hystrix dc or kaffir lime was used(10). These results are in accordance with the results of our recent study which also showed significant reduction in CFU in plaque in patients using Moringa Oleifera mouthwash.

As reported in previous studies this clinical trial showed that there was a significant increase in Plaque Index and bacterial load in plaque in patients undergoing Orthodontic treatment with fixed appliances over time(12), 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 and also improves the quality of dental plaque biofilm by

reducing the number of bacteria or bacterial load in plaque.

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 and bacterial load in plaque in turn all other associated side effects of fixed orthodontic appliances.

This clinical trial showed the positive effect of Moringa Oleifera mouthwash on bacterial load in plaque. With the use of Moringa Oleifera mouthwash for one month, there was statistically significant reduction in bacterial load in plaque, that proves the anti-bacterial effect of Moringa Oleifera herb. Bacterial load in plaque was almost the same in both the groups at the start of the study i.e. T0. After one month of the start of the orthodontic treatment there was statistically significant increase in bacterial load in plaque in both the groups showing that quality of oral hygiene and oral health decreases in patients undergoing orthodontic treatment with fixed appliances.

Fixed orthodontic appliances not only affect quantity of dental plaque but it also affects the quality of dental plaque. Bacterial load in plaque increases from T0 to T1. But the Bacterial load in Plaque significantly decreases from T1 to T2, i.e. one month after the start of mouthwash in the Moringa group. Whereas in the Control group bacterial load increases from T1 to T2. Showing that Moringa Oleifera extract has anti-bacterial properties and reduces the bacterial load in plaque thus improving the quality of bacteria(14).

Previous studies have shown that if bacterial load of plaque is less it prevents the development of gum diseases like gingivitis and it also prevents the progression of gingivitis to Periodontitis that can eventually lead to loss of teeth(15). More quantity of bad bacteria in the dental plaque biofilm also leads to white spot lesions and caries. Presence of high quantities of *Scardovia wiggisiae*, *Streptococcus Mutans* and *Propionibacterium acidifaciens*

are associated with causing white spot lesions and initial caries(16). As the caries progresses the microbial activity decreases. Thus, we can assume that if the Moringa Oleifera is effective in decreasing bacterial load in plaque it can effectively prevent the development of white spot lesions and eventually caries as well, proving its anti-cariogenic properties(17).

This clinical trial has shown the efficacy of Moringa Oleifera mouthwash in reducing bacterial load in dental plaque 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 bacterial load in plaque. Thus, improves the quality of dental plaque biofilm, this property reduces the chances of gingival inflammation that is somehow inevitable in patients undergoing orthodontic treatment with fixed appliances. 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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