Research Article

Efficiency of watermelon extract against oral microflora particularly *Lactobacillus* - An *in vitro* study

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ABSTRACT

Introduction: *Lactobacillus* is one of the bacteria responsible for the formation of dental caries and is one of the most common oral microfloras. It is acidogenic bacteria which grow only in an acidic environment and in regions where there is ready access for carbohydrates. Patients undergoing orthodontic treatment have alterations in their microflora which includes mainly the *Streptococcus mutans* and *Lactobacillus* species. The aim of this study is to find the effect of watermelon juice against oral microflora such as *Lactobacillus.* **Materials and Methods:** A known quantity of watermelon is obtained from the local market and is made into juice without adding any additives. The juice is then filtered and is centrifuged at the rate of 300 rotations per minutes for 15 min. Two test tubes with 5 ml of the clear watermelon extract are taken. To one test tube, 0.5 g of sucrose is added and mixed well. A test tube containing 5 ml of saline is taken as control group. The solutions are autoclaved, and suspension of *Lactobacillus* is freshly prepared and 10 µl of the same is transferred to every cuvette containing the autoclaved solution. Then after a holding period of one 24 h, 10 µl of the watermelon extract containing the bacteria is pipetted into nutrient agar culture plate and bacterial colony growth is studied after the incubation period of 24 h. **Results:** Results showed that there is a decrease in the bacterial colony count in the culture plate containing watermelon solution when compared with the control group containing saline. When compared with the watermelon solution containing sucrose, the colony count was high in the latter. **Conclusion:** Watermelon being an acidic fruit has a significant effect against acidogenic oral microflora such as *Lactobacillus* and can used as an additional aid for oral hygiene maintenance in patients undergoing orthodontic treatment.

KEY WORDS: *Lactobacillus*, Malocclusion, Oral flora, Oral hygiene, Orthodontics, Watermelon

INTRODUCTION

Oral cavity is the source for millions of micro-organisms. The most common microflora that harbors the oral cavity is the *Streptococcus* and the *Lactobacillus* species. Malocclusion is one of the most common dental conditions that we come across in our day-to-day practice. Facial esthetics plays a crucial rule in the social and psychological development of an individual.[1] Patients with malocclusion are more prone to inflammatory conditions such as gingivitis and periodontitis. This is because the patients find it difficult to manage their oral hygiene as the tooth surface becomes less self-cleansing which will lead to increased accumulation of plaque and calculus.

Due to increased esthetic demands, the number of people undergoing orthodontic treatment has increased in the past few years. People who are not satisfied with their appearance are the ones who seek orthodontic treatment.[2] The use of fixed appliance usually alters the oral microbiological profile of the patient. Patients undergoing orthodontic treatment are more prone to an increase in the level of bacteria such as *Streptococcus mutans* or *Lactobacillus*. *Lactobacillus* are Gram-positive, facultative anaerobic, round bacteria. *S. mutans* are one of the most common bacteria seen in oral environment and they belong to the *Streptococcaceae* family.

Although there are various oral hygiene measures available such as manual tooth brushes, interdental brushes, automated brushes, dental floss, and mouth rinses, patients find it difficult to manage their oral hygiene. This is due to the presence of components of fixed appliance such as the brackets, wires, bands, and elastomeric rings, so it is very essential to provide proper oral hygiene instructions to all the patients undergoing orthodontic treatment, along with

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other post-bonding instructions. The patient should follow strict oral hygiene protocol to maintain proper oral hygiene and prevent bacterial infection from accumulated plaque or calculus.

The use of home remedies and herbal products for oral hygiene maintenance has started becoming prevalent in the past few decades. Antimicrobial effect of fruit juices was studied earlier.[3] Watermelon is a trailing vine in the flowering plant family called Cucurbitaceae. It is grown in the subtropical and tropical areas of the world and is a special kind of a berry fruit called pepo. The fruits are edible and can be eaten raw. Watermelon is rich in Vitamin C and 90% of its content is water. The pH of plain watermelon juice lies between 5 and 5.2.

The aim of this study is to evaluate the ability of watermelon juice to maintain the bacterial colony count of *Lactobacillus* in the oral environment and thus evaluate its efficiency in maintaining the oral hygiene in patients undergoing orthodontic treatment.

MATERIALS AND METHODS

This is an *in vitro* study. A known quantity of fresh watermelon (250 g) was bought from the local market and made into juice without adding any additives such as sugar or water. The juice is filtered and transferred to two test tubes, and the test tubes are covered with cotton plugs. The tubes are then centrifuged at a speed of 300 rotations per minutes for 15 min. Then, the supernatant of the solution is transferred to another test tube and the bottom part is discarded. The process of centrifugation is repeated thrice to obtain a clear solution of the watermelon juice. After every centrifugation process, the bottom part of the solution is discarded and supernatant of the solution is used for the next centrifugation.

Then, the clear watermelon juice is transferred to two test tubes containing 5 ml of the solution each. A known quantity (0.5 g) of sucrose is measured using a digital weighing scale and is added to one of the test tubes. Sucrose crystals were ground into powder form before weighing it, and it was added to one of the test tubes and mixed well. A test tube containing 5 ml of saline is taken as a control group.

Thus, the samples were grouped into three as follows:
Group 1 - Test tube containing clear watermelon juice.
Group 2 -Test tube containing clear watermelon juice with sucrose.
Group 3 - Test tube containing saline (control group).

Three test tubes containing the solutions are autoclaved, and the solutions were transferred to sterile cuvettes. Each solution was transferred to two cuvettes containing 1 ml of the solution each. A fresh suspension of *Lactobacillus* bacteria was prepared from the individual colonies of the bacteria and was compared with McFarland standards. Then, 10 µl of the freshly prepared *Lactobacillus* bacterial suspension was added to the cuvettes containing the saline and the watermelon solutions. The cuvettes were left undisturbed for a holding period of 24 h.

After the holding period, 10 µl of the solutions from each of the cuvette is carefully pipetted out into a culture plate containing nutrient agar culture medium. The solution was streaked using sterile cotton swab and was incubated for 24 h to find the growth of bacterial colonies in the culture medium.

RESULTS

The number of bacterial colonies formed in the culture medium was counted after the incubation period of 24 hours and is tabulated [Table 1].

DISCUSSION

Oral hygiene maintenance is one of the crucial difficulties that are faced by the patients undergoing orthodontic treatment. This is due to the presence of various attachments of fixed orthodontic appliance. From the result, it can be seen that, when compared to the control group containing saline (Group 3), there is a significant reduction in the number of *Lactobacillus* bacterial colony count in the culture medium which had the Group 1 sample (plain watermelon juice). While comparing Group 2 (watermelon juice + sucrose) and Group 1, the sample containing sucrose had comparatively more bacterial colonies. This showed the effect of sucrose on the growth of the bacteria. Thus, the result showed the effect of watermelon juice against the oral bacteria such as *Lactobacillus* and it also showed the effect of carbohydrate in aiding the growth of bacteria. *Lactobacillus* being acidogenic bacteria should grow in an acidic environment, but the results obtained were contradictory. The discussion part would concentrate more on the malocclusion part and the presence of various methods of oral hygiene maintenance available and that are under study.

The increase in the prevalence of malocclusion and thus increase in need for orthodontic treatment were studied by various authors in their studies[4-7] which showed that there is an increased prevalence of Angle's Class 1 malocclusion. It included the patients having Class 1 molar relation with crowding or spacing. Apart from this, there was an increased prevalence of crowding in the lower anterior teeth in the subjects included in the study. It also stated that the most common reason the patient undergoing orthodontic treatment is the need for highly esthetic smile and facial profile. Although there was an increased prevalence of malocclusion in the subjects taken into consideration,

Table 1: Number of bacterial colonies found in sample groups

Groups	Number of bacterial colonies in sample 1	Number of bacterial colonies in sample 2
Group 1 (plain watermelon juice)	3	4
Group 2 (watermelon juice+sucrose)	7	9
Group 3 (saline control)	25	27

there were only few candidates who were willing for orthodontic treatment. They also studied the need for orthodontic treatment in the subjects. 37.5% of the subjects included in the study were in a greater need for orthodontic treatment.[7]

Patients undergoing orthodontic treatment are more prone to alterations in their oral microbiological profile. This is due to the presence of orthodontic attachments and auxiliaries on the tooth surfaces of patients undergoing orthodontic treatment. This will lead to increased accumulation of plaque and calculus and difficulty in oral hygiene maintenance. There are many studies which showed the alterations in the oral microflora in the patients undergoing fixed orthodontic treatment. Lara-Carrillo *et al*.[8] and Arab *et al*.[9] studied the effect of orthodontic treatment on saliva, plaque, and also the levels of microbial colony count in the oral cavity. They showed that there were major changes in the salivary properties such as an increase in stimulated flow rate, the buffer capacity, and salivary pH, which augment the anticaries activity of saliva. In contrast, an increase in the occult blood indicated that there was more gingival inflammation, which was because augmented by the retentive plaque on the tooth surfaces and the difficulty in maintaining a good oral hygiene. They studied both the microbiological and the non-microbiological parameters of saliva and stated that the presence of attachments leads to increased accumulation of plaque, thus resulting in alterations in the microbiological profile in the oral cavity of the patient. Although there were various studies showing the relationship between orthodontic treatment and microbial alterations, Amanda Freitas *et al*.[10] conducted a systematic review on the effect of orthodontic treatment on oral flora and showed that there was only moderate evidence for the alterations of the quantity and quality of oral microorganisms by orthodontic treatment. They reviewed the literature from various articles which studied the alterations in the bacterial count in the oral cavity after fixed orthodontic treatment. They took eight articles according to their inclusion criteria and took only four articles with moderate methodological quality into the review. Anyways, further study with recent research should be included to make the results of the review more reliable. A comprehensive study on the effect of orthodontic treatment on oral health status was studied by Cantekin *et al*.[11] which showed that decay-missing-filled index counts and the pulsatility index increased in a group of young dental patients who underwent orthodontic therapy, and thus, patients undergoing orthodontic treatment should follow very rigid oral hygiene protocols. Proper oral hygiene instructions should be given to the patients and the same should be reflected in the patient's oral hygiene which should be monitored during every appointment of the patient. Maret *et al*.[12] and Peros *et al*.[13] studied the effect of orthodontic treatment on salivary microbial and non-microbial parameters. Both the studies showed that there was a significant increase in the *Lactobacillus* and streptococcus bacterial colony counts. The effect of orthodontic treatment on Candida species and Enterobacterium species was also studied by Hägg *et al*.[14] It showed that there are a significant increase in the candidal bacteria count and increase in the periodontal index of the samples.

Maintaining oral hygiene becomes very difficult in the patients undergoing orthodontic treatment due to the presence of various attachments on the tooth surface. Antibacterial effect of different brushing frequencies on *Lactobacillus* and *S. mutans* in children undergoing orthodontic treatment was studied by Peros *et al*.[15] It showed that there is significant antimicrobial activity against *S. mutans* but there was no effect against *Lactobacillus* when fluoridated toothpaste is used thrice a day. Effect of chlorhexidine and the frequency of *Lactobacillus* in orthodontic patients were studied by Lundström and Krasse,[16] and it showed that there was a significant effect of chlorhexidine against *S. mutans* and the effect against *Lactobacillus* could not be demonstrated. The effectiveness of two different toothbrushes on oral hygiene in patients undergoing orthodontic treatment was studied by Kiliçoğlu *et al*.,[17] showing that the orthodontic toothbrushes were not superior to the conventional toothbrushes in maintaining the oral hygiene. Smiech-Slomkowska *et al*.[18] studied the effect of oral health education on levels of *Lactobacillus* and Streptococcus species, and it showed that there was no significant reduction in the same and stated that patients undergoing orthodontic treatment should be monitored periodically for dental caries. The microbial association between two different ligation methods in orthodontics was studied by Forsberg *et al*.[19] They stated that, although there was significant increase in the number of colonies after orthodontic treatment, there was no significant difference in the number bacterial colonies between the ligation done using stainless steel ligature wires and elastomeric rings.

Many studies were carried out for studying the reduction of microbiological colony count using

home dietary alterations and herbal products. The reduction of oral *Lactobacillus* by periodic restriction of carbohydrate was studied by Jay,[20] showing that though there was no significant reduction in the *Lactobacillus* count, and the caries activity was reduced which was also essential. Cildir *et al*.[21] conducted a study on the reduction of *S. mutans* bacteria in saliva in patients undergoing orthodontic treatment by daily consumption of yoghurt containing probiotic bacteria and stated that short-term consumption of fruit yoghurt daily containing *Bifidobacterium animalis* subsp. may reduce the levels of *mutans* streptococci in saliva. The antibacterial activity of *Acacia catechu* willd bark against *Streptococcus sanguis*, *Streptococcus mitis,* and *Lactobacillus acidophilus* was studied by Lakshmi and Kumar[22] and stated that these extracts had bactericidal action against *Lactobacillus,* and it was due to the phytochemicals present in it. The effect of traditional medicinal plant extracts and natural products against oral bacteria was studied by Palombo.[23] It was a review article and they discussed about the plant extracts or phytochemicals that inhibit the growth of oral pathogens, reduce the development of plaque accumulation on dental surfaces, influence the adhesion of bacteria to surfaces, and reduce the symptoms of diseases of oral cavity. The antibacterial activity of ethanolic root extract of *Glycyrrhiza glabra* against oral bacteria was studied by Geetha and Roy,[24] and they stated that the extract at different concentration had antibacterial activity against oral bacteria such as *L. acidophilus* and various *Streptococcus* species.

Although there were various oral hygiene measures available in the market, we decided to create a new adjunct to the same using watermelon juice and studying its antibacterial effect. Antibacterial activity of fruits against *Escherichia coli* was studied by Manzoor *et al.*[25] and stated that the extract of apricot had the maximum inhibition effect followed by the extract of watermelon. The various medicinal uses of watermelon were reviewed by Erhirhie and Ekene[26] and showed that *Citrullus lanatus* possesses numerous bioactivities from the natural sources which is of better advantage than the conventional therapies. The antibacterial activity of watermelon seed was studied by Adunola *et al*.,[27] and they stated that the antibacterial effects of *C. lanatus* (watermelon) seed extracts against the selected bacteria suggest that the extracts obtained by different extraction methods such as the cold maceration, Soxhlet extraction, as well as using methanol and chloroform methods had potential as antibacterial agents, especially against *Staphylococcus* species and *Pseudomonas aeruginosa.*

CONCLUSION

Clear watermelon extract obtained by centrifugation of plain watermelon juice showed a significant effect against one of the most common oral bacteria *Lactobacillus*. The presence of carbohydrate (sucrose) along with the watermelon increased the number of bacterial colonies in the culture medium. This showed the increased growth of the bacteria in the presence of carbohydrate. Although watermelon being an acidic fruit had a significant effect against the acidogenic bacteria *Lactobacillus*, the exact content of watermelon that is responsible for the same was not studied and future studies should concentrate on the same. Thus, the plain watermelon juice can be used as an adjunct to the additional oral hygiene aids and can be advised to be used by the patients undergoing orthodontic treatment. However, further studies in the patients will be needed to strongly agree with the same. Although there are various additional aids available for the maintenance of oral hygiene, proper education of the patient about the oral hygiene instructions, and their benefits, constant monitoring of the patient's oral hygiene is very essential to prevent any future damage to tooth or the periodontium.

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