ABSTRACT

**Purpose:** To develop an experimental mouthwash, neem extract added with honey and assess its anti-plaque, anti-inflammatory and antimicrobial activities. **Methods:** A mouthwash was formulated from aqueous extracts of neem to which honey was added. Sixty children aged 9-13 years were equally divided into two groups. Group 1 children were asked to rinse with 10ml of neem with honey mouthwash and group 2 with 0.2% of chlorhexidine mouthwash for 21 days. Gingival index, Plaque index, Lactobacilli counts in both groups from baseline up to 21 days postrinse were assessed. **Results:** Significant decreases in both gingival index, plaque index, *Streptococcus mutans* and *Lactobacilli* counts were assessed of both groups at baseline, 14th day and 21st day. Subjective and objective criteria regarding acceptability of both groups at baseline, 14th day and 21st day. Subjective and objective criteria regarding acceptability and unwanted side effects of the mouth wash were also assessed. **Conclusion:** Neem mouthwash with honey demonstrated effective antiplaque, anti-inflammatory and anti microbial properties comparable with 0.2% chlorhexidine digluconate.

KEYWORDS: Anti-plaque, anti-inflammatory, antimicrobial, honey, neem.

INTRODUCTION

Dental caries and disease of the periodontium are amongst the most common afflictions of oral diseases of mankind. Teeth and their supporting structures are subjected to infection by *Streptococcus* bacteria that causes cavities and gingivitis. Dental plaque has been proved by extensive research to be a paramount factor in initiation and progression of gingival and periodontal diseases. A direct relationship has been demonstrated between plaque levels and the severity of gingivitis. The most rationale methodology toward the prevention of periodontal diseases would be regular, effective removal of plaque by the personal oral hygiene protocol.

The most essential type of dental care begins at home. Daily oral hygiene plays a vital role in maintaining healthy teeth and gums. An ideal personal oral hygiene regimen would comprise of a combination of tooth brushing, flossing and use of a suitable mouthwash. Regardless of socioeconomic status, the degree of motivation and dexterity required for an optimal oral hygiene levels may be beyond the ability of the majority of patient. This is especially true in children, the majority of whom lack sufficient manual dexterity for effective tooth brushing till the early teenage. From this perspective, the utilization of antimicrobial mouth rinses has been considered a useful adjunct to oral hygiene.

Various chemical agents have been evaluated over the years with respect to their antimicrobial effects in the oral cavity; amongst the various chemical agents, the bisbiguanides constitute an important group. However, all are associated with side effects that prohibit regular long-term use. The search for alternative products continues and natural phytochemicals isolated from plant products used in traditional medicine are now considered as a good alternative to synthetic chemicals also which are safer, biodegradable and have fewer side effects. Therefore there is a need to develop new agents that are effective, safe and inexpensive.

Neem (*Azadirachta indica A. Juss*) is well known in...
India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activity. It is perhaps the most commonly used traditional medicinal plant of India. Almost all parts of the plant are endowed with medicinal property and have been used as traditional medicine or household remedies against various human ailments. Neem leaves exhibit a wide range of pharmacological activities and medicinal applications which is commonly used as an oral hygiene tool in different parts of the world, which has shown anti-plaque, anti-caries, and antibacterial effects. In our study since neem is very bitter, honey was added as a sweetener to increase patient compliance and acceptability.

Honey has been used as a source of nutrients as well as a medicine since ancient times. Honey is an effective broad-spectrum antibacterial agent. Honey’s antibacterial action is attributed to the presence of inhibitory factors such as flavonoids, hydrogen peroxide, low pH, and high osmolarity due to its sugar concentration. These factors play a major role in controlling inflammation and promoting microbial control and healing processes.

In our study, we sought to evaluate the anti bacterial, antiplaque and antigingival properties of neem extract in the form of a mouthwash in a group of school going children. We also sought to assess the subjective and objective criteria regarding acceptability and unwanted side effects of the two mouthwashes.

MATERIALS AND METHODS

Sampling

A total of 60 children aged 9-13 years of either gender, who fall under the inclusion criteria, were selected from Sri Swami Sadananda Saraswati Vidyalya, Mangalore. Children with poor to fair oral hygiene, mild to moderate gingival inflammation, and with DMFT/dft more than 3 were chosen for the study.

Ethical clearance was obtained from the ethical committee of the institution. Informed consent was obtained from the principal and parents prior to the study.

Preparation of the mouthwash

The preparation of the A. indica mouthrinse was carried out at the Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences, Mangalore. The leaves were collected from the gardens of St Aloysius College, Mangalore after taxonomic identification by a certified botanist Dr Suresh Chandra. Leaves were then dried under controlled parameters. Neem extract was prepared by macerating 20 g of dry powder with 100ml of sterile distilled water in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth and finally through Whatman No.1 filter and kept in an airtight amber colored container.

Formulation of the mouthwash included neem extract, natural honey as sweetener and compound sodium chloride mouthwash. The concentration of neem extract in the formulation was restricted to 25mg/g to fulfill the organoleptic (for patient compliance) properties of the final formulation, as neem is a bitter drug.

Characterization of the mouthwashes

Once the mouthrinse was developed, the prepared formulations were placed in the clear glass vials and checked for presence of any particulate matter or fiber by placing against the black and white background.

The pH was noted using pen pH meter (Model pH Tester 10) by bringing the electrode in contact with the surface of the formulation and allowing it to equilibrate for 1 min and reading was noted down.

The viscosity of the mouthwash was determined by using Brookfield Viscometer (Model DV-II plus Pro) with spindle no 61. The viscosity was measured using 25ml of mouthwash filled in a 50 ml clear glass beaker. The spindle was lowered perpendicular in the centre taking care that spindle does not touch the bottom of the beaker. The factors like temperature, pressure, and sample size which affect the viscosity were maintained. The viscosity measurement was done at room temperature and rotating the spindle at 100 rpm.

According to modified ICH guidelines, the stability studies were performed for all the formulations. Formulations were stored at 25 ± 2°C and 60 ± 5% RH using a stability chamber (Lab top instruments) and at 4 ± 2°C in a refrigerator (Whirlpool, India) and they were estimated periodically for 12 weeks. Their appearance and physical stability were inspected for a period of 3 months at interval of one month. As per ICH guidelines, the parameters like clarity, pH and viscosity were evaluated at the end of every month for a period of 3 months.

Commercially available 0.2% chlorhexidine gluconate mouthwash (chlorhexidine mouthwash) was used in our study.

All children were subjected to a thorough oral prophylaxis. After a period of 2 weeks, a single trained and calibrated investigator recorded the caries experience of the child using the DMFT/dft indices. Turesky - Gilmore - Glickman modification of the Quigley- Hein plaque index and Loes and Silness gingival index were used to measure and record the plaque and gingival scores. Children were examined, seated on an ordinary chair, under good illumination, either natural light or hand torch, using a sterile mouth mirror and CPI probe while taking protective cross infection control measures using disposable gloves and masks. Collection of Salivary samples and estimation of S. mutans and Lactobacilli colony count were done using unstimulated saliva, using sterile collection bottles.
sealed and transported immediately to the laboratory. All examinations were conducted by the same examiner.

The children were now randomly divided into 2 groups
- Group 1: 30 children given neem mouthwash with honey.
- Group 2: 30 children given chlorhexidine mouthwash (0.2%).

Children were asked to rinse their mouths with 10 ml of their respective.

Mouthwashes for 30 seconds, once daily, 1 hour after brushing, in the morning, for 3 weeks. 10 ml of the mouthwashes were dispensed by the investigators daily to each of the participants and mouth rinsing done under supervision during school hours.

Plaque and gingival indices were recorded at baseline and again at the end of 14 and 21 days, *S. Mutans* and *Lactobacilli* colony count were also similarly assessed. During the period of study, children followed their usual oral hygiene habits and were instructed to refrain from using commercial mouthrinses and inform if they initiated any antibiotic or anti-inflammatory drug therapy.[4] Subjective and objective criteria regarding the acceptability and unwanted side effects of the mouthwashes were assessed on the 14th and 21st day.[4] (Annexure 1).

Our study is a self funded study. We did not receive any specific grant from funding agencies in public, commercial or not-for-profit sectors.

**Statistical Analysis**

Mean and standard deviations were used to calculate mean scores of gingival and plaque indices of all groups at different time periods. Paired t-tests were used to assess the significance of changes in both indices and lactobacilli counts within each group between the different time periods (Intra group comparison) Critical P values of significance were set at 0.05 and a confidence of 95%. Changes in salivary *S. mutans* counts from baseline to the different time periods (Intra group comparison) were analyzed by the Wilcoxon signed rank test. ANOVA tests were used to identify significant differences between the percentage reduction of the indices and microbial counts of the study groups (Inter group comparison). All statistical analyses were carried out using the SPSS v20 software.

**RESULTS**

The clarity of the mouthwash and the values obtained for pH and viscosity at the end of the 1st month were seen to be maintained at the end of 3 months and there were no significant differences seen in the clarity, pH and viscosity in the neem with honey mouthwash even after the addition of honey. (Table 1).

Table 2 shows mean scores of gingival index and plaque index at different time intervals in both the groups. Statistically significant decreases in Gingival index and plaque index scores were observed in both groups from baseline to 14th day and 21st day (Table 2). In group 1 (neem with honey mouthwash) the gingival index decreased from 2.11±0.62 (baseline) to 1.16±0.51 (14th day) and remained the same on on the 21st day. The plaque index decreased from 3.89±2.06 (baseline) to 1.73±0.54 (14th day) and showed no further decrease till the 21st day. In group 2 (0.2% chlorhexidine) the gingival index decreased from 2.42±0.64 (baseline) to 1.12±0.35 (14th day) and further to 1.1±0.51 (21st day). The plaque index decreased from 4.2±1.76 (baseline) to 1.70±0.94 (14th day) and remained the same till 21st day. These changes in the gingival index and plaque index scores were found to be statistically significant (p<0.001). However, when the decreases in gingival index and plaque index scores from baseline upto 14th and 21st day were compared between group 1 and 2, we observed no statistically significant differences. (table 2). At the baseline, upto 76.6% of the children in Group 1 mouthwash exhibited moderate counts of *S. mutans* while 13.3% exhibited high counts and only 10% exhibited low counts (Table 3). At 14th day 56.6% of children showed low counts of *S. mutans* while 33.3% showed moderate counts and 10% showed high counts. Further, at the end of 21 days upto 86.6% of children showed low counts of *S. mutans* while 10% showed moderate counts and only 3.3% showed high counts which were statistically significant. In group 2 80% of the children exhibited moderate counts of *S. mutans* while 16.6% exhibited low counts and only 3.3% exhibited high counts. At 14th day 80% of children showed low counts of *S. mutans* while 20% showed moderate counts and 0% showed high counts. However, at the end of 21 days 93.3% of children showed low counts of *S. mutans* while only 6.6 % showed moderate counts and none showed high counts, all of which were statistically significant.

When Lactobacilli counts were assessed, at the baseline 80% of the children showed levels of < 10,000 CFU/ml (moderate caries activity) while 20% showed levels of <1,000,000 CFU/ml (marked caries activity) in group 1(Table 4). At the end of the 14th day, we observed that upto 80% of the children showed levels of >1000 CFU/ml (slight caries activity). At the end of 21 days all children exhibited levels of 0-1000 CFU/ml of lactobacilli (light or no caries activity) where as in group 2 80% of the children exhibited levels of <1,000 CFU/ml (moderate caries activity) while 20% showed levels of <1000 CFU/ml (slight caries activity). At the end of the 14th day, 66.7% showed levels of 0-1000 CFU/ml (light/no caries activity) while 33% showed levels of 000 CFU/ml (slight caries activity). At the end of 21 days all children showed levels of 0-1000 CFU/ml which is indicative of light/no caries activity.
No statistically significant differences in the percentage reductions of *Streptococcus mutans* and *Lactobacilli* counts were seen between group 1 and group 2 at any of the time intervals. [Table 5].

In our study, the subjective criteria scored by the children revealed that, bitter taste was experienced by six out of thirty children using neem with honey mouthwash. Objective criteria revealed staining of teeth in five children in the chlorhexidine group [Table 7]. Hence, the results of our study show that neem with honey mouthwash was acceptable in taste in most children and free of side effects such as staining of teeth, burning sensation and allergy.

Table 1: Changes in the clarity, pH and viscosity of neem with honey mouthwash at different time intervals.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Neem mouthwash</th>
<th>Neem with honey mouthwash</th>
<th>Neem mouthwash</th>
<th>Neem with honey mouthwash</th>
<th>Neem mouthwash</th>
<th>Neem with honey mouthwash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>pH(1-14)</td>
<td>9.24±0.001</td>
<td>9.23±0.004</td>
<td>9.25±0.002</td>
<td>9.26±0.003</td>
<td>9.23±0.008</td>
<td>9.22±0.005</td>
</tr>
<tr>
<td>Viscosity (cpi)</td>
<td>4.37±0.004</td>
<td>5.15±0.007</td>
<td>4.39±0.005</td>
<td>5.20±0.007</td>
<td>4.41±0.004</td>
<td>5.17±0.003</td>
</tr>
</tbody>
</table>

Table 2: Mean scores of Gingival index and Plaque index at different time intervals in both the groups.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Diff from baseline</td>
</tr>
<tr>
<td>baseline</td>
<td>2.11±0.62</td>
<td>-</td>
</tr>
<tr>
<td>14th day</td>
<td>1.16±0.51</td>
<td>0.95</td>
</tr>
<tr>
<td>21st day</td>
<td>1.16±0.51</td>
<td>0.95</td>
</tr>
<tr>
<td>baseline</td>
<td>2.42±0.64</td>
<td>-</td>
</tr>
<tr>
<td>14th day</td>
<td>1.12±0.35</td>
<td>1.3</td>
</tr>
<tr>
<td>21st day</td>
<td>1.16±0.51</td>
<td>1.26</td>
</tr>
</tbody>
</table>

P > 0.05 not significant.

Table 3: Changes in *S.mutans* count at baseline, 14th day and 21st day in Group 1 and group 2.

<table>
<thead>
<tr>
<th>Examination Interval</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Number of children</td>
<td>Percentage</td>
</tr>
<tr>
<td>baseline</td>
<td>3</td>
<td>10%</td>
</tr>
<tr>
<td>14th day</td>
<td>17</td>
<td>56.6%</td>
</tr>
<tr>
<td>21st day</td>
<td>26</td>
<td>86.6%</td>
</tr>
<tr>
<td>baseline</td>
<td>5</td>
<td>16.6%</td>
</tr>
<tr>
<td>14th day</td>
<td>24</td>
<td>80%</td>
</tr>
<tr>
<td>21st day</td>
<td>28</td>
<td>93.3%</td>
</tr>
</tbody>
</table>

Table 4: Changes in *Lactobacilli* count of Group 1 and Group 2 on baseline, 14th day and 21st day.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of <em>Lactobacilli</em> per ml saliva</td>
<td>Frequency</td>
</tr>
<tr>
<td>Baseline</td>
<td>0-1000</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>&lt;1,000,000</td>
<td>6</td>
</tr>
<tr>
<td>14th day</td>
<td>0-1000</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>&lt;1,000,000</td>
<td>5</td>
</tr>
<tr>
<td>21st day</td>
<td>0-1000</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>&lt;1,000</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td></td>
<td>&lt;1,000,000</td>
<td>&lt;1,000,000</td>
</tr>
</tbody>
</table>
Table 5: Inter group comparison of percentage reduction% of Streptococcus mutans and Lactobacilli counts in Group 1 and Group 2.

<table>
<thead>
<tr>
<th></th>
<th>Streptococcus mutans</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-14 days</td>
<td>0-21 days</td>
</tr>
<tr>
<td>Group 1</td>
<td>91.424</td>
<td>38.2821</td>
</tr>
<tr>
<td>Group 2</td>
<td>81.9310</td>
<td>42.9000</td>
</tr>
</tbody>
</table>

Difference between groups

<table>
<thead>
<tr>
<th>P value</th>
<th>T value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.061</td>
<td>1.314</td>
</tr>
</tbody>
</table>

Table 6: Subjective Criteria.

<table>
<thead>
<tr>
<th>Subjective criteria</th>
<th>Taste acceptability</th>
<th>Burning</th>
<th>Dryness/soreness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acceptable</td>
<td>Tolerable</td>
<td>Un acceptable</td>
</tr>
<tr>
<td>Group 1 H:30</td>
<td>20</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Group 2 H:30</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 7: Objective criteria.

<table>
<thead>
<tr>
<th>Objective criteria</th>
<th>Ulcer formation</th>
<th>Staining of teeth</th>
<th>Staining of tongue</th>
<th>Allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Group 1 H:30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2 H:30</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

A variety of chemotherapeutic agents have been examined for their ability to control oral microorganisms and to affect plaque formation. [30] Chlorhexidine (CHX) digluconate has a 40-year history in dental medicine [25] and is regarded as a “gold” standard in dentistry for the prevention of plaque and gingivitis, and against which other antiplaque agents are measured. Large reductions were found in plaque formation using chlorhexidine gluconate, applied topically or as a mouthrinse. [25, 31-25] Unfortunately, studies showed that these positive effects were accompanied by side-effects, the most disturbing being extrinsic tooth staining [36] and others such as unpleasant taste and burning sensation. [30] Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. [7]

The purpose of this study was to evaluate the effect of neem with honey mouthwash mouthwash on gingival inflammation, antiplaque activity and on the levels of salivary S. mutans and Lactobacillus in a group of children. Chlorhexidine di gluconate was taken as a benchmark control in our study in a concentration of 0.2% since it was the most commonly prescribed concentration. [37-40]

The neem mouthwash formulation was found to be clear without any fibrous matter or clouding (table 1). pH of the formulation was found to be more than 8.9 and less than 9.35 which indicates uniform pH without any significant deviation in pH. Viscosity ranged from 4.1-5.25. The addition of honey to the mouthwash increased the viscosity slightly; however, this was not a significant change. The stability study has been carried out as per ICH guidelines. [22-24]

In our study, we found statistically significant decreases in both gingival and plaque indices from the baseline to the 14th day and further up to the 21st day in both the groups (P<0.001) [table 2]. However, the mean scores of the gingival and plaque indices on the 14th day and 21st day in both groups remained the same. No significant differences were observed in the means scores of both indices between the two groups in any time interval. From this, we infer that both mouthwashes had significant but comparable antiplaque and anti inflammatory properties up to the 21st day post rinse.

Our results are consistent with a previous study [6] which compared the short term efficacy and safety of a mouth rinse based on leaves of neem tree on gingival inflammation and microbial plaque compared to 0.12% chlorhexidine. A small clinical trial from India suggested that a dental gel containing neem leaf extract (25mg/g) had significant reduction in plaque index and bacterial count. [3]

The anti bacterial and antiseptic properties of neem have been proved in various studies on health. [8,41] In dentistry A. indica has also demonstrated a good efficacy in the treatment of periodontal disorders. [42] For the last few years, there has been an increasing trend and awareness in neem research. [9]
**Streptococcus mutans** and *lactobacilli* species are the most common bacteria associated with plaque formation.\[43,44\] In our study when *S.mutans* counts were analyzed in both the groups, we found a statistically significant reduction in the bacterial counts from the baseline until the 14th day and 21st day post rinse ([p] < 0.001, Table 3). However, only neem mouthwash with honey group demonstrated a statistically significant reduction of *S.mutans* counts between the 14th day and 21st day post rinse. ([p] < 0.001). This could be attributed to the potent antibacterial properties of neem leaf extract which was used in its pure form in our study.

In the neem mouthwash with honey group, we observed, 76% children with moderate counts of *S.mutans*, 13.3% with high counts and 10% with low counts at the baseline. Remarkably, at the end of the 14th day, upto 56.6% children showed low counts while 10% children showed high counts. At the end of 21st day, 86.6% of children showed low counts while 10% showed moderate counts and only 3.3% showed high counts (Table 3). These results were in accordance with the study done by Pai et al\[2\] where microbial count of *S.mutans* in saliva was found to be reduced significantly. We found no significance differences in the percentage reduction of *S.mutans* between group 1 & 2, at any time interval ([p] > 0.05, Table 3).

When *Lactobacilli* counts were assessed in neem with honey mouthwash, we observed a steady decrease from the baseline to the 14th day and further upto the 21st day (Table 11).At the end of the intervention we observed that all children exhibited 0-1000 CFU/ml of *Lactobacilli* which indicates light or no caries activity. This result is not consistent with the result of a study by Vanka A et al\[2\] who found no inhibition of *Lactobacilli* growth.

From the above results, we observed that, at the start of the study, most of the children exhibited *Lactobacilli* counts of <100,000 CFU/ml in both groups. However, at the end of 14th day, the *lactobacilli* counts markedly reduced to < 10,000 CFU/ml in more than three-fourth of the children (80%-100%). Further, at the end of 21 days intervention, we observed that all the children showed very low levels of *lactobacilli* (0-1000 CFU/ml), which indicates light or no caries activity (Table 4).

From this we infer that both mouthwashes have highly significant antibacterial activity on *S.mutans* and *Lactobacilli*. The microbiological evaluation strongly supports the efficacy of the experimental mouthwash.

In our study, due to the bitter taste associated with both neem, honey was added as a sweetener to increase the patient compliance and acceptability. We assume that honey has been an additional factor for the efficacy of the antimicrobial activity of the neem mouthwash as honey has potent antibacterial activity,\[12,46,47\] effective against a very broad spectrum of species, and to have antifungal\[48,49\] properties as well.

We found that, at the beginning of the study, due to the bitterness of the mouthwashes, children were initially reluctant to participate in the study. After an interactive session with the children and after the promise of material incentives they willingly participated in the study. Three children dropped out of the study after a few days due to non-compliance and hence were excluded from the study.

The fact that the mouth rinses presently available in market are chemically based, costly, and have side-effects, restricts their use, especially in India. Today, cost-effective and easily available herbs as adjuvant to oral hygiene maintenance may have a far-reaching effect on the prevention as well as prevalence of oral diseases.\[50\]

The results of our study indicate that neem with honey mouthwash can be used as a suitable alternative to chlorhexidine mouthwash in children, as an adjunct in their regular oral hygiene maintenance.

**CONCLUSION**

Neem with honey mouthwash is stable under permissible conditions and demonstrated effective antiplaque, anti-inflammatory and anti microbial properties comparable with 0.2% chlorhexidine di gluconate. Hence neem mouthwash with honey can be used in children as an alternative to chlorhexidine mouthwash as part of their daily oral health care regimen. However, more studies with larger sample size should be planned to assess its efficacy, dosage, toxicity, exact concentrations, formulas for patient recommendation, and long-term effectiveness.

**ANNEXURE 1**

**SUBJECTIVE AND OBJECTIVE CRITERIA ASSESSMENT**

**Subjective Criteria**

1. **Taste Acceptibility**
   - Acceptable.
   - Tolerable.
   - Unacceptable

2. **Burning**
   - Present.
   - Absent.

3. **Dryness/soreness**
   - Present.
   - Absent.

**Objective Criteria**

1. **Ulcer formation**
   - Present.
   - Absent.
2. Staining of teeth
- Present.
- Absent.

3. Staining of tongue
- Present.
- Absent.

4. Allergy.
- Present.
- Absent.

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11. Balapannavar A, Sardana V, Singh M. Comparison of the effectiveness of 0.5% tea, 2% neem and 0.2% chlorhexidine mouthwashes on oral health: A randomized control trial. Indian J Dent Res, 2013; 24(1).
27. FDI core working group. IDJ, 1992; 42(2): 287-304.


