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ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL STUDY

EVALUATION OF ANTIMICROBIAL EFFECTIVENESS OF LICORICE AND TRIPHALA MOUTHWASHES AGAINST *STREPTOCOCCUS MUTANS*

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ABSTRACT

Background: Dental caries is one of the major public health burdens especially in developing countries like India. Mouth rinsing is the most cost effective method of preventing dental caries.

Aim: To assess the effectiveness of Licorice and Triphala mouth rinses against *streptococcus mutans* and to compare the effect of Licorice, Triphala and Chlorhexidine mouthwash.

Materials and Methods:A single blinded quasi experimental study was conducted among the 75 study subjects selected from a tertiary care hospital aged 18-30 years after obtaining ethical clearance. Aqueous extracts of Licorice and Triphala were prepared by maceration procedure. The mouth rinse was prepared by adding distilled water (15%) and peppermint oil. The study subjects were divided into 3 equal groups. Group A was provided with Licorice mouth wash, Group B with Triphala and Group C with Chlorhexidine and to safeguard against any bias, the examiner was not informed about the group allotment. The subjects were instructed on the procedure of using mouthwash. Baseline saliva sample was collected and inoculated in mitis salivarius agar and incubated at 37^0C for 24 hours. After which the colony count was counted and recorded. The saliva samples were again collected on the 7^{th} day and analyzed by the same procedure.

Results:R-commander 2.3-0 was used to analyze the data. Licorice, Triphala and Chlorhexidine groups showed a reduction of $3.36x10^4$, $3.48x10^4$ and $2.6x10^4$ respectively in mean *mutans streptococci* colony counts. So the mean mutans streptococci colony counts in all three groups decreased significantly. While comparing the antimicrobial effect of Licorice, Triphala and Chlorhexidine, it was concluded that there was no significant difference between the Licorice, *Triphala* and *Chlorhexidine* mouthwashes.

Conclusion: The study affirms that both the Licorice and Triphala mouthwashes have got similar antibacterial property as Chlorhexidine.

Key words: Antimicrobial activity, Chlorhexidine, Licorice, Streptococcus mutans, Triphala

Key Messages:

1. Both the Triphala and Licorice mouthrinses can reduce the *Streptococcus mutans* count in saliva when used at least for 7 days according to the study.
2. The antimicrobial effectiveness of Triphala mouthrise and Licorice mouthrinse was similar to Chlorhexidine mouthrinse.

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INTRODUCTION

The history of herbal medicines can be flourished from ancient civilization and their role as a primary source of medication is evident since then [1]. According to World Health Organization (WHO), 80% of the population of developing countries lean on traditional medicines, especially plant drugs, for their primary health care needs [2].

The multi factorial etiology of dental caries makes it an important public health problem in the entire world. The current preventive measures usually include the combination of dietary counseling, oral hygiene, and fluoride application. But none of the interventions focuses on Streptococcus mutans, the chief pathogen responsible for caries. For managing dental caries especially among the primary dentition, new methods of management need to be developed like a supplement for the present preventive method. It will be very useful for the children mainly in prevention of oral diseases when it is both antibacterial as well as acceptable by the children [3].

Chlorhexidine is the most common antimicrobial agent for prevention of dental caries especially in children. It is a cationic bis- biguanide and it is having a very broad antimicrobial spectrum. It is the commonly used over-the-counter mouth rinse. It is used alone and also as an adjunct to mechanical cleaning procedures. The most important property of this is its substantivity. In order to function for long time, it binds to the soft and hard tissues of the mouth.

But various disadvantages of chlorhexidine makes it unacceptable mainly for the children, such as staining, taste alteration and irritation to the soft tissues, which limit its long term use and indicates the need to develop alternatives without side effects [4].

Development of resistance against antibiotics and antiseptics is sprouting recently and it limits the application of many preventive measures. Therefore, there is a continuing need to search for new antimicrobial agents.

Developing countries, for example in rural India, dental care is usually not accessible instantly and even not affordable to them [1]. So, in such situations the use of cheap locally available products for prevention of oral health problems can do wonders.

Ayurvedic medicines can contribute to improve dental health and it provides more pure techniques to prevent and treat oral diseases [1]. Triphala is a pronounced poly herbal formulation in Ayurveda and literature shows that Triphala is useful to cure periodontal disease and for inhibition of Streptococcus counts [3].Few studies shows that Licorice possess strong antimicrobial activity against cariogenic bacterias[1].

The studies assessing the benefits of licorice in preventing dental caries are scanty. Hence, the present study was undertaken to assess the efficacy of the aqueous extracts of licorice and triphala against S. mutans and to compare the effect of Licorice and Triphala mouthrinses with commercially available Chlorhexidine mouthwash. Chlorhexidine is considered the gold standard and it is being used in many clinical trials of new mouth rinse formulations as a positive control [4].

MATERIAL AND METHODS

Study Design:

A single blinded quasi experimental study was conducted among 75 study participants in a

tertiary care hospital after obtaining ethical clearance from Institutional Ethics Committee. Since it is an exploratory study, sample size estimation was not done and samples were selected according to convenience. The participants belonged to 18- 30 years. They were divided equally into Group A, Group B and Group C as Licorice, Triphala and Chlorhexidine groups respectively. Subjects who gave consent to participate in the study and the subjects with DMFT score more than 5 were included in the study. Subjects who were under orthodontic treatment, who were using intra oral artificial prosthesis and those using any other mouth washes were excluded from the study.

Trial Drug

Preparation of licorice and triphala extracts (Maceration) and mouth wash

Licorice powder (2050 gms) and triplaha powder (1070 gms) was procured from Kottakkal Arya Vaidya Sala and Chlorhexidine mouthwashes from the pharmacy. Both licorice and triphala powders was soaked in 10% chloroform water separately for 6 hours with intermittent shaking and undisturbed for 18 hours. The active ingredients leached out in the solvent were subsequently filtered using several folds of muslin cloth and Whatman No. 1 filter paper. The filtrate for each extract was concentrated using china dish and gas burner. The extract obtained was diluted to 15% with distilled water and few drops of peppermint extract was added with to enhance the flavor[5].The samples as well as the mouthwashes was packed without labels, in 150 ml white plastic bottles at Karavali College of Pharmacy, Vamanjoor.

To safeguard against any bias, the examiner was not informed about the group allotment.

Examination procedure:

Informed consent was obtained from the subjects prior to the study. Intra examiner calibration was done before the start of the study. All subjects were instructed to continue their routine home oral hygiene procedures and to continue brushing twice daily during the study period.

On Day 1: Unstimulated saliva samples were collected at least 2 hours after meals in labeled saliva collection bottles for each patient.

They were then provided with a sample of licorice/ triphala/ chlorhexidine mouth wash and were advised on the technique/method to be followed.

The saliva collection bottles were stored at 4°C and then transported to microbiological laboratory in a refrigerated container. Samples were inoculated in mitis salivarius agar and incubated at 37^0C for 24 hours. After which the colony count was counted and recorded.

The study subjects were recalled for follow up after 7 days.

On Day 7: Unstimulated saliva samples was collected in labeled saliva collection bottles for each patient. Sample was inoculated in mitis salivarius agar and incubated at 37^0C for 24 hours. After which the colony count was counted and compared with the previous count.

RESULTS

Statistical analysis was done by using R-commander 2.3-0. Licorice group showed a reduction of $3.36x10^4$ with a p value of 0.0004,

triphala showed a reduction of $3.48x10^4$ with a p value of 0.0001 and chlorhexidine group showed a reduction of $2.6x10^4$ with a p value of 0.014 in mean *mutans streptococci* colony counts (Figures 1 and 2). So the mean mutans streptococci colony counts in all three groups decreased significantly. ANOVA test was conducted to compare the antimicrobial effect of licorice, triphala and chlorhexidine (p value= 0.966) and it was concluded that there was no significant difference between the licorice, *triphala* and *chlorhexidine* mouthwashes.

Table 1- Baseline and post CFU/ ml for Licorice, Triphala and Chlorhexidine mouthrinse.

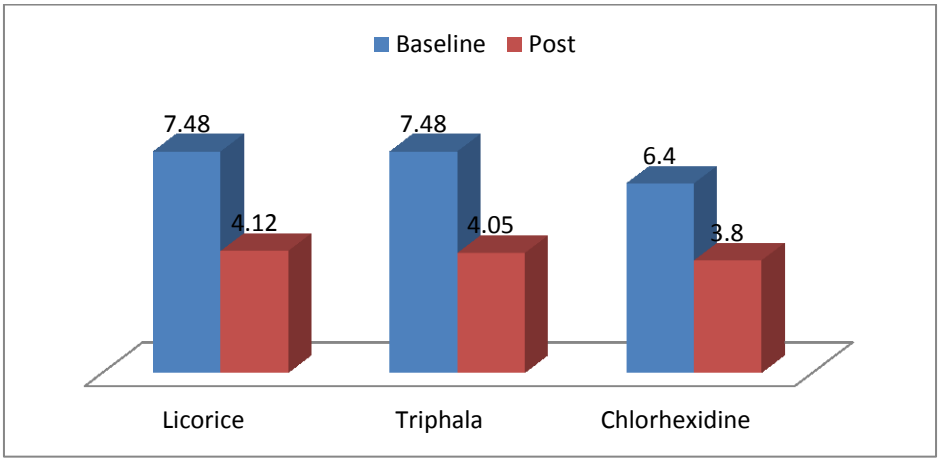
Group	Mean ± SD (CFU/ml)		N	p value
	Baseline	Post		
LICORICE	$7.48 \times 10^4 \pm 4.12 \times 10^4$	$4.12 \times 10^4 \pm 4.50x10^4$	25	0.0004
TRIPHALA	$7.48 \times 10^4 \pm 4.12 \times 10^4$	$4.05 \times 10^4 \pm 4.56 \times 10^4$	25	0.0001
CHX	$6.40 \times 10^4 \pm 4.5 \times 10^4$	$3.80 \times 10^4 \pm 4.34 \times 10^4$	25	0.014

SD: Standard Deviation, CHX: Chlorhexidine, CFU: Colony Forming Unit

Table 2- ANOVA test result comparing Licorice, Triphala and Chlorhexidine mouthrinses

	Sum of squares	df	F	p value
Between Group	$1.38x\ 10^8$	2	0.034	0.966

FIGURES:



CFU: Colony Forming Unit

Fig.1- Baseline and post CFU/ ml for Licorice, Triphala and Chlorhexidine mouthwashes.

Fig.2- Baseline and post intervention microbial colony forming units- Licorice

Fig.3- Baseline and post intervention microbial colony forming units- Triphala

DISCUSSION

There is an increasing interest throughout the oral health care profession in including herbals in oral health care products. Extensive studies are being done to find plant based alternatives for oral health. India is blessed with a great heritage of traditional knowledge on medicines. Many plant products have been reported to inhibit the growth of oral bacteria particularly Streptococcus mutans and thus prevent caries [6]

Licorice, (*Yashtimadhu* or Glycyrrhizaglabra) one of the major traditional medicinal plants grows in the various parts of the world. It has been utilized for medicinal purposes for almost four thousand years. A variety of pharmacological properties lies in the root of this plant like anti-inflammatory, antiviral, antimicrobial, anticancer and immunomodulatory actions and also hepato-protective and cardio protective effects too. Glycyrrhizol A is the prime compound found in the root extract of licorice with strong antimicrobial activity against cariogenic bacteria [1]. It is worthwhile in alimentary tract disorders as well as mouth ulcers [7].Two interventional studies have been carried out and indicated that application of Licorice roots extract to lollipop showed a noticeable decrease of cariogenic bacteria in oral cavity among majority of human subjects [8].

Triphala is an important polyherbal formulation in Ayurveda and it contains equal parts of three herbal fruits: Harada (Terminaliachebula, blackmyrobalan, The Buddha‟s Chosen Herb), Aaonla (Emblicaofficinalisor Indian gooseberry) and Bihara (Terminaliabellerica). It is useful in disease states like headache, dyspepsia, ascites and leucorrhoea and is also useful to purify blood. It carries anti-inflammatory, analgesic, anti-

arthritic, hypoglycemic and anti-aging properties. It is claimed to have antiviral and antibacterial effects also. Various reports states that it reduces the ill effects of oxidative stress. It prevents the growth of Gram positive and Gram negative bacteria [9].

The present study was designed to evaluate the anticariogenic properties of licorice and triphala to ascertain if it could be developed into a caries preventive regimen.

In this study chloroform aqueous extract of licorice and triphala were used to prepare mouthwash along with commercially available chlorhexidine. After the use of mouthwashes for one week, all the three groups showed significant reductions in CFU/ml.

According to the present study, the licorice group showed a reduction of $3.36x10^4$ CFU/ml (Table 1), which was significant. According to an in vitro study conducted by Jain E et al, the mean Streptococcus mutans colony count decreased significantly in both the aqueous as well as ethanolic extracts [5]. In the same study they also revealed that ethanolic extract of liquorice had better antimicrobial activity than the aqueous extracts. So it may be because of the better solvent action of alcohol than water [10]. Hu CH et al prepared lollipops made of licorice and they noticed marked reduction of cariogenic bacteria in oral cavity among most human subjects tested [6].

In the present study, the triphala group showed a significant reduction of $3.48x10^4$ CFU/ml in colony forming units (Table 1). Bajaj N and Tandon S also concluded that triphala mouthwash significantly reduced Streptococcus mutans count from baseline to the sixth and the ninth month [3]

Chlorhexidine group also showed a significant reduction of $2.6x10^4$ CFU/ml (Table 1). This was in accordance with the study done by Bajaj N and Tandon S wherin they observed a significant reduction of streptococcus mutans count after using chlorhexidine mouthwash for a period of six and nine months [3]. This was also supported by the study conducted by Emilson CG, where it was found that *Chlorhexidine* mouthwash reduced *Streptococcus mutans* counts for a period of 4–6 months [11].

While comparing the antimicrobial effects of licorice, triphala and chlorhexidine, the result was insignificant (Table 2). This result is in accordance with the study conducted by Bajaj N and Tandon S. They found out that the reduction in S. mutans count using triphala mouthwash was similar as in the chlorhexidine group [3].

An In vitro study by Sedighinia F et al showed that the antibacterial activity of chlorhexidine was not significantly greater than licorice[5].

In contrary, the conclusion of Gupta R et al according to an in vitro study conducted on triphala was that the inhibition zone of triphala was higher compared with chlorhexidine[12]. Similarly a study by Jain E et al showed that the antibacterial activity of ethanolic liquorice was significant compared to the chlorhexidine group [8].

Hence according to the present study it can be concluded that the licorice, *triphala* and *chlorhexidine* mouthwashes showed similar antimicrobial activity against Streptococcus mutans.

There are few limitations for the study. First, the study was of short duration as the study subjects used the prepared mouthwashes only for one week since the longer use of chlorhexidine may cause staining of the teeth and irritation to the mucosa. The duration could have been increased for more accurate conclusions. Secondly, both the plant extracts were prepared in aqueous medium. Alcoholic extract could have been used to improve the results because the solvent action of alcohol is better than water.

CONCLUSION

The licorice, triphala and chlorhexidine group showed a significant reduction in the streptococcus mutans colony count after one week of rinsing. It affirms the antimicrobial activity of licorice and triphala against Streptococcus mutans. The group wise comparison was also conducted to reveal the antimicrobial activity against Streptococcus mutans among the ayurvedic preparations as well as chlorhexidine. It pointed out a similar antimicrobial activity among the three groups. So it can be concluded that the licorice and the triphala mouthwashes has got an antimicrobial effect similar to chlorhexidine.

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