ORIGINAL RESEARCH

Cavity Disinfection with Herbal Disinfectants Licorice (Mulethi) and Munident Tablets in Children Aged 5–9 Years: A Randomized Controlled Trial

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Abstract

Background: Dental caries is still a foremost oral health issue affecting the population in developing countries. Many anticariogenic synthetic therapeutic agents have been used as cavity disinfectants, but with the rise in bacterial resistance, there is substantial concentration and an emergent trend in herbs and homeopathy.

Aim: This study compared the antibacterial efficacy of natural herbal agent licorice, commercially available Ayurvedic agent Munident tablets 500 mg, and normal saline as a cavity disinfectant.

Materials and methods: In this present study, two test groups were included, group I (licorice), group II (Munident crushable tablets), and group III (Normal Saline) was included as a control group. The children were randomly divided into 15 for each group with a cariogenic cavity not having any deep dental caries, pulp pathology, or any other intraoral pathology without systemic conditions. Two samples were collected by excavating cariogenic dentine predisinfection. After disinfection, the cavity with test agents, after collecting the samples sent to the microbiological laboratory of college for a total viable count of bacteria. The collected data were statistically analyzed with the Kruskal Wallis Test, Mann-Whitney *U* test, and suing Wilcoxon paired *t*-test.

Result: This study showed a statistically significant reduction in total viable count compared to pre and postdisinfection with test agents. Munident Crushable tablets were most effective amongst all the groups, whereas licorice has also displayed statistically significant results.

Conclusion: Ayurveda and herbal products can be effectively used as cavity disinfectants, helping eradicate the bacteria and diminishing secondary caries with longevity and success of the restoration.

Keywords: Antibacterial, Ayurveda, Herbal, Licorice, Munident.

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Introduction

The longevity and success of restoration depend upon the complete eradication of bacteria at the time of cavity preparation and preceding the insertion of any kind of restoration. The clinician has very challenging task while preparing the cavity in the carious tooth as it becomes difficult to completely eradicate the remnants of microorganisms in the cavity wall. These attached remnants of bacteria in the cavity walls, at the enamel-dentine junction, in dentinal tubules, or in the smear layer decreases the longevity and attainment of a good restoration. This finally leads to the development of secondary or recurrent caries, the sensitivity of tooth occurs postoperatively, and dislodgment of restoration after some time causing failure.[1,2] Previously, extensive mechanical cavity preparation was the commonly followed technique by clinicians. However, this causes the weakening of the tooth structure and affects the vitality of pulp, risking the life of a tooth. This type of dentistry was changed by Modern Dentistry, which projects toward the extension of prevention and conservation of teeth, minimum discomfort to the patient, and elimination of causative microorganisms.[3] Also, minimally invasive dentistry has achieved tremendous acceptance as ART is known for doing minimal invasion using hand instruments to excavate demineralized soft carious dentine and filling with adhesive restorative material creating the arrest mechanism for caries activity with minimum intervention.[4] With limitations of accessibility to the cavity with the instrument and clinician's fatigue, this procedure can lead to incomplete excavation of the remaining carious part, which could cause the development of secondary caries and could affect the success of the restoration.[5-7] Therefore, to overcome the limitations, the concept of using antibacterial agents for cavity disinfection came into dentistry.

Brannstrom and Nyborg, in the early 1970s, had done excellent work on antimicrobial agents and their mechanism of action on

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the origin of pulp, who highlighted the significance of eliminating bacteria attached on walls of the cavity, including enamel and dentine after excavation of caries using antibacterial agents and hence provided literature to use disinfection agents before insertion of restoration.[8] After their contribution, cavity cleaning with antibacterial agents for bacterial eradication has gained wide acceptance among dental clinicians.

The rise in commercially available synthetic antimicrobial agents has lowered the efficacy by making the pathogens more resistant and posturing the serious threat in successfully treating the diseases.[9,10] Hence, for 4–5 years, there has been an extensive interest and emerging trend in Ayurveda and Homeopathy by using plant extract as medicine.[11,12] Synthetic cavity disinfectants like chlorhexidine, sodium hypochlorite, Benzalkonium Chloride, Iodine-based disinfectants, etc., and other systems like lasers and ozone had reported pulp irritation, staining, metallic taste, and cytotoxicity due to inherent chemicals.[13] Also, the long-term use of synthetic antibacterial agents causes bacterial resistance, weak bond strength, and increased chances of micro-leakage. These shortcomings can be overcome by the use of herbal plant extracts like neem leaf, propolis, noni fruit, miswak, licorice, etc., which have advantages like antimicrobial activity, less cost, no significant cytotoxicity, and easy availability.[13]

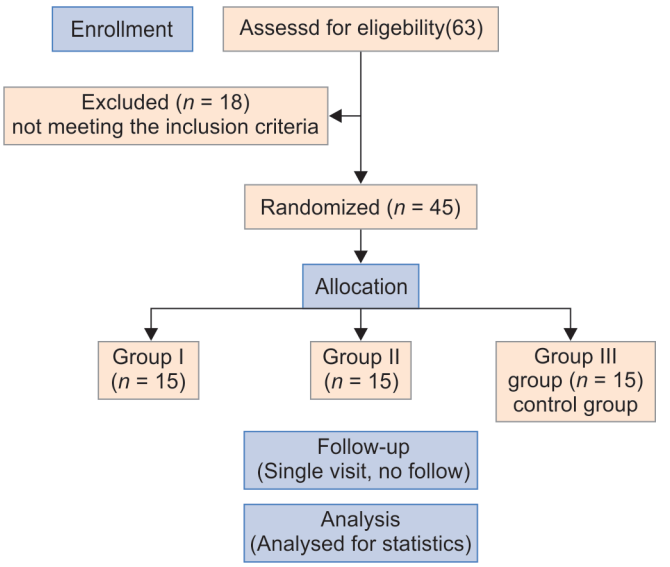
The munident crushable tablets have various components responsible for antimicrobial efficacy such as *Santalum album, Cyperus rotundus, Berberis aristata, Symplocos racemosa, Curcuma longa,* and *Cinnamomum zeylanicum*.[14] Munident tablets, as opposed to commercially available toothpaste, have all-natural ingredients, however, due to the absence of chemicals, Ayurveda claims to have fewer or no side effects. Several authors have mentioned the antimicrobial action of licorice in which the chief component glycyrrhizol A was found to show potent antimicrobial action against cariogenic bacteria.[15] According to FDA (The Food and Drug Administration) the licorice was recorded as the safe food flavoring and sweetening agent.[16] Hence, the study aimed to compare the antibacterial efficacy of herbal cavity disinfection between the three groups, that is, licorice (mulethi), muniment crushable tablets, and normal saline.

Materials and Methods

This study was carried out as a double-blind randomized controlled trial in the Department of Pediatric and Preventive Dentistry in collaboration with the Microbiological Department in a Dental Research Institute in Faridabad city, Haryana.

Ethical clearance was obtained (SRCDSR/ACAD/2022/10827) before starting the clinical study. The study participants were explained in detail the nature, purpose, and application of herbal agents to be used in the planned study, and written informed consent was obtained from the subjects for the same followed by WHO criteria. A total of 45 subjects (25 males and 20 females) within the age group of 5–9 years reporting to the OPD in the department in Faridabad city, Haryana were chosen for our study based on inclusion and exclusion criteria. Hence sample size was calculated using G Power Software (version 3.0.10). Based on the calculated effect size of 0.483, 5% level of precision, 95% confidence level, and 80% power of the study. The minimum sample size was calculated as 45, with 15 each. The samples were randomly allocated with the help of lottery method between the groups. The subjects with deep dentinal carious lesions giving no history of pain, clinically no signs of abscess, sinus, or fistula, and showing no radiographic signs of any pulpal involvement were included in the study (Flowchart 1).

Flowchart 1: Consort flow diagram for all the three groups



Inclusion criteria comprised of subjects willing to participate in the study, deciduous Molars and Occlusal or occluso-proximal lesion suitable for ART, dentinal lesions with no pulp involvement (confirmed by radiograph), absence of pain, no abscess and swelling, or adjacent soft tissue lesions or any intraoral pathology. The medically compromised patients and patients on antibiotics within 3 months were excluded from the study.

The total of 45 children (25 males and 20 females) aged 5–9 years was randomly divided into three groups: group I- licorice (mulethi), group II- Munident crushable tablets, and group III- Normal Saline (control group) 15 each (Table 1). All the subjects were instructed not to take any drink except water and any food for at least 1 hour before collecting dentine samples to diminish saliva stimulation and lessen the contamination from any food debris for better results.

Preparation of Licorice (Group I)

The commercially available Yastimadhu/Mulethi Powder- 500 G is also known as *Glycyrrhiza Glabra Linn- Liquorice*/Mulethi. An amount of 100 mg of Licorice agent was prepared freshly by adding 1 ml glycerol to form a gel just before performing the restoration procedure.[17]

Preparation of Munident Agent (Group II)

The commercially available munident- 100 Tablets by Muniyal Ayurveda for bleeding gums and Pyorrhea were chosen as the second group. An amount of 100 mg of munident Tablet (500 mg) crushed into powder form and mixed with 1 ml of glycerol to form a gel.

Normal Saline (Group III- Control Group)

Normal saline as a control group was filled in a 2 ml syringe and the cavity was rinsed with saline for 2 minutes.

Dentine Sample Collection

First Sample

The sample was collected with the help of a spoon excavator from the center of the lesion with proper instrument sterilization. The infected dentine was removed with the sharp spoon excavator

and the affected dentine was left behind (Fig. 1). The excavated dentine sample was then transferred to a test tube containing peptone water. After 1st sample, the prepared quantity of test agent was applied using a plastic filling instrument by the principal investigator. The application was done in each group onto the cavity for 2 minutes. After 2 minutes, the applied agent was washed with distilled water (Figs 2 – 4).

Second Sample

The second dentine sample was taken from the same excavation site with all three groups after disinfecting the cavity and transferred to peptone water (Fig. 5). After this, the glass ionomer cement (GC Fuji II) restoration was done in the respective tooth (Fig. 6). Finally, the collected samples in test tubes were sent to the microbiological lab of the college itself for microbial analysis for total viable count.

Microbiological Procedure

The collected dentine samples were stored in a refrigerated container at 4°C and immediately transferred to the microbiological laboratory within one hour of sample collection. Thereby, collected dentine samples were transported to brain heart infusion, and for the growth of microbes, it was incubated for the night. A tube shaker was used for homogeneous mixture, after which placed onto the plate surfaces comprising blood agar. Finally, all the sample was incubated with a culture plate for 24 hours at 37°C, followed by calculation of total viable bacterial colonies by visual assessment

Table 1: Distribution of samples among three groups

		N	*%*
Group I	Licorice (mulethi)	15	33.3%
Group II	Mundent crushable tablets	15	33.3%
Group III	Normal saline	15	33.3%
Total		45	100%

Fig. 1: Predisinfection dentine sample excavated from spoon excavator

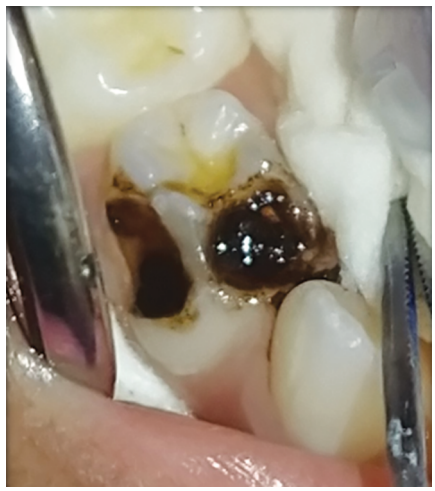


Fig. 3: Application of gel form of munident crushabe tablets

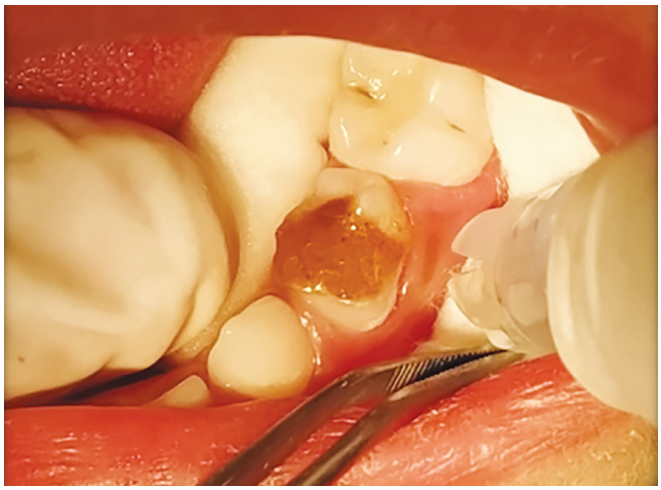


Fig. 2: Application of liquorice extract

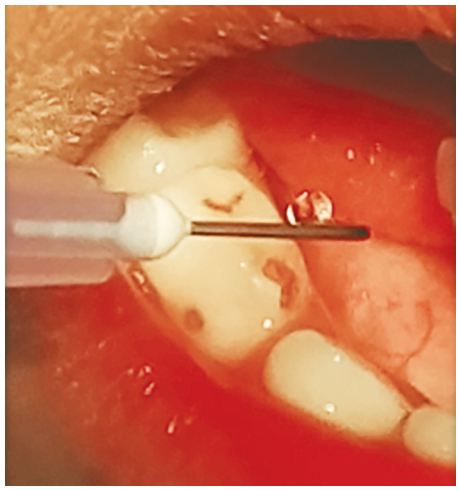


Fig. 4: Application of normal saline

using a HiMedia laboratories private limited Digital Colony Counter (LA6660) for statistical analysis.

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive data were reported for each variable. The summarized data was displayed using Tables and Graphs. Data were not generally distributed as tested using the Shapiro Wilk test ($p < 0.050$). Hence, a nonparametric test, Kruskal Wallis, was used for the comparison of two or more groups. The pairwise comparison was made using Mann Whitney *U* test, and the pairwise comparison was made using Wilcoxon paired *t*-test. A level of $p < 0.05$ was considered statistically significant.

Results

The predisinfection mean total viable count for groups I, II, and III were 15.00 ± 5.09, and 18.71 ± 5.38 and 16.52 ± 6.17; the mean total viable count reduced in all the three groups' postdisinfection, that is, 9.93 ± 3.76, 11.57 ± 3.18, and 14.22 ± 5.70 (Table 2).

The mean Total Viable Count reduction (pre-post) after cavity disinfection with test agents and control group was found to be 5.06±2.09 in group I, 7.13±2.97 in Group II- and 2.29±0.88 in Group III. At post disinfection, statistically significant difference was seen among the three groups. The maximum reduction of bacterial counts was seen with Group II, followed by Group I and least in control group i.e, Group III. On pair wise comparison, significant difference for the reduction in Viable count was seen among all pairs of groups (Table 3). On intra-group comparison, significant reduction in viable count was seen for difference for Group II and Group I. (Table 4)

This study displayed clinically relevant finding that all the groups had reduced the number of bacterial counts after applying agents before GIC restoration.

Discussion

In the present study, there was a definite reduction in total viable count after disinfecting the cavity with liquorice/mulethi) and munident crushable tablets. The age group of 5–9 was selected keeping into consideration the patient compliance and cooperation for the duration of the procedure as in this age group; children could keep their mouth open for the application time of the agents. Both liquorice and munident tablet have been found safe for use in children as mouth washes, toothpaste, irrigating solutions, and cavity disinfectant agents in various studies. In a study done by Jain et al., the toxicity of liquorice was performed using nematode *Caenorhabditis elegans*, stimulating the human model and found to be safe with no side effects for use in children.[19]

The munident tablets clinically found better results than others due to the presence of multiple ingredients with antimicrobial, anti-inflammatory, antioxidant, antifungal, antiviral, analgesic, wound cleanser and also help to relieve pain. The various constituent herbs present in the tablet which has beneficial antibacterial properties are *Santalum album* 30 mg, *Cyperus rotundus*: 20 mg, *Berberis aristata*: 20 mg, *Symplocos racemosa*: 20 mg, *Curcuma longa*: 20 mg, and *Cinnamomum zeylanicum*: 20 mg. Only one study performed by Shetty RN et al.[14] showed that munident (herbal) dentifrice had slightly better efficacy compared to formulated toothpaste but statistically found to be nonsignificant for gingival bleeding index and salivary S. mutans count. Though, further

Fig. 5: Dentine sample collection from spoon excavator

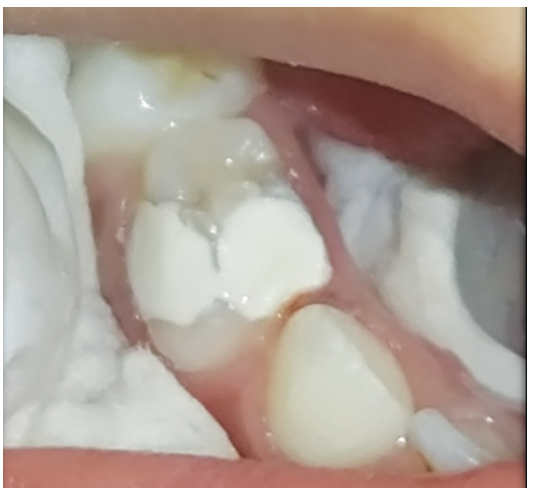


Fig. 6: GIC restoration done after cavity disinfection

Table 2: Comparison of mean of total viable count (TVC): CFU/mm among three groups: predisinfection and postdisinfection

		N	*Mean*	*Std. deviation*	*Chi-square value*	*p[a] value*	*p[b] value*
Pre	Group I	15	15.0064	5.09450	3.224	0.199,ns	NA
	Group II	15	18.7102	5.38926			
	Group III	15	16.5201	6.17109			
Post	Group I	15	9.9377	3.76702	3.704	0.033*, sig	I & II: 0.563, ns
	Group II	15	11.5729	3.18268			I & III,0.027,*, sig
	Group III	15	14.2258	5.70777			II & III: 0.229, ns

Table 3: Comparison of reduction of mean of total viable count (TVC): CFU/mm among three groups: predisinfection and postdisinfection

Pre-post	*N*	*Mean*	*Std. deviation*	*Chi-square value*	*p^a value*	*p^b value*
Group I	15	5.0687	2.09004	18.959	0.001*, sig	I & II: 0.032*, sig
Group II	15	7.1373	2.97829			I & III,0.003*, sig
Group III	15	2.2943	.88199			II & III: 0.001*, sig

Table 4: Intragroup comparision

	Pre			*Post*		
	N	*Mean*	*Std. deviation*	*Mean*	*Std. deviation*	*p-value*
Group I	15	15.0064	5.09450	9.9377	3.76702	0.001*, sig
Group II	15	18.7102	5.38926	11.5729	3.18268	0.001*, sig
Group III	15	16.5201	6.17109	14.2258	5.70777	0.052, non-sig

studies needed to be performed for munident for statistically significant results and for a better understanding of antibacterial action. Hence, the practice of using herbal dentifrice should be encouraged.

Chu Hong Hu et al. discovered that the extract of licorice roots contains a novel compound known as glycyrrhizol A, which has been proved to be a very effective antimicrobial agent against action against cariogenic bacteria.[15] Also, glycyrrhizin was its chief ingredient that inhibits the activity of mutans streptococci by acting on glucosyltransferase activity, which helps the bacteria to form biofilm from insoluble glucans.[17] Godbole E et al.[18] showed licorice had the highest antimicrobial activity than propolis at 60, 120, and 180 seconds. Differences in antimicrobial activity of licorice group at 60, 120 and 180 seconds were nonsignificant that is why in our study the average application time was taken as 2 minutes. In 2013, Jain E et al.[19] carried out a study that showed that rinsing with both ethanolic and aqueous extract decreases mutans streptococcus colony counts, elevates saliva pH, is well-accepted by children, and is nontoxic.

Normal saline is chemically inactive, which only physically flushes the debris from the cavity and the root canals. Normal saline is known to be biocompatible with soft tissues and even periapical tissues. It cannot remove the smear layer without any antimicrobial property; this can be explained by the least reduction in total viable count shown in the result.

Strengths

Previous studies have used licorice as a mouthwash, candy, lollipop, and irrigating material. Munident was only used in one study as toothpaste in the pediatric population, but not as a cavity disinfectant in patients. However, this present study is an *in vivo* study in which licorice and munident herbal agents have been used as cavity disinfectants to evaluate the antibacterial efficacy in the pediatric age group.

Limitations

Test agents were not tested against any specific strain which could provide a better understanding of the pathogens. Only total viable count was seen, specific bacteria were not isolated from the pre-excavated dentin samples. It is a single visit study; more studies with long follow-ups are needed for a detailed evaluation of antibacterial efficacy of licorice and munident.

Conclusion

Munident crushable tablets showed the maximum antimicrobial potential as compared to licorice which was statistically significant. Licorice/Mulethi and munident crushable tablets have shown an effective antibacterial efficacy in the reduction of microorganisms as compared to the control group. But, both extracts have shown a positive antimicrobial activity. Hence, both can be used as potential cavity cleaning agents. However, further scientifically sound clinical research and studies should be carried out with a larger sample size for natural antimicrobial agents with low toxicity and lack of microbial resistance in the prevention of dental caries.

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