Comparative efficacy of the Antiplaque Effect of Natural Honey Mouthwash and Chlorhexidine Mouthwash. A randomized controlled trial
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Abstract

Objectives: To compare the efficacy of natural Honey (10%) and chlorhexidine mouthwash (0.12%) on the dental plaque levels. Moreover, to evaluate the efficacy of natural Honey and chlorhexidine mouthwash on the level of dental plaque.

Methodology: This study was a single-centered randomized controlled trial conducted on participants attending the Dental OPD of Dar-ul-Sehat hospital in Karachi. A total of 60 participants were included in the trial from June 2020 to December 2020. The sample size was calculated using openepi after entering the mean and SD of the honey and chlorhexidine groups. The study included sixty male and female participants ranging in age from 18 to 25 years, and those with medical illness, missing teeth, and antimicrobial history were excluded from the study. The subjects were randomly assigned to two groups: Natural Honey or Chlorhexidine gluconate mouthwash. The data was collected at the baseline and 14th day; the plaque was revealed using a disclosing solution. Their scores were recorded at four sites per tooth using the Silness-Loe plaque index criteria. Later, statistical analysis was performed to compare the effects of the two groups. P ≤ 0.05 was considered statistically significant.

Results: Our findings revealed that both groups effectively lowered the plaque score. However, it showed a more significant reduction in plaque indices in the honey group on Day 14 when compared to chlorhexidine mouthwash.

Conclusion: Honey has been identified as a potent antibacterial agent with therapeutic properties. Based on the findings of this study, it is possible to conclude that gargling with the original honey 10% solution is effective in lowering dental plaque score. Honey can be used in relation to conventional treatments to protect dental plaque and gingivitis. More research will be needed to back up these preliminary findings. Moreover, the synergistic effect of Chlorhexidine with natural Honey must be investigated.

Keywords: Natural Honey, Plaque, Chlorhexidine, Mouthwash, Antibacterial.

IRB: Approved by the ethics committee of Liaquat College of Medicine and Dentistry, Darul Sehat Hospital.


Dental caries and periodontitis are two of the major dental conditions caused by plaque deposition that have abundantly afflicted the population of Pakistan³. According to the World Health Organization, 10-15% of the world's population suffers from severe periodontitis caused by dental plaque⁴.

Dental plaque is a host-associated matrix-enclosed biofilm containing various microbes that adhere to the tooth's surface or other surfaces⁵. Microbes contribute to calculus formation, which triggers inflammatory responses associated with periodontal disease progression in soft (gingiva, periodontal ligament, connective tissue, junctional epithelium) and hard tissues (cementum, alveolar bone). Periodontal disease progresses due to the mutual interaction of bacteria and host defense response⁶. As a result, dental plaque is

Introduction

Scientific research in the medical field has shown that health originates in the oral cavity. These days, good oral health is about more than just tooth health; it is a basic foundation for our general health and well-beingⁱ. Over 3.5 billion people have been affected by oral infections worldwide, with untreated dental caries being the most common health condition².

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likely to cause various issues, including caries and periodontal diseases, which damage the tooth's supporting structure and eventually lead to tooth loss.

The Mechanical approach to supragingival plaque control tools includes toothbrushes, floss, wood sticks, and interdental brushes. However, it is believed that the level of motivation and skill required to use these oral hygiene products effectively is beyond the majority of patients' abilities. As a result, to address the potential deficiencies of daily self-performed oral hygiene, a chemical plaque control approach in the form of mouthwashes is preferable.

Chlorhexidine is the "gold standard" antiplaque agent. However, it is not a "Magic Bullet" due to side effects such as tooth staining, taste disturbance, and so on. It has been successfully used by dental professionals and pharmaceutical companies for over three decades, establishing it as a good control against which the effectiveness of alternative antiplaque agents should be measured.

Nature has been a source of traditional remedies for thousands of years, and plant-based systems play an important role among them. Honey is one such natural product. According to research, the antibacterial effect of Honey can be attributed to the presence of hydrogen peroxide, flavonoids, and bee defensin-1, among other things. When these antibacterial properties are diluted, they become even more potent.

The rationale is to compare the efficacy of natural honey based preparation with commercially available Chlorhexidine-based mouthwash in preventing dental plaque formation because Honey is readily available, cost-effective, and organic. This study will contribute to the collection of data from our community. The positive outcome may lead to developing a new method of oral hygiene maintenance with fewer side effects. We will be able to provide the most effective antiplaque agent. As a result, the current study was designed to compare the antiplaque efficacy of honey and chlorhexidine gluconate mouthwash (0.12%).

The study aims to evaluate the antiplaque effect of natural honey mouthwash, the antiplaque effect of chlorhexidine gluconate mouthwash, and the antiplaque effect of natural honey mouthwash versus chlorhexidine mouthwash.

**Material and Methods**

An open-label, randomized controlled trial (NCT05258955) was used to evaluate the effect of both mouthwashes on the level of dental plaque in young adults. A total of 60 new patients who presented to the Periodontology department with complaints of dental stains and bleeding gums, ranging from 18 to 25 years, were enrolled. A single-center study was conducted on participants attending the department of Periodontology, Dental OPD of Dar-ul-Sehat hospital in Karachi. The participants were included in the trial from June 2020 to December 2020.

Randomization is done by using the Opaque sealed envelope method. The patients were divided into two groups. The group name is written on paper that has been double-folded and placed inside the envelope to prevent identification by Transillumination. These envelopes were prepared and sealed by personnel other than the principal investigator and were signed on the back to ensure that no one tampered with them before allocating. After completing the inclusion criteria and providing informed consent, patients were asked to choose an envelope, after which they were assigned to a specific group.

The institutional review board approved the study protocol of Liaquat College of Medicine and Dentistry (Reference number: Ref.No.EC/11/20). All participants signed a written informed consent form and were then enrolled in this study.

The patients with age between 18-25, who had 28 retained teeth excluding wisdom, were included while patients with any medical illness which impact the oral cavity missing teeth due to extraction, faulty fillings or denture wearer, pocket depth more than 3 mm, history of consumption of antimicrobials in past 6 month, a non-cooperative patient who are not willing to maintain their oral hygiene, patient with a habit of chewing betel nut or smoking were excluded.

Discontinuation criteria: exaggeration of periodontal
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problem during the study, if the patient fails to adhere to the protocol for more than two days if the patient required to take antimicrobial due to any illness.

After obtaining consent and informing participants about the nature of the study, they were divided into two study groups. The sealed opaque envelope method was used for randomization. Each group consisted of 30 people. Group A: Chlorhexidine Mouthwash. Group B: Natural Honey Mouthwash.

The sample size was calculated using open epi after entering the mean and SD of the honey and chlorhexidine groups on the 15th day, 2.85±0.44 and 2.40±0.51, respectively\(^\text{13}\).

Sample Size Formula for Difference in Means

\[
n = \frac{2\sigma^2(Z_\beta + Z_{\alpha/2})^2}{\text{difference}^2}
\]

Where \(Z_{\alpha/2}\) is the critical value of the Normal distribution at \(\alpha/2\), \(Z_\beta\) is the critical value of the Normal distribution at, \(\alpha2\) is the population variance, and \(d\) is the difference we would like to detect.

A minimum sample size of 27 is required in each group for 54. Because there was a 10% chance of error, the sample size was set at 30 in each group for the two groups’ total sample size of 60.

The Silness and Loe index was used to assess the level of plaque. Plaque deposits were measured on teeth 16, 12, and 24 in the upper arch and on teeth 44, 32, and 36 in the lower arch. The principal investigator calculated the initial baseline score before scaling and polishing to return the baseline score to zero.

An investigator who was not involved in the assessment of outcome measures obtained commercially available chlorhexidine gluconate (0.12 percent) mouthwash and natural Honey. Natural Sidr Honey was purchased from the Islamic Shahad Center.

Patients were given dark bottles labeled 1 and 2 for groups A and B, respectively. The first contains 0.12% chlorhexidine gluconate, while the second is a natural honey-based preparation. The mouthwash composition was dispensed in 450 ml quantities in a coded bottle. Honey mouthwash was prepared by diluting it with lukewarm water. To make 100ml of 10% honey solution, 10ml Honey is diluted in 90ml lukewarm water.

Patients were instructed to thoroughly swish the oral cavity twice daily with 10 ml of solution for at least 60 seconds each time. All study participants were advised to use a modified bass method technique to maintain oral hygiene. Furthermore, participants were asked to refrain from using any other type of mouth rinse during this time. After two weeks, patients were recalled, and plaque levels were measured using a periodontal probe and plaque disclosing tablets. The principal investigator obtained the plaque disclosing tablet from PD Produits Dentairs SA. The colored area showed the presence of a plaque deposit on the tooth surface. The scores were recorded using the plaque index criteria.

The data was analyzed using SPSS 21.0. The numeric variable was represented by mean, standard deviation, and the categorical variable was represented by frequency and percentage. The Shapiro–Wilk test was used to confirm the normal distribution. To compare the pre-treatment and post-treatment results the paired t-test was used. The Independent Samples t-Test was used to compare the mean values of two groups, and a statistically significant p-value of <0.05 was used.

Results

Initially, 70 participants were tested for eligibility in this trial; however, 10 were exempted, including those who refused to sign the consent form or discontinue intervention (\(n= 5\)) and did not return for follow-up (\(n= 5\)). As previously stated, the study included a total of 60 participants. At random, all eligible participants were assigned to two interventional groups. Each group has 30 participants, as depicted in Figure 1.

According to the findings of our study, the mean age of participants in Group A was 23.53 ± 2.60, and the mean age of participants in Group B was 24.0 ± 3.76. The majority of the participants in Groups A and B were male.
(20 and 22 respectively), with the remainder being female (10 and 08), as shown in Table 1.

Group A was given Chlorhexidine mouthwash, and Group B was given Natural Honey. There was no significant difference between the two treatment groups before the treatment (p=0.230). After 14 days of intervention, antiplaque levels in both treatment groups were significantly reduced (p≤0.001).

However, the Natural Honey Mouthwash group showed slightly more prominent results than Chlorhexidine Mouthwash after 14 days of intervention. Significant changes exist between the two groups after treatment (p=0.048), as shown in Table 2.

Table 1: Demographic Data of the Studies Participants.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in Years</td>
<td>23.53 ± 2.60</td>
<td>24.0 ± 3.76</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 20</td>
<td>Female 10</td>
</tr>
<tr>
<td></td>
<td>Female 22</td>
<td>Female 08</td>
</tr>
</tbody>
</table>

*Age is presented in Mean ± Standard Deviation (n) – Frequency of Gender
Group A was given Chlorhexidine mouthwash (0.12%)
Group B was given Natural Honey mouthwash (10%)

Table 2: Comparison of chlorhexidine mouthwash and natural honey mouthwash on plaque index.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline Plaque Index</th>
<th>14th Day Plaque Index</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.86 ± 0.29</td>
<td>1.28 ± 0.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>1.89 ± 0.18</td>
<td>1.18 ± 0.17</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>p-value</td>
<td>0.54</td>
<td>0.048</td>
<td></td>
</tr>
</tbody>
</table>

*All the variables were presented in Mean ± Standard Deviation. The paired t-test for comparison between baseline plaque index and 14th Day plaque index. Independent t-test to test between two unrelated groups, i.e., a) between baselines of both groups and b) between 14th-day plaque of both groups (p-value<0.05).
Discussion
Dental plaque is a multifaceted, unique, but extremely variable structural entity formed by the colonization of microbes integrated into a gelatinous extracellular matrix on the surface of teeth, restorations, and other oral cavity structures. Microbial products of dental plaque biofilm have been shown to activate host defense mechanisms, leading to hard and soft tissue damage. Mechanical control of the dental plaque biofilm is required to prevent and manage caries and periodontitis. Because mechanical plaque control methods may be ineffective and challenging in preventing periodontal diseases, adding chemical agents to tooth brushing and flossing may provide relevant benefits. As a result, Chlorhexidine mouthwash has been the most widely used antiplaque agent, with studies demonstrating its efficacy. Chlorhexidine produces its antibacterial effect by disrupting prokaryotic cell membranes by damaging cytoplasmic components, causing increased permeability and cell lysis.

Van Ketel initially discovered the antibacterial properties of Honey in 1892. Recent research on this natural product honey that is expected to be more safe has shown that in vitro can eliminate a wide range of bacteria.

The existing study was carried out to compare the effectiveness of Natural Honey (10%) with Chlorhexidine gluconate (0.12%) on the levels of dental plaque. The findings of this study reveal that when administered twice daily using proper brushing technique, both mouth rinses containing 0.12% chlorhexidine and 10% honey were found to be clinically effective in preventing plaque buildup. Therefore, the antibacterial activity of natural Honey is comparable to that of Chlorhexidine in terms of plaque reduction. These findings corroborate those of Jain et al. The adhesion of S. mutans bacteria to tooth surfaces is now well understood to be the first stage in developing dental plaque. In an experiment, Badet and his colleague established that Honey at a concentration of 10% could influence the formation of an S. mutans biofilm. Nayak et al. observed parallel results with various types of honey.

When Honey and Chlorhexidine mouthwash were evaluated against dental plaque in the current study, there was a noteworthy difference, with honey mouthwash (10%) showing somewhat better results in terms of reducing dental plaque. Jain et al. reported similar results, claiming that natural Honey reduced plaque by a higher proportion. Furthermore, Ahmadi et al. discovered that Honey has the strongest antibacterial action at 100% concentration.

Honey has a variety of actions that contribute to its antibacterial properties. Among them are low PH, osmotic effect, and hydrogen peroxide produced by enzymes in Honey. Honey's high osmotic property helps it extract water from microbial cells and kill them. All saturated sugar syrups, including Honey, have a high osmolarity that inhibits microbial growth. Additionally, Honey, due to its high sugar content and low pH (bacteriostatic action), inhibits the growth and kills bacteria with hydrogen peroxide and other antibacterial agents. Therefore, Honey is said to effectively prevent bacterial growth and decrease the number of acids produced in the case of dental plaque.

Furthermore, hydrogen peroxide is the primary antibacterial substance produced by the enzymatic reaction of honey. These findings reinforce the findings of the current study by elucidating the mechanisms assumed to be responsible for the plaque-reducing benefits of Natural Honey. The honey group in this study had a higher reduction in plaque score, which could be due to its antibacterial effect, which also reduced the amount of supragingival plaque. In our study, Natural Honey exhibited a statistically significant plaque-reducing effect compared to Chlorhexidine mouthwash.

Natural products, such as Honey recently gained as a plaque management agent. They could be used as long-term antiplaque agents in maintenance therapy or as low-cost alternatives to conventional products. They could also be used by individuals experiencing side effects from chlorhexidine therapy. Further prospective clinical studies with a larger sample evaluating honey mouthwashes’ anti-inflammatory efficacy will help clarify the role of honey mouthwashes in plaque control. Extended duration clinical trials with larger
sample sizes and microbiological investigations are needed to validate Honey as an effective plaque reducing agent.

Conclusion
Using a plaque-inhibitory mouthwash as a supplement to teeth brushing could significantly improve an individual's oral health. Our objective was to compare natural honey mouthwash and Chlorhexidine mouthwash as an effective antiplaque agent. Whereas results have shown that both the products were equally effective in plaque reduction, natural Honey was found to be more effective as a plaque control agent than Chlorhexidine.

Conflict of Interest
Authors have no conflict of interest and no grant/funding from any organization.

References
11. Jain A, Bhaskar DJ, Gupta D, Agali C, Gupta V, Gupta RK et al. Comparative evaluation of Honey, chlorhexidine gluconate (0.2%) and combination of xylitol and chlorhexidine mouthwash (0.2%) on the clinical level of dental plaque: A 30 days randomized control trial. Perspect Clin Res. 2015;6(1):53.

