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ORIGINAL RESEARCH

Anticaries Efficacy of Liquorice Lollipop: An *ex vivo* Study

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ABSTRACT

Aims: To investigate the anti-bacterial efficacy of liquorice lollipop and also to investigate the remineralizing potential of the extract.

Materials and methods: An interventional study was carried out on 20 volunteers aged between 18 and 21 years. Antibacterial efficacy was investigated by evaluating the bacterial counts of salivary *Streptococcus mutans* and *Lactobacilli* before and after the consumption of liquorice lollipops for 10 days while the remineralization potential was studied with scanning electron microscopy (SEM) analysis of the enamel block embedded on a Hawley's appliance worn by volunteers for 14 days after consumption of the liquorice lollipop.

Results: A reduction in the bacterial counts of *S. mutans* which was statistically significant was observed. However, *Lactobacillus* growth was not observed before or after use of liquorice lollipop. No structural or morphological changes were observed on the enamel surface through SEM imaging.

Conclusion: Thus, liquorice lollipop was effective in reducing *S. mutans* but did not show to possess a remineralizing potential.

Clinical significance: The unique approach of a lollipop delivery system to reduce *S. mutans* count in children is also observed to be palatable for children. This raises hopes of a simple and effective way to deliver a targeted intervention to young children who are at risk for dental caries.

Keywords: Adolescent, Antibacterial, Anticarious, Herbal, Liquorice, Remineralizing, Scanning electron microscope analysis.

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INTRODUCTION

Dental caries still remains the most common dental problem to affect individuals across the globe. With the gradual change in lifestyle trends and food habits, adults and especially children are becoming more susceptible to caries. Thus, there is a pressing need to develop new natural and innovative antimicrobial agents for the treatment of dental caries.[1,2] Herbal extracts in the form of leaves, fruits, flowers, seeds, roots, and barks have been studied by researchers and have shown to possess anti-inflammatory, antibiotic, analgesic, and antinociceptive properties. Validation of the uses of these different therapeutic modalities is particularly important to help serve as a potent, safer, and cost-effective solution to dental diseases.[3]

Root extracts like ginger, garlic, turmeric, and liquorice (*mulethi*) are traditionally used in India and have been known to have an antibacterial potential. The antibacterial efficacy of liquorice has been studied by various researchers, but the effective mode of administration of the extract by which the optimal antibacterial potential can be attained has not been established.[4] Literature reveals that liquorice has an inhibitory effect on *S. mutans* and the modes of administration reported are either as mouthwashes or through a lollipop delivery system.[5,6] The novel approach of incorporation of the liquorice extract with active compound *Glycyrrhizol* A into a lollipop delivery system had been devised by Hu et al,[7] and a reduction in salivary *S. mutans* levels postconsumption of the liquorice lollipops has been observed and it has also been reported that the mode of delivery was well accepted by children.

The potential of liquorice lollipop has been proven but has not yet been investigated in the Indian scenario. Further, the remineralizing potential of liquorice, if

any, has also not been investigated. Thus, the scope of this study is to investigate the antibacterial efficacy of liquorice lollipops among volunteers and to investigate if the extract has a remineralizing potential. The hypothesis is that there is no difference in the bacterial counts of salivary *S. mutans* and *Lactobacilli* before and after the consumption of liquorice, and there is no remineralizing effect of liquorice on the enamel surface.

MATERIALS AND METHODS

The study was carried out in the Department of Pedodontics and Preventive Dentistry, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India, from March 2017 to September 2017. The volunteers were selected from the undergraduate students of the institution and were in the age group of 18 to 21 years.

The exclusion criteria included adolescents who were on any antibiotic regimen for the past 3 months, undergoing any orthodontic treatment, and if suffering from any systemic disease or condition. All volunteers of the study had signed consent for the same. The sample size determination was done in accordance with a previous study by Hu et al,[7] power of 80%, error of 5%, dropout of 10%, and confidence level of 5%.

The liquorice lollipops were procured from the LOLOZ™ Company who had fabricated the lollipops to contain 7 to 15 mg of the active compound, *Glycyrrhizol* A. The genotoxicity and cytotoxicity of this liquorice lollipop had been earlier studied[7] and is reported to be safe for children and adults. The recommended regimen is twice daily for 10 days for an antibacterial effect.

Assessment of Antibacterial Efficacy

Unstimulated saliva (2–2.5 mL) was collected for the purpose of evaluating the antibacterial efficacy of liquorice extract. The samples were collected between 8 and 10 am in accordance with the circadian rhythm, and at least 1 hour after the consumption of any food or drink. The collected samples were transported to the laboratory within 3 to 4 hours for processing and estimation of the bacterial counts of *S. mutans* and *Lactobacillus*. Saliva samples were inoculated into mitis salivarius bacitracin (MSB agar) and Rogosa agar for specific bacterial culturing and identification of colony characteristics, and counting was done by digital colony counter. Saliva collection was performed twice during the study period. Saliva was first recorded at baseline before start of the liquorice regimen to evaluate the salivary bacterial status of the subject. The liquorice regimen was then followed for a period of 10 days. Similar procedure was performed again to evaluate the salivary bacterial status posttreatment.

Assessment of Remineralizing Potential

Twenty premolars extracted for orthodontic purpose were obtained from the Oral Surgery Department of the same institution. The radicular part of each tooth was removed and the coronal part was then sectioned along the long axis of the tooth into two portions of buccal and lingual using a high-speed diamond-tipped disk. Further, the tooth was sectioned into three sections of occlusal, middle, and cervical third using the high-speed diamond-tipped disk. Four enamel specimens were prepared from each tooth and only the buccal sections were selected for the study (Fig. 1). Each enamel block was standardized to 3 × 2 mm in size.

The prepared enamel blocks were further subjected to demineralization by suspending each section of the enamel block into a glass bottle containing 20 mL of demineralization solution for 72 hours in an incubator at a temperature of 35°C.[8] The composition of the demineralizing solution used was $CaCl_2$ = 2.2 mM, NaH_2PO_4 = 2.2 mM, lactic acid = 0.05 M, fluoride = 0.2 ppm. Solution was adjusted with 50% NaOH to a pH of 4.5.[9] The pH was recorded each time with litmus paper before subjecting the enamel blocks to demineralization.

Maxillary and mandibular alginate impressions were recorded in order to fabricate a Hawley's appliance for each subject. Once the Hawley's appliances were fabricated, a window measuring 5 × 5 mm was prepared in the palatal aspect of the acrylic. The demineralized enamel blocks were incorporated into this prepared window with cold cure acrylic (Fig. 2). The incorporated appliances were sterilized by 3% ethylene oxide gas sterilizer. The subjects were instructed to wear the appliance while consumption and 20 minutes postconsumption of the lollipop. When not worn, the Hawley's appliance was stored in a plastic container. The liquorice lollipops were consumed for 14 days. Post the treatment regimen, the enamel blocks were subjected to SEM analysis for evaluation of remineralization.

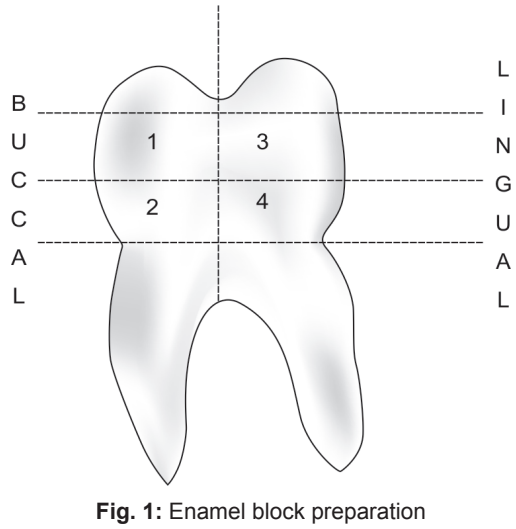


Fig. 1: Enamel block preparation

Fig. 2: LOLOZ liquorice lollipops used in the study

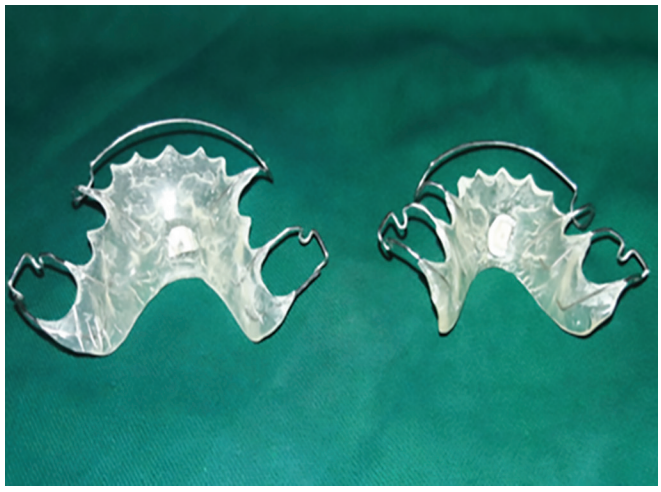


Fig. 3: Hawley's appliance with the incorporated enamel blocks

ETHICAL AND HUMANE CONSIDERATIONS

The study was approved by the ethical review board University Ethics Committee for Human Trials of the university and is in accordance with the Declaration of Helsinki. The commercially produced liquorice lollipop was procured from LOLOZ™ 601 SW 2nd Ave, Portland, OR (Fig. 3).

Data were subjected to statistical analysis performed using Statistical Package for the Social Sciences software version 20.0. Paired Student's t-test was performed as analytical statistics to measure the *S. mutans* count before and after consumption of liquorice.

RESULTS

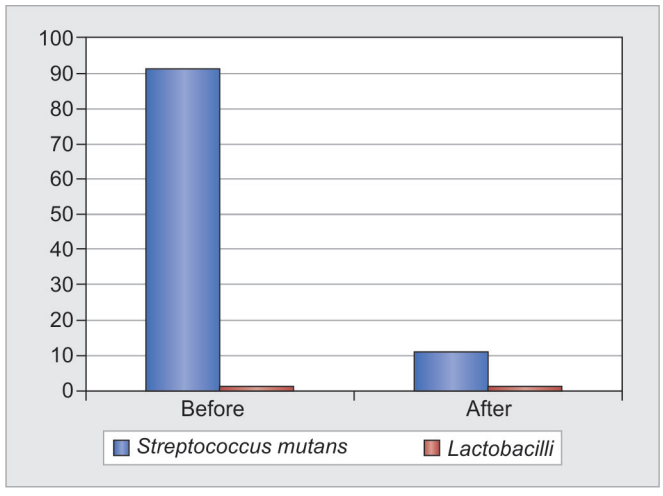
A considerable reduction in the *S. mutans* count was observed from 91.70 ± 63.814 to 11.20 ± 11.448 colony-forming units (CFU)/100 mL, which was statistically significant with p-value <0.05 (Table 1), suggesting that consumption of liquorice lollipops showed antibacterial potential against *S. mutans*. However, *Lactobacillus* growth was not observed before or after use of liquorice lollipop (Graph 1).

Figures 4A and 5A show the initial enamel lesion created on the surface with significant porosity. The porosity observed was assumed to help in penetration of solution ion constituents and allows a greater area for reaction of enamel mineral. The morphological evaluation of the surface enamel subjected to the action of liquorice root extract was observed to have no change worthy of observations (Figs 4B and 5B). The structural features

Table 1: Difference in *S. mutans* count (CFU/100 mL) among subjects before and after the use of liquorice lollipops

	Mean ± SD	*t-value*	*p-value*
Before	91.70 ± 63.814	4.427	0.002*
After	11.20 ± 11.448		

SD: Standard deviation; *<0.05



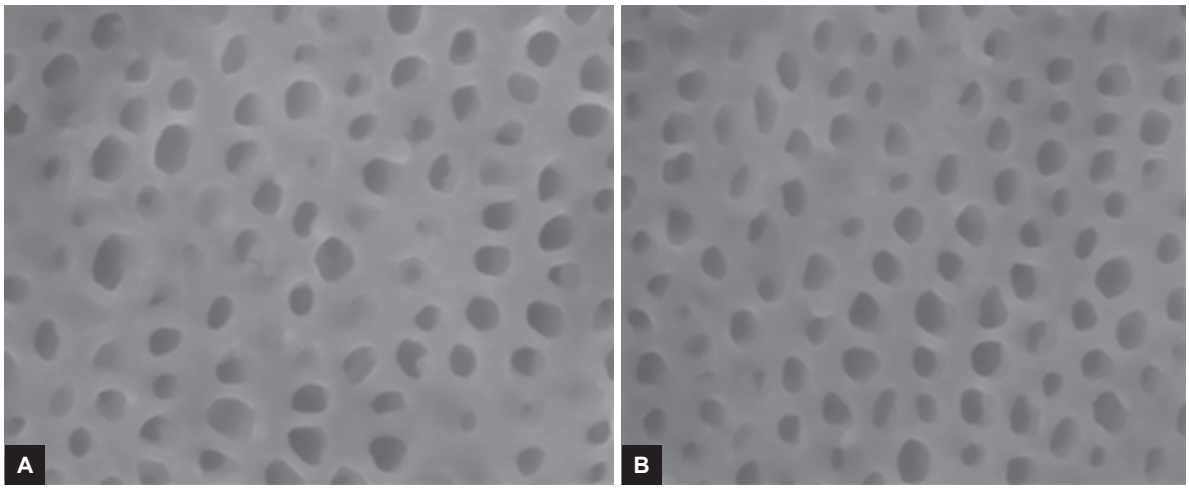
Graph 1: Difference in CFU counts before and after treatment

observed at 5,000× and 10,000× magnifications remained the same with no presence of any mineral deposition or collagen formation, which is suggestive of the fact that liquorice root extract did not show any remineralizing potential following the study period of 14 days.

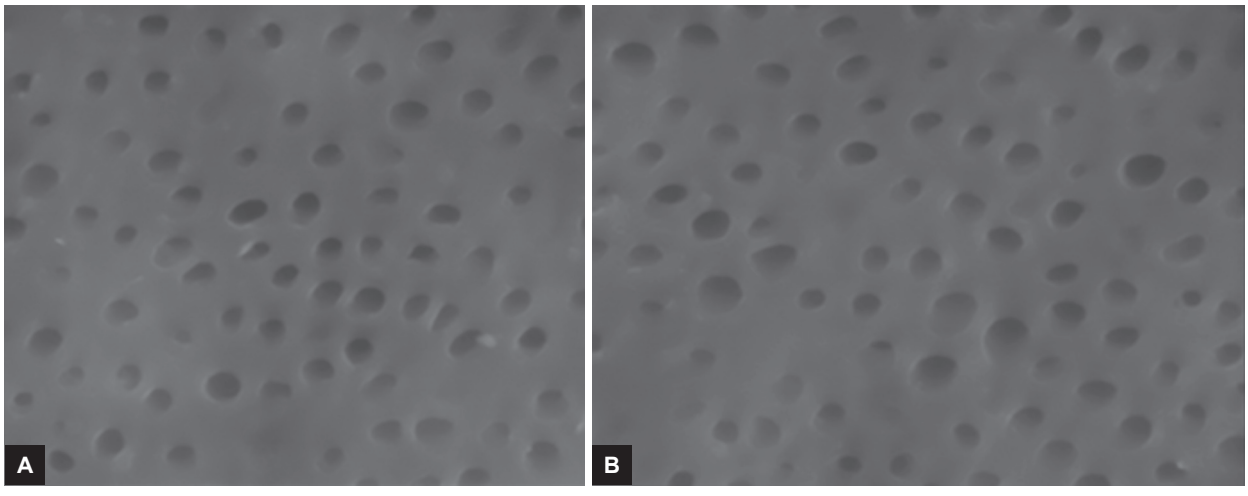
DISCUSSION

Several researchers have investigated the chemical constituents and biological activities of liquorice roots (*Glycyrrhiza glabra*) and many phenolic compounds have been identified that are shown to have an inhibitory effect against bacterial growth. Literature reveals the antibacterial role of polyphenols on cariogenic streptococci, suggesting the mechanism of action as a direct effect against bacteria or an interaction with microbial membrane proteins, thus inhibiting the adherence of bacterial cells to the tooth surface or also by inhibiting glucosyl transferase and amylase enzymes that disrupt the glucose mechanism.

A sugar-free orange-flavored lollipop has been developed containing the novel compound *Glycyrrhizol A* against



Figs 4A and B: Scanning electron microscopy analysis of specimen—pre- and posttreatment



Figs 5A and B: Scanning electron microscopy analysis of specimen—pre- and posttreatment

S. mutans in children who are at risk for caries. A study by Peters et al[5] to determine the antibacterial effect of consumption of the liquorice lollipop twice daily for 3 weeks reported a significant decrease in *S. mutans* count and also observed a steeper decrease in children who were at a higher risk for caries. A similar study was carried out by Hu et al,[7] in which two pilot human studies were conducted to observe the effect of the developed liquorice lollipop against *S. mutans*. They concluded that a brief twice-daily application for 10 days led to a marked reduction of cariogenic bacteria in the oral cavity among the human subjects tested.

Among the Indian population also, the antibacterial effect of liquorice has been studied, and a similar inhibitory effect on *S. mutans* by the ethanolic extract of liquorice has been reported.[6,10]

In our study, we followed regimen of twice daily for 10 days and observed a significant reduction in the *S. mutans* count, which was in accordance with previous studies. But, the present study had certain limitations that, the age group comprised of adolescents, which makes it not generalizable to pediatric patients. The sampling was convenient and also salivary and dietary parameters were not taken into consideration which could be confounding factors.

Regarding the *Lactobacillus* species not showing any growth in the culture media in this study, it could be inferred that, *Lactobacilli* usually comprise less than 1% of the total cultivable microbiota and a strong correlation has been established between the *Lactobacillus* count and caries. The higher the decayed, missing, and filled teeth (DMFT) index, the higher would be the number of subjects harboring a high *Lactobacillus* count.[11,12] Thus, the growth is dependent on the DMFT status of the subject as well as the sensitivity of the culture method used.

Pre- and posttreatment, the enamel blocks were analyzed by SEM. Scanning electron microscope allows assessing qualitatively the process of demineralization through the observation of specific morphological and structural features, such as opening and size of the tubules, the presence/absence of peritubular mineral, and the evolution of the organic network of collagen.[13] The remineralization potential of liquorice has not been studied much. But, flavanoid compounds, such as

1-methoxyficifolinol, Licorisoflavan A, and 6, 8-diprenylgenistein have been isolated from liquorice root extracts (*G. glabra*).[14] Flavanoids have been reported to increase the availability of calcium and phosphate ions, increasing the possibility of remineralization.

So, in our study, we made an attempt to investigate the remineralizing potential of liquorice extract through SEM analysis, and the results show that liquorice root extracts do not show any collagen formation/remineralizing potential. In the literature, studies regarding the remineralization potential of any material reveal that the material should be studied for a period of 7, 14, 21, and 28 days to observe structural and morphological changes on the enamel surface. But we studied the material only for a period of 14 days which could be one of the reasons for the material not showing any remineralizing ability.[15] Thus, we suggest that a study for more than 14 days must be carried out before concluding that liquorice root extract has no remineralizing potential.

The observed adverse effect associated with excessive intake of glycyrrhizin has been termed as liquorice-induced pseudoaldosteronism. It consists of a triad of increased blood pressure, hypernatremia, and hypokalemia. Glycyrrhizin blocks the activity of the enzyme 11β-hydroxysteroid dehydrogenase type II, which converts cortisol to inactive cortisone. Cortisol, in turn, binds to mineralocorticoid receptors, promoting sodium reabsorption, potassium excretion, and hypertension. Nowadays, this is becoming a more frequent phenomenon with increased use of liquorice as flavorants. The World Health Organization, however, recommends a maximum daily dose of 100 mg/kg of liquorice containing *glycyrrhizin* as the safely level. The regimen to be followed for optimal antibacterial efficacy of the liquorice lollipop is twice daily for 10 days as described by the manufacturer. Thus, considering the amount of glycyrrhizin in each lollipop and the recommended daily dosage, the intake of glycyrrhizin from these lollipops can be stated as well within the safety levels if consumed under proper guidance.

A recent study has determined the optimal concentration of deglycyrrhizinated liquorice root extract with minimum inhibitory concentration against *S. mutans* as 4 to 8 µg/mL and minimum bactericidal concentration against *S. mutans* as 8 to 16 µg/mL.[16] These findings help provide an alternative to the glycyrrhizin compound and thus avoid their adverse effects. Further investigations on the deglycyrrhizinated liquorice extract are required before it can be used clinically.

CONCLUSION

Thus, we would like to conclude that liquorice is effective in reducing the salivary *S. mutans* counts, but liquorice root extract did not show any remineralizing potential. It is a known fact that children love sweets and confectionary. Overindulgence in high sugar-containing food items, along with improper oral hygiene methods, leads to children being at a higher risk for caries. Liquorice shows to have an antibacterial efficacy and at the same time is palatable for children.

CLINICAL SIGNIFICANCE

The unique approach of a lollipop delivery system counteracts this problem as it is in the form of a candy that is sugar-free and is shown to have an antibacterial efficacy while at the same time being esthetic and palatable for children. This raises hope of a simple and effective way to deliver a targeted intervention to young children who are at risk for dental caries.

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