A Comparative Study on the Effect of *Spilanthes acmella* and Chlorhexidine (0.2%) Mouth Rinses on Salivary *Streptococcus mutans* Count in Children

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Authors’ contributions

All the authors have contributed to the study design, data collection, analysis and drafting of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

In the wake of an increased interest in finding natural alternatives for chemical medicinal products, this study assesses and compares the effects and acceptance of *Spilanthes acmella*, a widely used plant in various traditional systems of medicine, with Chlorhexidine (0.2%) mouth rinse on the dental caries causing pathogen *Streptococcus mutans* colonizing the oral cavity of children. The study included 40 healthy children in the age group of 8-12 years based on inclusion and exclusion criteria. In the beginning of the study, non-stimulated whole salivary samples were tested for the baseline count of colony forming units (CFU) of *Streptococcus mutans* using Mitis Salivarius Agar supplemented with potassium tellurite. The same process was repeated 24 hours after 15 days of regular use of mouth rinses twice daily. Results showed that there was statistically highly significant decrease in CFU in the post rinse phase in both the groups. *S. acmella* showed greater acceptance.
by study subjects when compared with the Chlorhexidine mouth rinse. It can be concluded based on the results of this study that S. acmella has beneficial effects against S. mutans and can be a potential option for preventive measures against dental caries, although more studies are needed for its validation.

Keywords: Chlorhexidine; Spilanthes acmella; mouthwash; dental caries; Streptococcus mutans.

1. INTRODUCTION

Lately, an increased popularity of natural and plant-based products has been noticed in the healthcare field globally. Evidence shows that as high as 80% of the population worldwide benefit from medicinal plants and indigenous systems of medicine to meet their primary healthcare requirements [1]. The growing interest in drugs of plant origin are attributed to the perceived advantages of greater biocompatibility, lesser toxicity and better access compared to synthetic medicines [2,3]. The field of dentistry is not excluded from this phytotherapeutic upsurge. Role of phytochemical mouthwashes as anti-cariogenic or cariostatic agents have been widely studied [4,5,6].

Spilanthes acmella, is a plant which is widely used in various indigenous healthcare practices in the Indian subcontinent. It is known as the anti-toothache plant owing to the anaesthetic effect produced on chewing or placing crushed flower heads at the site of pain [7,8]. Phytochemical analysis has shown the presence of the isobutylamide constituent ‘spilanthol’ and triterpenoids [7,8]. The anaesthetic property is attributed to alkalamides which are similar to certain endogenous cannabinoid cerebral neurotransmitters [8]. Despite the time-tested benefits on oral diseases affirmed by alternative systems of medicine, it is important to scientifically validate the efficacy and safety as a preliminary step in adoption of the product to mainstream dentistry.

Factors like lack of dexterity, individual motivation and monitoring, limit the effectiveness of tooth brushing which necessitates the use of chemotherapeutic agents for control of plaque in children [9]. Chlorhexidine is considered as the ‘gold standard’ mouth rinse due to its broad-spectrum anti-microbial effect and substantivity [10].

The most common plaque-mediated disease in children is dental caries, which is also one of the most common childhood diseases. Oral microorganisms present in dental plaque are considered crucial for the initiation and progression of dental caries. The most commonly implicated bacterial species in its etiology is Streptococcus mutans for its initiation.

The aim of this study is to assess and compare the effects and acceptance of Spilanthes acmella with Chlorhexidine (0.2%) mouth rinse on dental caries causing pathogen Streptococcus mutans colonizing the oral cavity of children.

2. METHODOLOGY

The investigation was a double-blind, comparative interventional study on two randomized parallel groups of children who used either a Spilanthes acmella mouth rinse or a commercially available Chlorhexidine (0.2%) mouth rinse twice daily for a 15-day period. Healthy children aged 8-12 years with four or more restored, decayed and/or missing teeth (DMFT/dmft ≥4), no history of recent antibiotic usage or fluoride therapy were selected from a residential school which ensured a uniform dietary pattern for the participants. Subjects who did not meet these criteria were excluded from the study. The participants were randomly divided into two groups of 20 each by lottery method. Commercially available Chlorhexidine (0.2%) mouth rinse (Periex from Dentaids) and Spilanthes acmella mouth rinse prepared using Soxhlet extraction were used in the study. Unlabelled identical bottles containing the mouth rinses and 10ml measuring cups were provided to each subject for easy dispensing of the solution. The children were trained to rinse the mouth for one minute using five milliliters of the respective mouth rinse twice daily for 15 days, once after breakfast in the morning and once at night after dinner. Non-stimulated whole salivary samples were tested for the colony forming units (CFU) of Streptococcus mutans both at baseline and at the end of 15 days using the same technique.

2.1 Preparation of Spilanthes acmella Mouth Rinse

Spilanthes acmella flowers were collected from the medicinal garden of KMCT Ayurveda Medical College. Identification and authentication of the
specimens were done at the same institution (Fig. 1).

Flower heads of S. acmella were air dried and subjected to coarse grating to produce a coarse powder of uniform texture at the National Institute of Pharmacy, Kozhikode. The extract was then obtained with ethanol using a Soxhlet apparatus (Fig. 2).

The desired concentration of the plant extract (10%) was made by dissolving in dimethyl sulfoxide. Authorized additive, peppermint flavour (1g/l), and sodium saccharine (1g/l) a sweetening agent were used to formulate the mouth rinse [11].

2.2 Microbiological Method

The saliva samples were collected in the morning on an empty stomach, adhering to precautions to avoid any bias in concentration due to circadian rhythm or contamination with food debris [12,13]. The samples were inoculated within 30 minutes after collection. All the saliva samples were serially diluted using physiological saline to obtain minus three (-3) concentration in order to obtain working salivary samples. Using a reusable inoculating loop, 0.1 ml saliva sample was spread by the streaking method on Mitis Salivarius agar enriched with potassium tellurite. The inoculating loop was sterilized by flaming the loop. The plates were incubated for 48 hours at 37ºC in the incubation chamber to obtain maximum growth of microbial colonies. The colony forming units (CFU) of S. mutans were identified by the morphology, size and colour. The colonies had blue (gum drop) appearance. Counting was done with the help of a microbiologist using a digital colony counter. The colony count was expressed as the number of colony forming units per millimeter (CFU/ml) of saliva. Semi-quantification of the number of colonies was done by multiplying the actual colony count with $1 \times 10^2$ to adjust for the dilution factor [14]. The process was done at baseline and repeated on the 15th day after commencing the intervention.

2.3 Statistical Analysis

The data was entered into Microsoft Excel and analyzed using SPSS software version 25 and subjected to descriptive statistics, paired t-test (for comparison of intragroup differences in pre-rinse and post-rinse phases).

3. RESULTS

Table 1 shows the descriptive statistics of study subjects.

Table 2 assesses the intragroup variation in pre and post rinse CFU counts by paired t test. Mean colonies of S. mutans at baseline for Chlorhexidine was $5.570 \times 10^7 \pm 2.86$ which reduced to $0.510 \times 10^6 \pm 0.22$ after 15 days of intervention. This difference was statistically highly significant ($p<0.001$). S. acmella mouth rinse showed reduction of colony forming units from $4.736 \times 10^7 \pm 1.63$ to $3.200 \times 10^6 \pm 1.33$ within the same time frame , which was also statistically highly significant ($p <0.001$). So, when pre-rinse and post-rinse CFU were compared, there was a highly significant reduction of colony forming units for both Chlorhexidine and S. acmella groups ($p <0.001$).

Pre-rinse mean CFU for S. acmella mouth rinse was $4.736 \times 10^7 \pm 1.63$ against $5.572 \times 10^7 \pm 2.86$ for Chlorhexidine. Post-rinse values in both groups showed lesser mean CFU count, $3.200 \times 10^6 \pm 1.33$ for S. acmella and $0.510 \times 10^6 \pm 0.22$ for Chlorhexidine. This difference was statistically significant ($p=0.001$).

Table 4 shows the percentage of the study subjects in each mouth rinse group giving a positive (YES) and negative (NO) response to the questions asked regarding flavour, smell and willingness to rinse respectively.

The subjects in the S. acmella group showed better acceptance for the flavour (60%) and willingness to use (65%) compared to 45% and 40% respectively for Chlorhexidine. With regards to smell of mouth rinses, Chlorhexidine showed better acceptance with 75% participants favouring this over S. acmella mouth rinse (60%).

To summarize the results, mean differential colony counts in pre-rinse and post-rinse phases showed very highly significant fall in the colony counts of Streptococcus mutans in both the mouth rinse groups ($P<0.001$) though reduction in Chlorhexidine group was greater compared to S. acmella group.
Fig. 1. *Spilanthes acmella* flowers

Fig. 2. Preparation of ethanolic extract of *S. acmella* using a Soxhlet apparatus

Table 1. Descriptive statistics of study subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Age Mean and Standard deviation values</th>
<th>DMFT+dmf score Mean and Standard deviation values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>10.8 ± 1.04</td>
<td>14.51 ± 5.47</td>
</tr>
<tr>
<td><em>S. acmella</em> mouth rinse</td>
<td>10.20 ± 1.40</td>
<td>11.75 ± 4.52</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the CFU observed in pre and post rinse phases (paired t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Pre-rinse mean±SD (10 CFU/ml)</th>
<th>Post-rinse mean±SD (10 CFU/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>20</td>
<td>5.570±2.86</td>
<td>0.510±0.22</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><em>S. acmella</em> mouth rinse</td>
<td>20</td>
<td>4.736±1.63</td>
<td>3.200±1.33</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)
Fig. 3. Baseline sample (Chlorhexidine)

Fig. 4. Post-rinse sample (Chlorhexidine)

Fig. 5. Baseline sample (S. acmella)

Fig. 6. Post-rinse sample (S. acmella)

Table 3. Comparison of chlorhexidine mouth rinse with S. acmella mouth rinse using the unpaired t-test

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Pre-rinse mean±SD (107 CFU/ml)</th>
<th>Post-rinse mean±SD (106 CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>20</td>
<td>5.572±2.86</td>
<td>0.510±0.22</td>
</tr>
<tr>
<td>S. acmella mouth rinse</td>
<td>20</td>
<td>4.736±1.63</td>
<td>3.200±1.33</td>
</tr>
<tr>
<td>t value</td>
<td></td>
<td>5.038</td>
<td>2.513</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&lt;0.001**</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Table 4. Responses of the children to various questions regarding the acceptability of mouth rinses

<table>
<thead>
<tr>
<th>Question</th>
<th>Chlorhexidine N=20</th>
<th>S. acmella N=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavour</td>
<td>YES=09 (45%)</td>
<td>YES=12 (60%)</td>
</tr>
<tr>
<td></td>
<td>NO=11 (55%)</td>
<td>NO=08 (40%)</td>
</tr>
<tr>
<td>Smell</td>
<td>YES=15 (75%)</td>
<td>YES=12 (60%)</td>
</tr>
<tr>
<td></td>
<td>NO=5 (25%)</td>
<td>NO=8 (40%)</td>
</tr>
<tr>
<td>Willingness</td>
<td>YES=08 (40%)</td>
<td>YES=13 (65%)</td>
</tr>
<tr>
<td>to rinse</td>
<td>NO=12 (60%)</td>
<td>NO=7 (35%)</td>
</tr>
</tbody>
</table>
4. DISCUSSION

Chlorhexidine has been widely used as an antiplaque and antigingivitis agent since many decades [15]. The results of this study were in accordance with a similar study assessing the effectiveness of 0.2% Chlorhexidine mouthwash on Streptococcus colony counts [9]. Many studies over the years have affirmed the effectiveness of Chlorhexidine mouthwash against S. mutans species [9,16,17,18]. Brookes et al. state that Chlorhexidine mouthwashes can be bacteriostatic or bactericidal depending upon the concentration [19]. Despite being a popular antiseptic mouthwash, there are many disadvantages for Chlorhexidine. Long term use has been found to result in brownish discoloration of the teeth, irritation of oral mucosa, and bitter taste [9,19]. Hence, there is a need to develop an alternative mouth wash which will have comparable benefits as Chlorhexidine without having its adverse effects.

The results of this comparative interventional study showed that both S. acmella mouth rinse and Chlorhexidine exhibited significant reductions in salivary Streptococcus mutans counts upon comparing the baseline values with the posttreatment values after the 15 day intervention.

Spilanthes acmella is a plant of great importance in the African and Indian traditional pharmacopoeia [7,8,20]. Phytochemical studies have reported the presence of various bioactive metabolites which have antipyretic, anti-inflammatory, analgesic, local anesthetic and antimicrobial activities [7,8]. Despite its widespread use in traditional systems of medicine as the anti-toothache plant, scientific studies validating its use in dentistry are scarce. A comparative evaluation of the antibacterial and antifungal activity of S. acmella and calcium hydroxide on root canal pathogens have exhibited remarkable results, thus suggesting the potential use of S. acmella as an intracanal medicament in endodontics [21]. To the best of our knowledge, this is the first study to scientifically extrapolate the effect of S. acmella on the dental caries causing pathogen Streptococcus mutans. Our study shows that significant reduction was noted in the colony forming units of salivary Streptococcus mutans. It was also found that the general acceptance to use was greater for S. acmella mouth rinse in comparison with Chlorhexidine.

5. CONCLUSION

Although the reduction of the Streptococcus mutans colony count in the chlorhexidine group was greater compared to the S. acmella group, in the current scenario where researchers are interested in scientifically validating natural substances as alternatives for the control of caries in terms of antimicrobial response and lower associated risks, this study provides a foundation for further scientific and clinical research with regards to the anti-cariogenic properties of S. acmella. However, more studies with higher quality is required before full recommendations can be made with regards to incorporating this product into preventive protocols.

CONSENT AND ETHICAL APPROVAL

The study protocol was reviewed and approved by the Institutional Ethical Committee, KMCT Dental College, Kerala, India and registered with the Clinical Trial Registry of India. An informed consent was obtained from the caretakers and authorities of a residential school after detailed explanation of the nature of the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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