



ASSESSING THE EFFECTIVENESS OF LIQUORICE ROOT EXTRACT LOLLIPOPOP IN REDUCING THE S.MUTANS COUNT IN SALIVA IN CHILDREN AGED 6-12 YRS-A PILOT STUDY

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ABSTRACT

To determine the effectiveness of Liquorice root extract lollipops in reducing the S.mutans count in the saliva of children aged 6-12 yrs using both Culture plating method and Dentocult. Study design; Pilot study Methods; Supervised administration of Liquorice root extract lollipops was given twice daily for 10 days to 10 children aged 6-12 yrs in a primary school setting. Baseline counts and the post intervention counts were compared using Culture plating methods and Dentocult method. Statistics were carried out using non parametric analysis. Results; The post intervention counts when compared to baseline values showed a significant reduction in the S.mutans colonies. Conclusion; Liquorice promises a simple yet effective caries prevention method for high risk children. But further studies need to be carried out in a larger group of population.

KEYWORDS: antimicrobial therapy, liquorice root lollipop, Streptococcus mutans



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INTRODUCTION

Dental caries is the most prevalent oral disease [1]. It can occur in young as well as old and carries no gender predilection. Miller proposed that oral bacteria in the presence of fermentable carbohydrates produced acids that dissolved the tooth structure[2]. Research has shown that caries is a site specific dietomicrobial disease and its primary pathogens are Streptococcus mutans and other non Streptococcus species like Lactobacillus and Veillonella species. Caries risk assessment is determining the probability of development of new carious lesions in a given period of time. So the proper anti caries measures can be provided for such patients. The usual anti caries treatment is by the usage of fluorides, pit and fissure sealants and by reducing the intake of cariogenic foods.[3] Research has also been done on anti microbial drugs which prevent the colonization or growth of S.mutans[4]. One of them is liquorice. Liquorice is the name applied to the extract from roots and stolons of glycyrrhiza species. It is an herb native to the Mediterranean and certain areas of Asia having varied application in chronic hepatitis, peptic ulcer and lichen planus. Its usage dates back centuries[5,6]. References of its use have been made by Pliny the elder and Theophrastus.[6] It is also known to have a known anti microbial effect against Streptococcus mutans which is the primary cause of decay.Liquorice has been processed into different forms for exerting its anti microbial effect like toothpastes, lozenges, mouthwashes and lollipops. The later being the most effective form. Lollipops have been developed with the active component of liquorice- glycyrrhizin A, which has an anti-adherent effect on S.mutans[7]. Though it is a known fact that liquorice has an anti microbial effect on S.mutans, research is still in a primitive stage in that aspect. The exact mechanism by which liquorice reduces the S.mutans count is not clear [8]. Only one pilot study has been done in American population to assess the effectiveness of liquorice in reduction of Streptococcus mutans in young children in a pre-school setting [9]. No clinical study has been carried out in Indian population.

Hence this pilot study was carried out to assess the effectiveness of liquorice root extract lollipop in reducing the S.mutans count in saliva of children aged 6-12 years.

MATERIALS AND METHODS

This study was planned and carried out in the Department of Pediatric Dentistry in association with the Department of Microbiology in Saveetha Dental College. The study design, protocol, and informed consent were approved by the Institutional Review Board of the institution involved. Permission was obtained from the school authorities in the Government Senior Secondary school, Puliyanbedu. Sixty children were screened by two examiners under daylight using mouth mirror and explorer. Ten subjects were selected for the study with the following inclusion criteria DMFS/dmfs score of >3[10]. A written description was forwarded to the parents explaining the purpose of the study along with the written consent form. All the parents consented for their wards to participate in the study. The 10 children were assigned into the test and the control group by means of random selection of a colored bead from a sealed pouch. Children of the test group and the control group were given brushing instructions and a diet diary to monitor their intake of cariogenic foods.

Saliva collection

Baseline saliva sample of group A and B was collected. Stimulated oral fluid was obtained by having the children chew paraffin wax for 1-2 mins, instructed to tilt their heads forward, and spit continuously for 3 mins in a sterile container. The containers were retrieved and labelled[11]. After the saliva collection, the samples were placed inside an ice box and immediately transported to the Microbiology lab. Ten agar plates were streaked by means of a wire loop. After streaking the plates they were incubated for a period of 24 hours at 37 C. The presence of S.mutans in the plate was confirmed by raised frosted glass appearance of the colonies. Subsequent stimulated saliva was

collected after 10 days of lollipop distribution [12].

Saliva collection for dentocult

The round-tipped dentocult test strip was placed against the saliva on the patient's tongue. Sterile microbrushes were used to collect plaque samples for the control and study groups from the following four sites: a. buccal surface of the maxillary canine, b. labial surface of the maxillary incisor, c. labial surface of the mandibular incisor and d. lingual surface of the mandibular left canine. These samples were spread thoroughly but gently on the four sites of the rough surface of the strip. The strips were then placed in a selective culture broth with the smooth surfaces clipped and attached to the cap. The vials were then labeled and incubated in an upright position at 37°C for 48 hrs with the cap opened one quarter of a turn to allow growth of the organisms. After incubation, the presence of *Streptococcus mutans* was confirmed by detecting light-blue to dark-blue, raised colonies on the inoculated surface of the strip. Colonies suspended in the culture broth were excluded from the evaluation. The results were evaluated according to the manufacturers' chart. [13,14]

Class 0: <10,000 CFU/ml

Class 1: <100,000 CFU/ml

Class 2: 100,000-1000 000 CFU/ml

Class 3: >1000 000 CFU/ml

Lollipop distribution

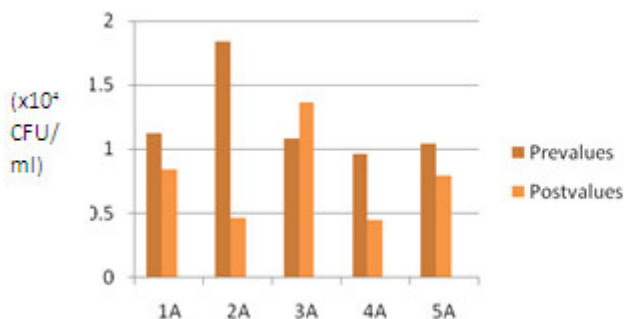
Group A students were given the liquorice root extract lollipop, twice daily for a period of ten days at a time frame of approximately 9 am and 2 pm. There were no eating restrictions before and after the lollipop intake. The children were monitored by the investigators till they successfully finished the lollipop, defined as holding the lollipop in the mouth for ten minutes

Statistical analysis

The pre intervention and post intervention *S.mutans* count were tabulated and compared. Non parametric tests were applied.

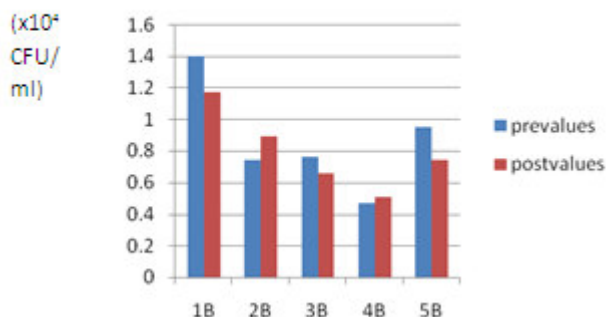
RESULTS

Non parametric tests were applied for the statistical analysis. Mean differences between the samples is 0.25. Wilcoxon signed test ranks showed a more number of reduction in the post values of *S.mutans* colony count when compared to pre values (negative ranks=7; where post values < pre values). Also there was a reduction observed in the post values in the 25th, 50th, 75th percentile when compared to the pre-intervention values.



(A) Data representing activity of Liquorice against *S.mutans*-Test Group;

No. of CFUs/ml of *S.mutans* were taken for evaluation from the salivary samples collected. Pre and Post exposure values were recorded for both test and control group. The given data (1A,2A,4A,5A) clearly shows a reduction in the CFUs/ml of *S.mutans* post exposure to liquorice lollipops.

**(B) Data representing the control group;**

Pre and post values do not show much difference in this group (2B,3B,4B). Reduction seen in 1B, 5B samples of the control group can be contributed by the Hawthorne effect.

Values obtained from dentocult showed reduction in the test group than in the control group. But definitive analysis could not be obtained.

DISCUSSION

Research has proved the role of *S. mutans* in the initiation of dental caries. Reduction of oral flora which are said to play a part in the development of caries have been proven beneficial. Various anticaries measures have been introduced and of recent findings show Liquorice possessing a strong antimicrobial activity against *S. mutans*. This is the first clinical trial carried out in the Indian population, using Liquorice root extract, in a simple yet effective prevention protocol manner. Lollipops were chosen to administer the active ingredient in a caries prone population to prove the concept. The use of a control group and stimulated saliva collection using paraffin wax has not been done in the previous studies. The results suggest the use of a twice-a-day regimen of liquorice extract lollipops for 10 days significantly reduced the CFU/ml of *S. mutans*. No adverse effects were documented. When

given the lollipops the children liked the flavor and were compliant. The limitations of this study include a very less study population and also Hawthorne effect [15] must be brought into consideration. Rebound [9] seen in previous studies was not taken into consideration. This might be resolved in the future by using a larger number of study population where parametric studies can be applied.

CONCLUSION

Although Liquorice lollipops cannot be used as a treatment adjunct as of now, it proves to be a good preventive measure especially for children. Further studies have to be carried out in this field so that new doors can be opened in the research of oral health care.

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