AkiNik

International Journal of Herbal Medicine
Available online at www.florajournal.com

IJHM International Journal of Herbal Medicine

ISSN 2321-2187
IJHM 2013; 1 (4): 50-54

Received: 24-10-2013
Accepted: 28-10-2013

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Efficacy of a Herbal Mouthrinse on Oral Microbial Load in Down Syndrome Children

Priya Subramaniam, Tulika Gupta

ABSTRACT

The efficacy of a herbal mouth rinse on the oral microbial load in Down syndrome children. The study design consisted of thirty Down syndrome children aged between 7 and 13 years from Divya down Development Trust in Bangalore. Children were divided into 2 groups consisting of 15 children in each group. Group A were given herbal mouth rinse and Group B were given chlorhexidine mouth rinse, both of which were alcohol free. Mouth rinsing was carried out under supervision. Microbial samples were collected from the dorsum of the tongue at baseline and at different time intervals. The samples were subjected to culturing and quantitative analysis for aerobic and anaerobic micro-organism. Polymerase chain reaction assay was done for the detection of *Porphyromonas gingivalis*. Significant difference was observed between baseline and following 1 hour of rinsing in aerobic microbial load in both groups. Polymerase Chain Reaction assay also showed marked decrease in *Porphyromonas gingivalis* with both mouth rinses. Herbal mouth rinse can be a suitable alternative for use in Down syndrome children.

Keywords: Herbal Mouthrinse, Down Syndrome Children.

1. Introduction

All that man needs for a healthy living has been provided in nature and it is a challenge for science to find it. Ayurveda is an ancient healing system that originated in India more than 5,000 years ago and relies on herbs for maintaining good health. Vedic philosophy believes that human beings are all part of nature and there is connection between the universe and human beings [1].

Nowadays multiple drug resistance has developed due to the commercially available antimicrobial drugs. This has prompted scientists to divert their interest to phytochemistry and thus herbal medicine [2].

Greatest challenge in dentistry is the control of dental biofilm. Although mechanical methods are considered effective, they are inadequate in certain individuals, such as Down syndrome children. Down syndrome children exhibit early colonization by various pathogens like *Porphyromonas gingivalis, Prevotella intermedia, Streptococcus salivarius, Staphylococcus aureus, Klebsiella, Streptococcus mutans* which can leads to early onset periodontitis [3]. These children also have a compromised host status, fragile periodontal condition, pseudo-macroglossia, fissured tongue, malocclusion and innumerable depressions on the dorsum of tongue. These abnormal oral conditions create an ideal niche for harbouring microorganisms and food debris, which in turn acts as a substrate for metabolism and growth of aerobic and anaerobic microorganisms.

Down syndrome children also present with decreased manual dexterity and cognitive impairment which can lead to difficulty in brushing and tongue cleaning. These obstacles have led to the introduction of simple adjuvant methods like mouth rinses for use in these children [4]. Although chlorhexidine mouthrinse has proved to be effective it has been reported to have numerous side effects on long term use [5]. The use of a natural alternative could be as beneficial with fewer side effects.

Therefore, this present study was conducted to evaluate the efficacy of a herbal mouth rinse on oral microbial load in Down syndrome children

2. Materials and Methods

Thirty non-institutionalised Down syndrome children from Divya down Development Trust for Down syndrome children in Bangalore aged between 7–13 years were included. The nature of the study was explained to both parents and the school authorities and their written consent was taken. Ethical clearance was also obtained from the institution ethical review board.

Exclusion criteria

- Non–cooperative children
- Children without parent consent
- Children with mental retardation were excluded

Prior to commencement of the study, Down syndrome children were asked to gather in a large room. Demonstration of mouth rinsing was done by the investigator. The children were trained for 3 days to swish their mouth with 10 ml of distilled water. The children were then divided into 2 groups of 15 children each. Two alcohol free mouthrinses were selected.

Children from Group A were given 10 ml of herbal mouth rinse (HiOra-K, Himalaya Herbal Health Care) and children from Group B were given 10 ml of 0.20 w/v chlorhexidinegluconate mouth rinse (OROGARD mouthwash, Alkem Laboratories Ltd) to rinse their mouth for 1minute.

Baseline microbial samples were collected from the dorsum of tongue using tongue swab. Breakfast was provided at the school for these children at 8.30 a.m., thirty minutes following breakfast under the supervision of the investigator mouth rinsing was done. The children were monitored and instructed not to eat or drink for the following one hour.

Microbial samples from the tongue were collected immediately after using the mouth rinse. This procedure was repeated at 30 minutes and at 1 hour. Samples were placed in sterile vials containing 1ml of thioglycollate broth and were transferred in icebox at 4 ºC immediately to the laboratory for culturing and quantitative analysis of aerobic and anaerobic microorganisms.

Samples obtained at baseline and following 1 hour of rinsing were finally subjected to Polymerase Chain Reaction method for detection of *Porphyromonas gingivalis.*

For culturing, each sample was diluted with normal saline and was streaked on blood agar (for aerobic count) and thioglycollate agar plate (for anaerobic count) and was incubated at 37 ºC for 7 days. A digital colony counter was used for counting of aerobic and anaerobic microorganisms and was expressed in cfu/ml.

The detection of *Porphyromonas gingivalis* was performed using polymerase chain reaction (PCR) method [6]. Bacterial genomic DNA was isolated using DNA isolation kit containing the prepared solutions for cell lysis, RNase–treatment, protein precipitation, and DNA hydration. The RNase treated cell lysate was washed with isopropanol (600 µl) and ethanol (600 µl) following protein precipitation. PCR was performed with a total volume of 25 µl, which consisted of PCR beads, 0.8 µM of each primer, 2 to 5 µl of the template DNA mixture from the baseline and final microbial sample from the dorsum of the tongue and sterile distilled water. The amplification reaction was done by thermo cycling with the following cycling parameters: an initial denaturation at 95 ºC for 5 minutes , then 35 cycles consisting of 94 ºC for 30 seconds , 55 ºC to 60 ºC for 30 seconds , and 72 ºC for 30 seconds, followed by a final extension at 72 ºC for 7 minutes. Positive and negative controls were included in each PCR set and in each sample processing. The PCR products were then subjected to electrophoresis in a Tri-acetate EDTA buffer and analysed [6].

Data obtained for both mouth rinses was subjected to statistical analysis. Group wise comparison was made using Mann Whitney U test. Intra group comparison was made using Wilcoxon matched pairs test. A p value of <0.05 was considered as statistically significant. Analysis was carried out using the SPSS - Version 16 software.

3. Results

In both groups there was reduction in aerobic and anaerobic microbial load following use of mouth rinses [Tables 1 and 2; Figures. 1 and 2]. Their education in aerobic micro flora was significant ($p<0.05$) in both groups. Polymerase Chain Reaction assay showed marked decrease in *Porphyromonas gingivalis* after administration of mouth rinse in both groups (Fig. 3)

Table 1: Percentage of reduction in aerobic microbial load with herbal and chlorhexidine mouth rinses

Time following rinsing	Percentage reduction with herbal Mouth rinse (%)	Percentage reduction with chlorhexidine Mouth rinse (%)	p-value
Immediately	82.57	74.93	0.01*
30 minutes	20.30	60.10	0.00*
60 minutes	46.8	3.54	0.03*

*$p<0.05$ is significant

Table 2: Percentage of reduction in anaerobic microbial load with herbal and chlorhexidine mouthrinses

Time following rinsing	Percentage reduction with herbal Mouthrinse (%)	Percentage reduction with chlorhexidine Mouthrinse (%)	p- value
Immediately	50.0	36.58	0.20
30 minutes	5.26	25.70	0.72
60 minutes	11.10	37.80	0.11

*$p<0.05$ is significant

4. Discussion

Periodontal disease is a serious and morbid oral condition in Down syndrome children. It is characterised by severe gingival inflammation and loss of periodontal attachment. Down syndrome children also present with difficulties in controlling dental biofilm due to their altered neuro motor development which leads to compromised oral health. These obstacles can be resolved by using simple methods like mouth rinsing addition to tooth brushing in order to control the dental biofilm and gingival inflammation.

Mouthwashes have been used for centuries for medicinal and

cosmetic purposes. Mouthwashes have the ability to deliver therapeutic ingredients and benefits to all accessible surfaces of mouth including interproximal surfaces.

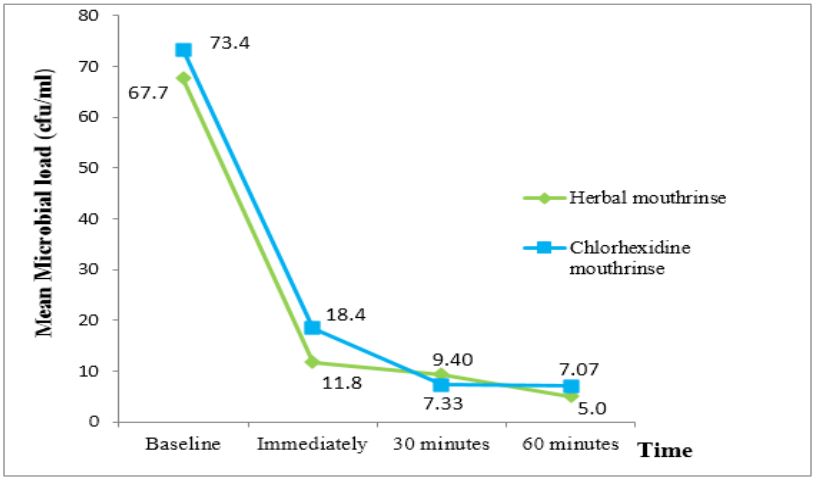

Fig 1: Effect of herbal and chlorhexidine mouth rinses on aerobic oral microbial load

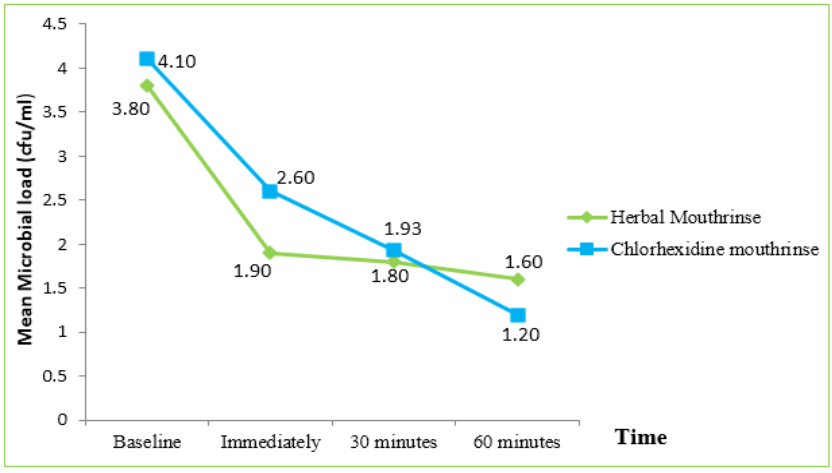

Fig 2: Effect of herbal and chlorhexidine mouth rinses on anaerobic oral microbial load

They also remain effective for extended period of time depending on their composition.

Development in alternative medicine research has led to development of herbal mouth rinses. Antimicrobials of plant origin are known to have enormous therapeutic potential. They are effective in the treatment of infectious disease while simultaneously mitigating many of the side effects.

Consequently, the incorporation of herbal extracts with antibacterial property into mouth rinses has been proposed as a potential prophylactic method of reducing plaque mediated diseases [7]. Natural herbs like tulsi, neem, and clove oil have been scientifically proven to be safe and effective against various oral health problems [7].

The present study showed significant decrease in aerobic microbial load after using herbal mouthrinse. This antibacterial effect could be due to various constituents in herbal mouth rinse.

Basil or tulsi (*Ocimum sanctum*) showed antibacterial property due to its high content of linolenic acid which inhibits DNA replication by cross linking with amino group of bacterial enzyme and thus inhibits growth [8]. It also has an excellent anti – inflammatory property as it has potential to inhibit both cyclo-oxygenase and lipo-oxygenase pathway of arachidonic acid metabolism [8].

Agarwal *et al* assessed the effect of 0.2% chlorhexidine mouth rinse, Listerine mouth rinse and 4% tulsi extract mouth rinse and concluded that tulsi is as effective as Chlorhexidine and Listerine in reducing the salivary *S. mutans* levels [9].

Clove (*Syzygium aromaticum*) is known for its analgesic, antiseptic, antifungal and antibacterial property. This antimicrobial property is due to cell lysis by disrupting protein and lipid composition of cell membrane [10]. In a study by Serfaty *et al* stated that, clove oil has been used successfully to treat gingivitis and hence reduces gingival inflammation and is helpful in reducing plaque scores over a period of time [11].

Mint (Mentha) has antibacterial property because it kills the plasmid containing bacteria due to its increased sensitivity to menthol [12]. A herbal mouthwash containing mint oil and clove oil was found to have good antimicrobial property against *Streptococcus mutans*, and *Streptococcus aureus* [13].

Nutmeg (*Myristica fragrans*) and fennel (*Foeniculum vulgare*) have antifungal and antibacterial activity as their essential oil can travel through the cell wall and cytoplasmic membrane , disrupt the structures of different layer of polysaccharides , fatty acids and phospholipid and permeabilize them [14,15].

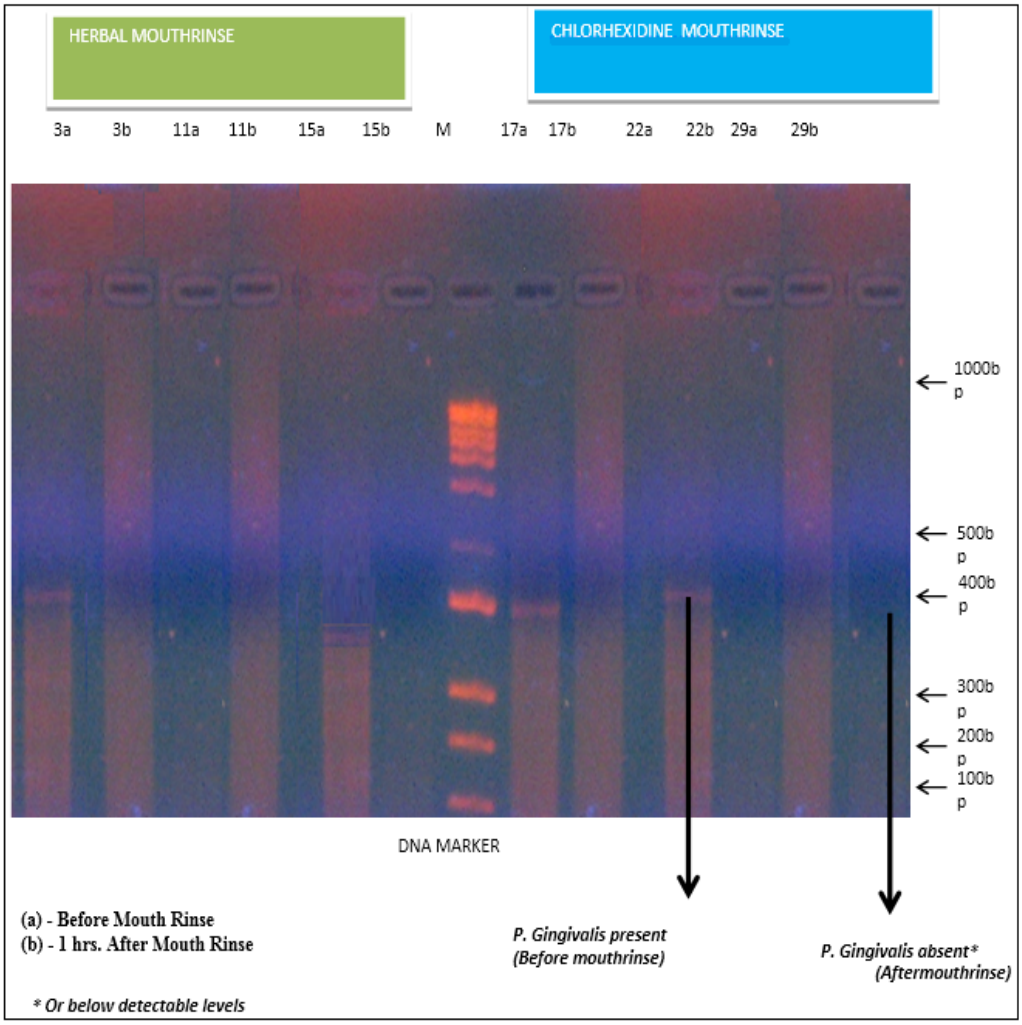

Fig 3: Polymerase chain reaction showing marked decrease in *Porphyromonas gingivalis*

Ethanol extracts in *Myristica fragrans* exhibited good antibacterial property against both gram-positive Streptococcus mutans, Streptococcus salivarius and Gram-negative periodontopathic bacteria *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* [16].

In the present study, chlorhexidine was more effective against anaerobic micro-organisms especially *Porphyromonas gingivalis.* Due to its increased substantivity, chlorhexidine adheres and damages the bacterial cell wall leading to osmotic imbalance, precipitation of cytoplasm and thus resulting in cell death [17].

Though chlorhexidine was found to be more effective on aerobic and anaerobic micro- organisms, well documented side effects of chlorhexidine like tooth staining, taste alteration, and development of resistant micro-organisms, limits its use in special children [5]. Therefore, herbal mouth rinse can be considered as a potential plaque inhibitor and can serve as an alternative in patients with special health care needs.

However our study should be interpreted in the light of certain limitations as it consists of a small sample size and short follow-up period. Further research on beneficial effects of the individual herbal components on saliva, teeth and oral mucosa would enable clinicians to select an appropriate mouth rinse for special children.

5. Conclusion

Herbal mouth rinse was effective in reducing the oral microbial load in Down syndrome children. It can be considered as an alternative mouth rinse to chlorhexidine for use in Down syndrome children.

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