"Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial"

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For the award of the degree of

Doctor of Philosophy

FACULTY OF DENTISTRY

(PUBLIC HEALTH DENTISTRY)

By

Dr. Sankeshwari Roopali Manohar MDS.

(Registration No: KLE/Ph.D/13-14/ DOUN13006)

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Date ***Dr Roopali Sankeshwari***

ABBREVIATIONS

AUC	-	Area Under Curve
BHI	-	Brain Heart Infusion
BSRC	-	Basic Science Research Centre
CaF	-	Calcium Fluoride
CAST	-	Caries Assessment Spectrum and Treatment
CFU/ml	-	Colony Forming Unit per millimeter
CTRI	-	Clinical Trial Registry of India
CV	-	Combination Varnish
dmft	-	decayed, missing and filled teeth
E LOC	-	External Locus of Control
ECC	-	Early Childhood Caries
ECM	-	Electrical Conductance recording
ERK	-	Ekstrand
FOTI	-	Fibre Optic Trans Illumination
FV	-	Fluoride varnish
GLP	-	Good Laboratory Practices
GRAS	-	Generally Regarded As Safe

I LOC	-	Internal Locus of Control
ICDAS	-	International Caries Detection and Assessment System
IL	-	Initial Lesions
IP	-	Indian Pharmacopia
ITT	-	Intention to treat analysis
LoC	-	Locus of Control
mg	-	Milligrams
MIC	-	Minimal Inhibitory Concentration
mm	-	millimeter
MS	-	*Mutans Streptococci*
MSA	-	Mitis Salivarus Agar
NaF	-	Sodium Fluoride
nm	-	Nanometer
NS	-	Non Significant
OHIS	-	Oral Hygiene Index Score
Pa S	-	Pascal seconds
PCR	-	Polymerase Chain Reaction
ppm	-	parts per million

PUFA	-	Pulpal involvement, Ulceration, Fistula and Abscess
QLF	-	Quantitative Light Fluorescence
RI	-	Refractive Index
RTF	-	Reduced Transport Fluid
S.mutans	-	*Streptococcus mutans*
S.Sobrinus	-	*Streptococcus sobrinus*
SECC	-	Severe Early Childhood Caries
SEM	-	Scanning Electron Microscopy
SES	-	Socio Economic Status
SiSta	-	Site and Stage
SNMC	-	Stronger Neo minophagen C
SPSS	-	Statistical Package for Social Sciences
UnviSS	-	Universal Scoring System
US FDA	-	United States, Food and Drug Administration
WHO	-	World Health Organization
µl	-	Microlitre

ABSTRACT

"Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial"

Background – Dental caries is widespread oral health problem with prevalence reaching as high as 80% in some places. Even infants and toddlers are not spared and when this younger group gets infected with caries it is called Early Childhood Caries. Modern medicine, especially fluoride is known for caries prevention since early 1980's. With rejuvenating interest in herbal medicine, the present research focused on Licorice, root of traditional medicinal plant. Literature so far is affirmative about role of licorice in oral health promotion.

Aim – To compare and evaluate effectiveness of indigenously prepared Licorice varnish, Combination varnish with fluoride varnish on initial lesions of Early childhood caries and their antibacterial activity against *S.mutans*. To assess if parental Locus of Control is associated with caries experience of their children.

Methodology – In vitro study was conducted to know antibacterial activity of Licorice root extract which was used to prepare licorice varnish and combination varnish. Varnishes were compared for their physical parameters and antibacterial activity against standard strain of *S.mutans*. Invivo study was an interventional parallel three arm study conducted to which 198 children were randomly assigned. Study lasted for 1 year with 4 varnish application at 3monthly interval .Preschool children were selected from various preschools and randomly assigned to the group. To be eligible for trial child had to have initial lesion as identified by Diagnodent and

Nyvad's index, should have dmft ranging from 3-6 and whose parents must give consent. Parents responded to Validated questionnaire regarding Demographic profile, Locus of control along with oral hygiene practices. Along with initial lesion, Nyvad's index, unstimulated salivary sample was collected, dmft and OHIS were recorded. Examination was performed at baseline and post intervention after 1 year. Study was registered at CTRI before initiation of trial. **Statistical analysis** – Data was analyzed using tests like – Chisquare, Mann whitney, Wilcosxon sign rank,Logistic regression and Intention To Treat analysis.

Results –Licorice extract had MIC of 2mg/ml. Physical parameters like pH, viscosity, shelf life, rate of evaporation and film forming ability were comparable between three varnishes. Prevalence rate of ECC was 73.21% whereas, initial lesions was 48.64% which was equally distributed among males and females. Children of Parents who had Internal LOC had 1.8times lesser chance of developing ECC.

At baseline age, gender, oral hygiene practices, initial lesions, dmft experience, *S.mutans* were equally distributed among three groups. Median Diagnodent scores changed from 4 to 2.5 in Fluoride varnish group, from 3 to 2 in both Licorice varnish and Combination varnish when baseline scores were compared with post intervention scores. Remineralisation of initial lesions was seen in 67.9%, 91.6% and 83.6% in Fluoride varnish, Licorice varnish and Combination varnish respectively which was significantly different. Mean Salivary *Streptococcus mutans* scores changed from 3.2±0.5 to 3.1±0.4 ($p>0.05$) in Fluoride varnish group, 3.29±0.8 to 3.06±0.35 ($p<0.05$) in Licorice varnish group and 3.11±0.87 to 2.81±0.48 ($p<0.05$) in Combination varnish group when baseline scores were compared with Post intervention scores. Logistic regression revealed oral hygiene and position of tooth as

significant factors for successful outcome of the intervention. There were 39 dropouts in the study. Intention to treat results was similar to per protocol results. Oral hygiene of the students improved in a significant manner from the baseline in Licorice and Combination group.

Conclusion – All the three varnishes were effective in remineralising the initial lesion however Licorice varnish was significantly better of the three, followed by Combination and Fluoride varnish. When the antibacterial activity against *S.mutans* was compared no significant result was seen with Fluoride varnish and significant difference was seen with Licorice and Combination varnish.

Thus Licorice varnish and combination varnish were significantly better than Fluoride varnish for prevention of ECC. Children whose parents had Internal LoC were more likely to be caries free.

Keywords: Licorice, Early Childhood Caries, Fluoride Varnish, Preschool children, Combination Varnish.

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"Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial"

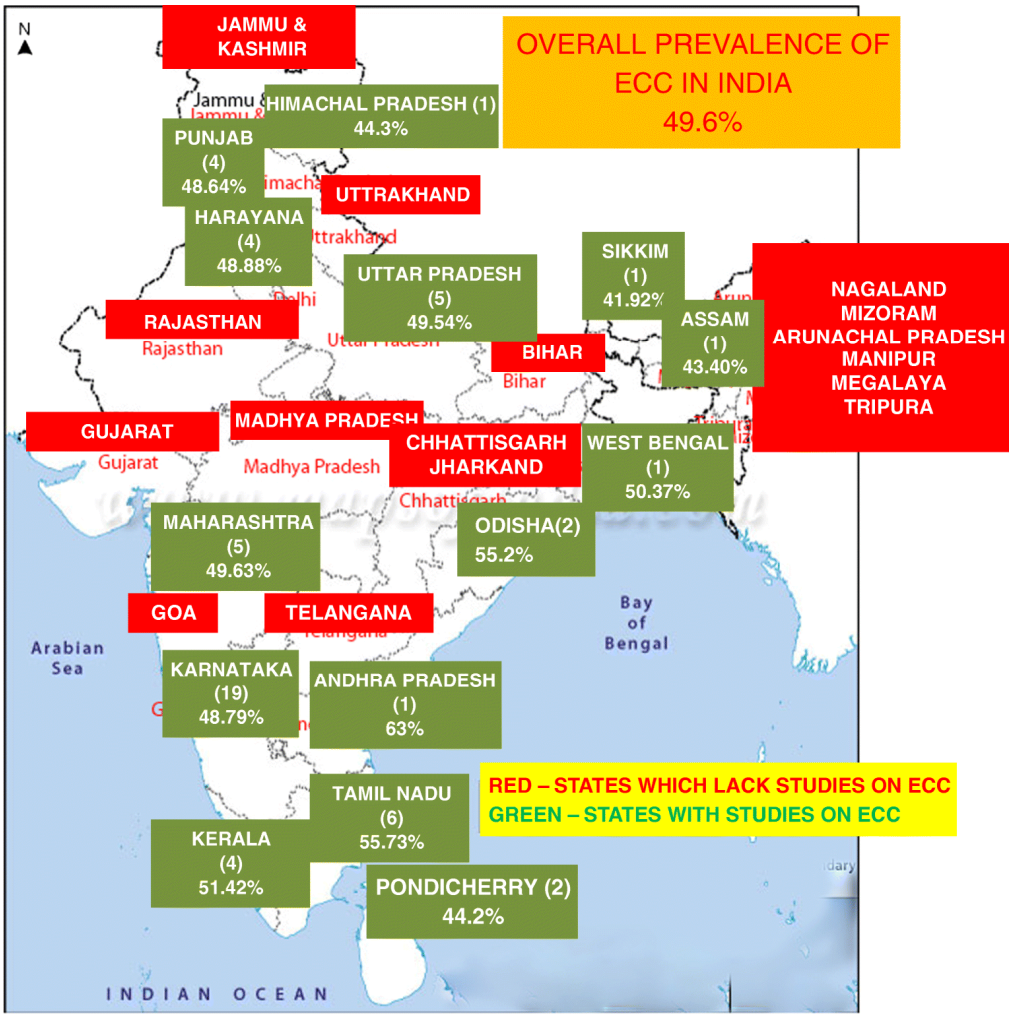
1.1Background

Dental caries is common age old oral health problem. It results due to an interaction of food substrate, tooth surface and cariogenic micro organism. When there is an interaction of micro organisms with tooth surface and food substrate for a sufficient duration of time, it paves way for occurrence of dental caries. Among various oral health problems, the most widely prevalent and widely reported is dental caries. It has no bar on any age, gender or social class and thus is prevalent throughout the world. [1-3]

Early Childhood Caries (ECC) is defined as "presence of any decayed tooth, filled tooth or tooth missing due to decay in a child less than 71 months of age. If the same condition is seen in a toddler who is less than 3 years old, then the condition is known as severe early childhood caries (SECC)".[4] Majority of the children who are socio economically backward in terms of poorly educated parents, belonging to minority groups and whose income is below poverty line are the ones who may suffer from the disease.[5-6] ECC is prevalent throughout the world in developing and developed nations.

In 2-3 years old, approximately 12 to 27% of the children are affected.[1,2,7] Prevalence rate in 4 to 6 year old, ranges from 27 - 48%.[3,8]

Indian reports on ECC prevalence -A systematic review of ECC prevalence rate in India scenario has been reported by Akila et al[9] as shown below.



Reference - Akila et al[9]

In Indian scenario prevalence of ECC has been 27% to 48%.[10-12] Prevalence rate of Belagavi was 63%. [13] Over all prevalence rate of India is 49.6% [9]. Most of the caries which has been reported is all related to untreated disease. ECC can have catastrophic effect on growth and development of a child as well as quality of life when it is left untreated.[14-15] ECC negatively affects speech, eating habits, sleep and risk of admission to hospitals with consequent absenteeism in school, adding to the

inability of the child to learn.[16] Due to failure of steps taken to prevent disease, the disease progresses to cavitated lesion which if left untreated rapidly spreads to other teeth throughout the mouth. The practical problems which arise during management of ECC are that, there are multiple teeth which needs attention of the specialist and since compliance is limited in toddlers, many a times general anesthesia is required, nevertheless to say, hospital stay becomes mandatory and thus the hospital bills escalates all of which adds to the economic burden.[17]

Etiology of ECC It is well recognized and is frequently related to poor feeding habits with excessive exposure to sugars, microbiological risk factors like *Streptococcus mutans* and *Streptococcus sobrinus*, socio cultural attitudes, failure in maintaining oral hygiene and innate features of tooth morphology.[18]

Clinical presentation – Human oral cavity is host to number of micro organisms and not all are pathogenic. However when the population of cariogenic flora increases, a person per se becomes more prone to develop dental caries. It is due to the acid producing capacity of these cariogenic micro organisms. Sucrose of the oral cavity is converted to lactic acid by these micro organisms which leads to reduction in salivary pH and thus posing a threat of demineralization. As soon as salivary pH returns to normal, demineralization stops and if saliva is saturated with inorganic contents like Potasstium, Fluoride and Calcium, the remineralization process begins. When demineralization outweighs remineralization, it leads to initiation of dental caries. Clinically it begins with visible signs in enamel with subsurface lesions which can be viewed as white spot located towards gingival margins.[18] These initial lesions cavitate within a short span of time which is a unique feature attributed to ECC.

ECC differs from other dental caries mainly due to two reasons- It occurs in young individuals in whom immunity is not well established and it occurs in newly erupted teeth which have not remineralized completely [19] and hence toddlers are prone to rapid progression of the disease. Since treatment and management modalities are expensive options, we should focus our attention in the preventive aspect of ECC.

Prevention of ECC – For preventing ECC, we need to identify child at risk and the tooth which is prone for ECC. Diagnosing initial lesion is a major step which can be taken towards prevention of ECC. Today technology has enabled us to identify initial lesion or white spot lesion. Initial lesion can be defined as a lesion which has not entered the stage of cavitation.

There are various options available to detect initial lesion such as – visual detection, tactile detection, caries indices, Radiography, Transillumination, Laser fluorescence, Light induced fluorescence, electrical characteristics and photothermal radiometry.[20] Every technique has its own pros and cons. Amongst all these techniques a widely used objective detection method is Diagnodent.

Diagnodent works on laser fluorescence and aids in detection of dental caries even in initial stage of non cavitation. Although it has good sensitivity, certain precautions need to be taken to overcome the chances of getting high false positive values. [21]

Fluoride varnish – Fluoride varnish has played a pivotal role in remineralization and is considered as standard of comparison for prevention of caries. Cochrane database of past three decades has unanimously concluded fluoride varnish to be the best preventive tool available for the prevention of ECC.[22-23] Fluoride works in multiple

ways – it increases resistance of tooth, helps in remineralization of demineralized areas as well as inhibits *S.mutans*.

Of all the topical fluorides available only 2.26% fluoride varnish is safer regimen in children who study in preschools.[24] Applying Varnish is easy procedure and it works even in moist media. Since varnish is highly concentrated topical fluoride, very small quantity of varnish is required to cover the entire dentition.

Chances of toxicity are reduced when proper protocols are followed; hence it is the modality of choice for topical fluoride application in very young children. However it does have its drawbacks like it is an expensive preventive modality, requires a professional for its application, toxicity can occur if product not handled with care. The current study was initiated with an intention to find an alternative to fluoride varnish which could overcome these drawbacks.

With this intention we searched the literature of Ayurveda and came across a frequently used medicinal plant called **Licorice- *Glycyrrhizia glabra*.**

Literature review suggested the use of licorice for many general ailments. It has many beneficial effects on general health [25] anticariogenic property of licorice have been known for more than 3 decades now.

This anticariogenicity has been basically attributed to its capacity to inhibit the *Streptococcus mutan's* adherence with tooth surface. This is achieved by its direct action on glucosyltransferase which is a key enzyme required for biofilm formation.[26] Thus it would be interesting to know if Licorice could be used for preventing ECC.

Apart from diet, cariogenic bacteria and oral hygiene, there are some psychosocial factors which have been linked with ECC and one such contributing factor is parental psychology towards caries prevalence or caries prevention. Among various theories which have been reviewed, Locus Of Control(LOC) theory has been widely reported. This theory tries to measure degree to which an individual believes himself or some external person to be responsible for his health.

There are three variants of LOC- Internal LOC , people who believe that they themselves are the ultimate people responsible for their health and they can take measures to prevent disease, External LOC – people who believe that professionals, doctors are responsible for their health and they have hardly any role to play whereas Chance LOC group believes that no matter what they do, disease or health is all predestined and cannot be altered.[27]

Thus there is need to assess if parental LOC has any association with ECC either in its prevalence or disease progression in their young ones.

1.2 REVIEW OF LITERATURE

In 1962 Dr.Ellias Fass published the pioneering work on caries in infancy which was called as “nursing bottle mouth”. Since that first description nursing bottle mouth has been called by various names like baby bottle tooth decay. In 1978, attention was focused on typical form of caries seen in younger children by the joint committee of American academy of Paediatric dentists and American academy of Paediatrics by coining the term “Nursing bottle caries.” The term “early childhood caries” was recommended at an international conference of Centers for disease control in 1994, as the specialists in the field were of the opinion that the association of only the baby bottle usage and occurrence of ECC did not give a true depiction of the disease etiology. Frequent exposure to cariogenic foods along with early habitation of caries promoting bacteria also played major role in the etiology of the disease.[28]

Classification

Dental caries results due to the existence of cariogenic micro organisms which are very adherent to the tooth structure. They utilize tooth space to convert oral carbohydrates to lactic acid which sets the ideal environmental conditions conducive for initiation of tooth decay.[29] ECC is the “presence of one or more decayed (non cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child who is less than six year old. In child younger than 3 years old, the condition is called severe ECC. For children aged 3 to 5 years any dmft score greater than 4 in 3year old, dmft score greater than 6 in 5year old constitutes Severe Early Childhood Caries.[4] A systematic review on ECC was reported by Vadiakas. [30]

Since there was no consensus among researchers on the definition of ECC, the existing literature was searched particularly for nomenclature, disease definition, prevalence and various risk factors thought to be related to the disease.

Today ECC is pertinently used to define dental caries experience in preschool children where as in earlier days it was thought due to faulty feeding practices per se. One of the pre requisite for occurrence of ECC is early establishment of bacteria specifically *Streptococcus mutans.* Bacteria could be acquired both by horizontal transmission and vertical transmission to the infant. This coupled with excessive exposure to cariogenic diet predisposes an individual for ECC. Research so far suggests that children of minority groups, socio economically backward class with less than optimum parental lifestyle practices are at more risk of acquiring the disease. The study pointed out the fact that there is dearth of scientifically sound, meticulously conducted studies in the literature which explore the interaction of bacteria, disease progression, quality of saliva and other socio behavioural patterns which could be the determinants of the disease.[30]

Stages of dental caries – Dental caries begins with a locally demineralized white spot lesion which progresses to cavitated lesion and if left untreated, leads to complete destruction of hard structure. Caries lesions develop within both paediatric and adult dentition.

Caries occurs in areas of tooth, where the plaque builds up without any interference from plaque control measures. Hence the areas which are more prone to develop plaque and may go unnoticed during routine oral hygiene measures are the sites, most susceptible for caries. If plaque buildup is left undisturbed for 14 days, a whitish opaque area develops which is evident after air drying the tooth surface. The

exact reason for the occurrence of white spot lesion with intact outer surface is not clearly understood, but it is presumed that salivary stateherin which helps in reducing demineralization and since these molecules are of larger size, they are unable to penetrate the tooth surface thus giving protection only to the outer enamel layer.[31]

Identification of caries lesion- Gomez [32] conducted a systematic review on more than 124 articles on performance of detection techniques for incipient lesions. Of the total 124 articles which were reviewed, 42 were included which described more than 85 different methods of identifying non cavitated lesions. However among the methods evaluated were – Visual, caries lesion activity assessments, Radiographic, Fibre Optic Transillumination (FOTI), Laser Fluorescence, Electrical Conductance and as a benchmark comparator "Histological assessment" was considered for in-vitro studies or clinical/ visual validation of in-vivo studies. The strength of the evidence for Diagnodent was rated as poor as the mean score of the studies on a scale from 0-100 obtained was 53.The rating was considered as poor as the standard error values ranged from 0.16 to 0.96.

Monitoring of carious lesion

Dirks[33] has reported a classic longitudinal study where he categorized buccal surfaces of premolars in 8 year old Dutch children into visible sound and white spot noncavitated and cavitated lesions. Surface changes in enamel were observed in different types of caries – Pit and fissure caries, proximal caries and free smooth surface caries.

There was rapid progression of caries in pits and fissure lesions; however 26-48% of the proximal surface caries were arrested, remineralized. The cervical lesions

in free smooth surface caries showed white spot opaque spots. Authors concluded that If no visible changes occur in 1and half year s time, then the lesion was less likely to progress to cavitations. However few lesions did progress to cavitations very rapidly. Remineralisation and recrystallization or both would have contributed to the arrest of white spot lesions.

Carvalho et al [34] divided caries lesions detected in different anatomical sites of occlusal surfaces in erupting teeth. Active lesions have an opaque enamel which has a dull whitish surface; Opaque area with a Shiny appearance with various grades of brownish discolouration (arrested lesion). Dull-whitish enamel with localised surface destruction (active lesion with cavitation). If no changes in translucency were noted, the site was classified as sound.

Ekstrand et al.[35] used the ERK system and found that under clinical conditions, it could predict the depth of lesions in third molars scheduled for extraction on Danish military conscripts and reliably assess the activity of those lesions. The non-cavitated lesions (scores 1 and 2) were separated into those which were whitish and those which were brownish, and the gold standard used for activity assessment was the pH in the lesion assessed after extraction. Whitish and cavitated lesions were most commonly classified as active, while the brownish non-cavitated lesions were usually seen in arrested caries. Thus, this study supported the assertion that surfaces with opaque enamel and a dull-whitish surface were active lesions and those lesions with shiny, brownish surfaces were arrested lesions, but irrespective of their activity status, they were equally deep, either confined to the enamel, or if in dentine restricted to the outer third of the tooth. This formed basis of ICDAS criteria.

White spot lesion- Kidd and Fejerskov[36] used this term to explain the process of caries and also to name the end result of interaction of the microbes with tooth structure and food debris. The dental tissues – enamel, dentin and cementum are the important oral fixed solid surfaces to which micro organisms attach through the pellicle. Interaction among primary and secondary invaders among themselves play a major role for the decayed lesion to progress. The areas of tooth where the plaque gets stagnated and continues to grow undisturbed, lays the foundation for the dental caries process to begin and all those areas where the plaque biofilm stagnates and remains undisturbed are prone for dental caries. When the biofilm is removed and oral hygiene is initiated the lesion may get arrested. If the plaque continues to grow then the lesion becomes active carious lesion. The diagnosis of caries lesion includes identifying the lesion, predicting its depth so that appropriate treatment can be carried out. When the plaque remains undisturbed for more than 14 days the enamel changes become visible clinically after the tooth is air dried. After almost 28days the changes in enamel surface can be appreciated before air drying the tooth, the lesion being opaque with a matte surface. Thus the surface the lesion formation at the very initial phase of the disease process by losing its minerals. The porosity of the subsurface lesion can be turned to some advantage by the researcher. Before examining the tooth it has to be cleaned. With the help of the vision and three way syringes the lesion's depth can be assessed. The lesion which appears only on air drying is probably present in the outer enamel; where as the one which appears on wet tooth has reached dentin through enamel. The reason as to why white spot or initial lesion appears opaque is an optical phenomenon and can be attributed to differences in refractive indices of enamel, air and water. Refractive index of enamel is 1.52. In the subsurface lesion, enamel has pores and the space in these pores is occupied by water which has refractive index of

1.33.The difference in refractive index between enamel and water affects the light scattering and thus the lesion looks opaque. When the surface is dried air replaces water. Air has refractive index of 1. Thus now the refractive index of air greatly differs from that of enamel compared to difference between enamel and water and thus the lesion becomes much more obvious. It is to be noted that the dentist should be very careful in probing such a lesion as jabbing a sharp instrument into the lesion can lead to break in the thin surface of lesion leading to cavitations.

Diagnosis of early carious stage or initial lesion (Manton 2013) [20]

Manton conducted a research to summarize various ways in which early or initial carious lesions could be detected. He has pointed on

- Visual detection – Before beginning the examination, the tooth has to be thoroughly cleaned and dried, supplemented with adequate lighting[37]. The initial lesion can be defined as a lesion which has a surface layer intact above the subsurface lesion and hence is amenable to be treated by minimal invasive dentistry procedures.[38]
- Tactile detection- This a 100 year old practice of identifying a dental caries.

But since past two decades this method has been restricted to evaluate fissural and smooth surface caries. Research states that there is minimal information obtained by tactile examination whereas conversely there is every possibility that the instrument may cause iatrogenic injury.[39]

- Indices to measure lesion activity caries indices- various indices have been used to record initial carious lesions the important ones being Nyvad's index, ICDAS index, PUFA, CAST, UNIVISS and Ekstrand.

- Nyvad's index [40] - Nyvad and co workers described a set of scores for diagnosis of carious lesions both cavitated and non cavitated. They also assessed the reliability as well as the reproducibility of these scores in a population with high caries experience. They defined 10 diagnostic codes as part of the index as shown below.

Score	Criteria
0	Sound
1	Active (Intact)
2	Active (Surface discontinuity)
3	Active (Cavity)
4	Inactive (Surface intact)
5	Inactive (surface discontinuity)
6	Inactive (cavity)
7	Filling
8	Filling with active caries
9	Filling with inactive caries

ICDAS (International Caries Detection and Assessment System) It was proposed by Pitts. [41] This particular index helps to measure carious lesions which are active and inactive.

ICDAS I and ICDAS II criteria have included the research findings of Ekstrand. The criteria of ICDAS and its scorings are presented in table I. It includes two components- detection of caries and activity of the caries

Table I. Criteria for caries detection using visual methods (ICDAS II) and SiSta classification from Lasfargues Colon 2010 [38].

ICDAS Code	Criteria for visual lesion detection	Degree of severity of lesion	SiSta Stage	Therapeutic options
0	Sound surface			Not necessary
1	Earliest optical change, visible on drying enamel	Demineralisation in outer third of enamel	0	Minimal intervention, non invasive care, remineralisation or sealant
2	Clear enamel change,white or brown blemishes visible without drying	Demineralising reaching the inner third of enamel possibly ADJ		
3	Localized break in enamel	Demineralisation in outer third of dentine	1 and 2	Minimal intervention , adhesive ultra conservative restoration
4	Underline dark shadow from dentin with or without localized enamel breakdown	Demineralisation of middle third of dentine ,no weakening of dental crown structure		
5	Distinct cavity with visible dentin	Demineralisation of middle third of dentine , weakening of dental crown structure	3 and 4	Operative dental care, functional crown restoration with or without cusp coverage
6	Extensive distinct cavity with visible dentin	Demineralisation of inner third of dentine ,undermine of cusp structure and support		

ICDAS II

Dikmen [42] has reported about ICDAS II criteria and its development. He has also compared Nyvad's index with ICDAS II criteria.

Difference between ICDAS I and ICDAS II.

	ICDAS I	ICDAS II
Primary coronal caries	One digit system	It has 2 digit system First one is related to restoration of teeth and is in the range of 0 to 9 Second digit ranges from 0 to 6 which is used for coding caries
Caries activity	Does not include caries activity of the lesion	Includes caries activity of the lesion

Difference between Nyvad's index with ICDAS II criteria.

Nyvad criteria	ICDAS II criteria
A single score is mentioned for caries lesion activity and severity	Two separate scores are used one for severity of the lesion and other for caries activity assement
It is used on teeth which are covered with plaque	Tooth is cleaned before applying the criteria
A sharp probe is used for caries detection	Ball ended probe is recommended.

ICDAS criteria consists of two categories: coronal primary caries and root caries.

Coding for caries detection and coding for caries activity needs to be marked separately. In ICDAS II for coding the primary caries, two digit codes are used.The first digit describes codes from 0-9 which are used for restoring teeth and second digit describes caries which is coded from 0-6. Details of these scores are shown below

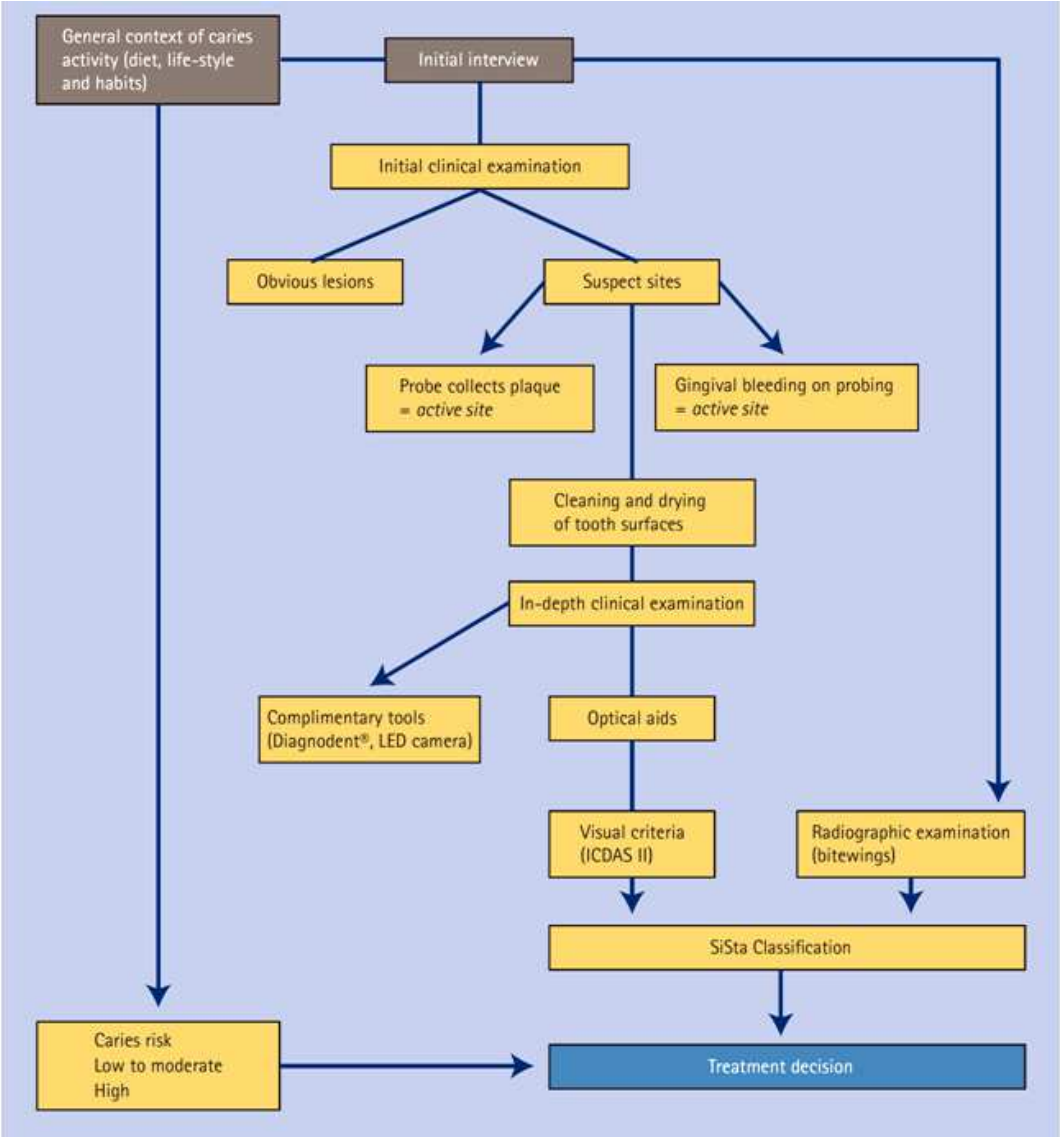
0	Surface not restored or sealed	5	Stainless steel crown
1	Sealant, partial	6	Porcelain or gold or PFM crown or veneer
2	Sealant, full	7	Lost or broken restoration
3	Tooth colored restoration	8	Temporary restoration
4	Amalgam restoration	9	Used for the following conditions ⇨ **96:** Tooth surface cannot be examined **97:** Tooth missing because of caries **98:** Tooth missing for reasons other than caries **99:** Unerupted

Table 2. *Description of the second digit that is used for coding the coronal primary caries (18, 21)*

0	Sound
1	First visual change in enamel
2	Distinct visual change in enamel
3	Localized enamel breakdown (without clinical visual signs of dentinal involvement)
4	Underlying dark shadow from dentin
5	Distinct cavity with visible dentin
6	Extensive distinct cavity with visible dentin

Reference (Dikmen 2015) [42]

Diagnosis of caries in a systematic method has 3 stages- Lesion detection, assessment of its depth and its activity level as described by N. B. Pitts [43] Before undertaking an examination, the practitioner will have noted the general context of caries activity.However due attention must be given to age groups,lifestyle habits,long term medication, brushing and oral hygiene habits apart from use of fluorides and other nutritional information.



Reference - Guerrieri A et al[38]

Validation of ICDAS II in primary teeth was conducted by Shoaib et al [44] where the authors reported good inter examiner and intra examiner reliability for assessing occlusal and approximal tooth surfaces in primary dentition.

Radiography – It is the oldest investigative aid dating back to more than 100 years, being used for diagnosis of dental caries. However when the dental caries is in its earliest stage, radiography may be of little value. Radiography has low specificity especially with occlusal caries as is reported by Ricketts. [45]

Hintze et al(46) conducted a study comparing the FOTI, Radiography and visual examination for detection of proximal lesions in young adults.. The sensitivities of visual examination for dental caries ranged from 0.12-0.50; for FOTI it ranged from 0.00-0.08; and for Radiography it ranged from 0.56-0.69.Specificity was found to excellent among all examiners for all the methods. However reliablility was least with FOTI, hence it was concluded that combination of two to three methods should be used for accurate diagnosis of decayed teeth rather than depending on only one technique.

Along with radiographic techniques and other investigative aids,even the experience of the clinician matter when it comes to diagnosing initial lesions.

Diniz et al [47] compared the accuracy in diagnosing dental caries from stage D1 to D3 among final year dental students and dentists with 5-7 years of experience. The evaluations were compared with gold standard which was obtained by sectioning of the tooth and by histological preparation. Dentists performed better than students in every parameter which was assessed and this difference was observed to be significant.

Transillumination- it relates to positioning of light source opposite to the side tooth either buccal and lingual and usually used technique is FOTI. It works on the principle that a normal tooth has greater index of light transimisson than a decayed tooth. It means that whenever a tooth is decayed, photons from the light source get scattered and are captured on the image.[48] However it has a major disadvantage that the imaging of the tooth is not available. This drawback was overcome with the development of digital FOTI. [49]

Fluorescent techniques

Laser fluorescence- Diagnodent

Detection and diagnosing of initial lesions with visual examination although has high specificity but suffers from low sensitivity. The literature review suggests about draw backs of other methods like tactile sensation- it can transfer micro organisms from one site to another apart from causing iatrogenic injury of the tooth, where there was every possibility of remineralization. Radiography dose pose the risk of exposure to the radiation. Hence a diagnostic tool 'Diagnodent' was developed by Kavo (KaVo,Bibeach Germany) introduced in 1998, was intended as a method by initial lesions can be quantified accurately. It works on quantitative laser induced fluorescence and works on the principle that decayed dental lesions show elevated fluorescent level when they are studied under red light. [50]

Diagnodent has been studies widely in the literature as the instrument is economical and is easily available. It is a non invasive method, very handy to use, gives display of the current lesion condition and hence can aid to monitor lesion progression or regression. It works on red light fluorescence of 655nm which is emitted by 1 mm tip. This is carried by two intra oral tips one releases the light and other collects the consequential fluorescence. A fibre optic bundle consisting of two components transmits laser beam into the tooth while the second bundle carries information of the fluorescence of the tooth to the sensor which quantifies and gives the display reading.

Advantages of Diagnodent are-

- The tip has small diameter of 1mm. Since the tip is small, various anles of the tooth can be measured.
- Extensive research has been done on Diagnodent and reports are available which correlate Diagnodent reading with histological reading.
- Diagnodent shows the result in numbers and gives information of the current highest reading and overall peak value of the examination.

Bader et al[51] reported systematic review and assessed reliability of the Diagnodent when used as a diagnostic instrument. The author reviewed more than 115 articles. Both in-vivo and in-vitro studies were considered. Compared to visual assessment method, higher sensitivity was reported for Diagnodent for detection of caries. However it has lower specificity value for detection of caries.

Review had more in vitro studies than in vivo studies, thus applicability of the results to in vivo situation becomes questionable. Though Diagnodent is more sensitive than visual examination alone, it does have some limitations- it carries a risk of high positive values. Thus it becomes crucial that

- Proper angulations of the tip and tooth surface be achieved before examining the tooth.
- Surface to be examined should be clean and dry, and
- The instrument must be calibrated as per manufacturer instructions.

Some reports claim that Diagnodent do not measure intrinsic changes in enamel but they measure only the degree of bacterial activity. Bacteria produce porphyrins which illustrate the level of fluorescence which is greater than the surrounding tooth structure and can give false positive reading.[52,48]

Diagnodent device and Diagnodent pen were compared with each other for their abilities in quantifying the smooth surface caries in an in-vitro study where authors concluded that Diagnodent device and Diagnodent pen, both are similar as they have excellent agreement. However a study reported that Diagnodent device and Diagnodent pen did not give consistent results and hence the authors cautioned that it should be used as an accessory tool only in clinical practice.[53] Diagnodent pen showed higher values of fluorescent and the cut off values published for Diagnodent device cannot be applied for Diagnodent pen. Kavvadia et al [54] compared white spot lesions which were in very initial stages as well as in moderate stages, using Diagnodent and Vistaproof. However both performed better for moderate caries than the milder caries.

In another study examiners were compared for identifying early carious lesions using ICDAS and two quantitative laser fluorescence devices (QLF) Diagnodent and Sporolife. Training and calibration of examiners was completed and their performances were assessed. They performed well with the ICDAS index. However Diagnodent and Sporolife did not contribute to better diagnosis of early enamel lesions.[55]

Visible light induced fluorescence- QLF Qunatitative light induced fluorescence systems include Vistaproof and Soprolife. Their working principle is similar to Diagnodent except that their wavelength of excitation is different. It uses a light of

405nm and a specific software that analyses the fluorescence given out by the tissues.[50] They work on the difference value of auto fluorescence between normal enamel, demineralized enamel and dentine. Demineralized enamel appears darker than the adjacent sound tooth structure. It uses a blue light for imaging objects. Decayed dentine appears red. Unlike Diagnodent it has an inbuilt camera and hence can record images which are of value in longitudinal studies. It is self calibrating and can capture two dimensional image of tooth surface along with its approximate score of decay. This particular software shows the area of the tooth that produces fluorescence which ranges from green with 510nm wavelength to 680nm wavelength of red. The outcome value ranges from 0-3, with higher values relating to severe lesions . Severity is calculated as the ratio between intensities of red to green colour fluorescence. When the ratio of red to green fluorescence was higher in comparison to sound tooth, the software diagnosed it as caries lesion.[56] Better sensitivity was observed with Fluoroscence camera, Diagnodent pen and ICDAS II, however better specificity was observed with bitewing and Laser fluorescence. The best performance was shown by the combination of Bitewing and ICDAS II for identifying decay of the occlusal surface.

Detection system based on measurement of electrical current

All materials have some electrical characteristics and these properties change as the constituent of the substance changes. Similarly the electrical characteristics of dental structure change as the mineral content changes. The concentration of fluids and electrolytes are of utmost importance when substances are assessed for their conductivity.[35] To simplify the concept, caries results in increased porosity of the enamel as well as dentin. So when the pores increase in frequency, they have higher

fluid content compared to sound tooth structure. Electrical impedance or electrical resistance detects this difference.[48]

Electronic caries monitor ECM

It works on alternating current which tries to assess the total resistance offered by the tooth tissue. When measuring ECM properties of a tooth, its probe is held directly on the tooth for duration of 5 seconds, during which compressed air gets ejected from the probe's tip. The tip collects information about drying profile that provides useful information related to carious lesion. However there are some concerns with this technique

- Does the tip measure pore volume?
- If the lesion is remineralized, then how does it measure the pore depth?
- Can morphological complexity be of importance in measurement of conductivity?

Carie scan is an instrument which can scan fluctuations in impedance/resistance values of a tissue and can estimate probable mineral content of tooth. However there are some factors which can influence this reading like hydration status of the tooth, thickness of the tissue to be assessed and temperature of the tissue.[20]

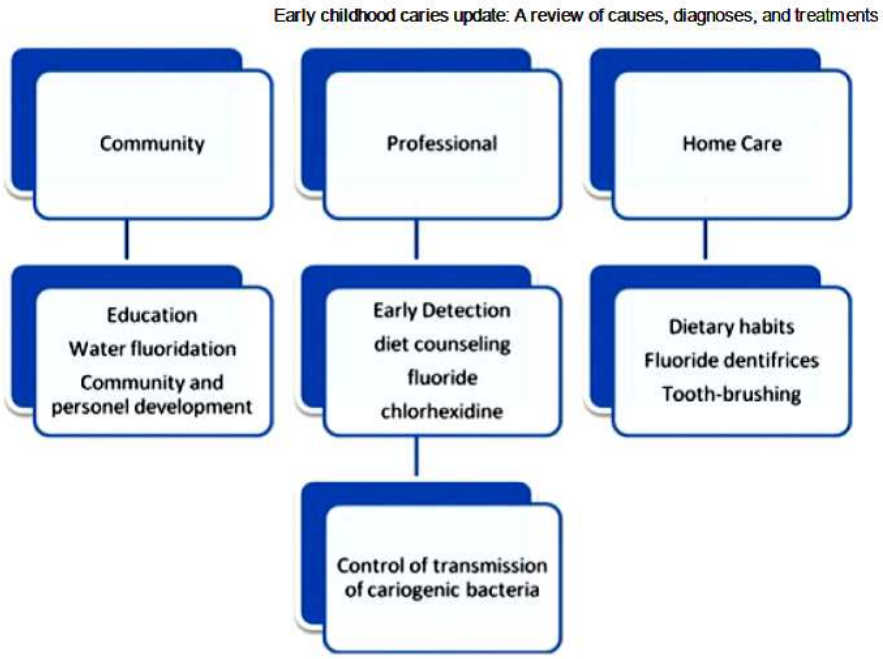
Photothermal radiometery It is a new system released recently which uses laser based photothermal radiometry which detects luminescence differences and also changes in temperature to quantify mineralization changes. Images are stored in a software and a number is generated which explains the features of the dental caries lesion.

Reproducibility

Obtaining reproducibility among examiners can be an arduous task as every practitioner tends to evolve his expertise based on individual experiences. The time basically number of years spent by examiner in patient care contributes to the experience of the examiner. Examiners with more experience tend to have more sensitivity, specificity and better reproducibility than those with less experience.[20]

Prevention of ECC

Prevention of disease spares a person from its suffering and also saves time and money which would be otherwise spent on its treatment. Prevention of a disease is more of a moral and ethical obligation for health professionals and should be more prioritized than the treatment aspect. ECC can be prevented in three ways as is depicted in the figure below



Reference Colak et al. 2013.[17]

1. **Preventing transmission of bacteria especially *S.mutans* from mother to baby** As part of primordial prevention we can prevent new born babies from the vertical transmission of *Streptococcus mutans* from its mother. This is possible by educating mother about various aspects which include the following measures.

- The mother has to first reduce the bacterial count in her oral cavity.

  This reduces the chances of getting bacteria getting transferred to the off spring. Bacterial load was successfully reduced by using chlorhexidine gluconate either as mouth rinse, gel, dentifrices or varnish.[57]

- Reduce the transmission of bacteria that cause the tooth decay. Reduce the sharing of spoons, foods and drinks to reduce the vertical transfer of bacteria to baby. Mothers should not share tooth brushes and not lick the pacifiers before putting it in baby's mouth.[17]

2. **Oral health education** – We have a better understanding of dental caries today and we acknowledge the fact that sugar is an essential element for caries initiation. Therefore professionals should spend some time in counseling parents and advising them to modify dietary habits.[58] Dentist should team up with pediatricians, nurses, physicians and together they can work as a team to reduce the burden of this disease. Paediatric dentists of American academy have given recommendation on anticipatory guidance, bottle feeding habits for prevention of decay in early childhood. [59]
3. **Fluoride** The use of fluorides for its preventive role began in 19th century and today it is considered as gold standard for preventing tooth decay. Fluoride is present in wide variety of fruits, vegetables, soils and natural resources.

Fluoride is available in dental products like fluoride mouth rinse, fluoride gel, and paste for prevention of tooth decay.

Fluoride varnish were initially introduced in 1988 in USA to reduce sensitivity around exposed roots, and after crown cutting procedures but were used as" caries preventive agent" as an off label product. Initially it was not approved by US FDA for caries prevention, but now is being recommended world wide as an ideal prophylactic measure especially for caries prone young patient's especially preschool children.[60] The popularity of the product is due to the ease and safety of the application procedure. It can be applied even in moist media and varnish sets even when saliva is present and forms a thin film on the tooth surface. The treatment of entire dentition typically requires only 0.3 to 0.5ml of varnish which contains 0.3 to 0.5mg of fluoride. Dosage of varnish(5% sodium fluoride) prescribed for primary dentition is 0.25ml,for mixed dentition is 0.40 ml and 0.5ml for permanent dentition.[61] For infants 0.10ml is required. The application steps involve cleaning the tooth surface, applying varnish using applicator tip and drying. It forms a thin yellow colored film on drying. Varnish is retained on teeth for 24-48 hrs during which time fluoride is released for reaction with the enamel.

- The first fluoride varnish Duraphat contained 50mg NaF/ml or a concentration of 2.2% F ion. It was developed in Germany by Schmidt. A varnish containing silane fluoride in a polyurethane polymer was introduced in 1970, Fluor protector, Schann Liechtenstein.

- In 1990's two other fluoride varnishes have been discussed in literature: Bifluorid 12® (Voco Chemie GmbH, Cuxhafen, Germany) (containing

2.71% wt as sodium fluoride and 2.92 as calcium fluoride) (Attin et al, 1995) and Carex® (containing 1.80% of sodium fluoride) (Haugejorden and Nord, 1991). A review was reported on Fluoride varnish by (Strohmenger & Brambilla 2001) [62] for prevention of dental caries.

Action of fluoride varnish

Fluoride acts in more than one way to give protection against caries. The fluoride ion acts on Enolase enzyme, interferes with metabolism of anaerobic cariogenic bacteria. This leads to reduction in the count of cariogenic bacteria and reduces the pathogenicity of dental biofilm.[63] Initially systemic fluoride was considered as the best method to reduce caries. In this process, fluoride ion gets incorporated with the hard tissue of tooth and strengthens crystalline structure. It also reduces carbonated apatite and magnesium whitlockite in enamel, which have lower critical pH than enamel. However research in 1990's pointed that this method was not a significant method for caries prevention.[64, 65]

Today most of the researchers have consensus that presence of small quantity of fluoride in micro environment of the tooth for prolonged duration can enhance uptake of fluoride ion during remineralization phases and prevents loss of minerals during demineralization phases.[62] When varnish is applied to tooth, leads to uptake of firmly bound fluoride by enamel and forms crystalline fluoroapatite. When Topical fluorides interact with saliva calcium fluoride is formed. This is generally thought as not so desirable action, as it easily dissolves away after application. But studies in 1980's have reaffirmed that calcium fluoride is stabilized by pellicle proteins with secondary phosphates at neutral pH. Whenever salivary pH nears critical pH Fluoride is released from CaF2 thus acting as a reservoir of fluoride and acts as an important mechanism for caries preventive action of fluorides [66].

FLUORIDE 'S EFFECT ON REMINERALIZATION AND *S.MUTANS*

So far 19 fluoride varnish reviews, [67] one systematic review [68] and 3 meta analyses [69-71] have been published in English language. Most of them reported fluoride varnish and its effect on permanent teeth of 10-15 year old school children. They concluded that fluoride had protective effect against dental caries; however no consensus was reached on research pertaining to primary teeth. Weintraub reported that addition of fluoride varnish to the regimen along with parental counseling was effective in bringing down the incidence of new caries lesion. Odds ratio reported for fluoride varnish was 2.2 [71]

When comparison was done to evaluate whether varnish is better or sealant, authors could not reach any conclusion due to low quality evidence obtained from research in spite of the fact that authors tried to update a systematic review.[72, 73] Lenzi et al [74] conducted a meta analysis to analyze the effectiveness of topical fluorides- gels and varnishes and their potential in remineralizning the white spot lesions both in pediatric and permanent dentition. They concluded that white spot lesions were effectively remineralized by professionally applied fluoride varnish; however no consensus was reached for fluoride gel.

Gao S et al [22] have reported a systematic review along with meta analysis which describes how topical fluorides are effective in remineralising enamel lesions and arresting dentine caries. Authors screened 2177 papers and found 17 clinical trials which met their inclusion criteria. Ten of these studies tested sodium fluoride gel, topical fluoride like silver diamine fluoride, silicon tetrafluoride's remineralising effect on enamel carious lesions while seven reported the role of silver diamine fluoride in combating the progression of dentine caries. Meta analysis performed on 4

sodium fluoride varnish studies corroborated that remineralisation of enamel lesions was effectively obtained by 5% sodium fluoride, whereas dentine caries was arrested by use of 38% silver diamine fluoride and thus it can help in arresting dentine caries. However silver diamine is associated with staining of the teeth which can be prevented by using nano silver fluoride an innovative product. However it is noteworthy that only one study in the review assessed dimension change in early enamel lesion.[75]

An invitro study was conducted by Lippert [76] where the author compared 10 different commercially available Acclean, Butler, Cavity shield, Enamel Pro, MI Varnish, Nupro, Patterson, Profluorid, Vanish and Vella varnishes. Fluoride released in saliva and under other acidic conditions were compared and it was noted that fluoride released from varnishes differed among various brands. Fluoride release from varnishes was tested in various conditions and significantly more fluoride was released when acidic pH was maintained. This research can help the practitioner in framing guidelines about avoiding acidic food and drink for patients who have been treated with varnish.

Systematic review conducted by Carvalho [77] in which more than 513 articles were screened and a total of 8 articles were selected. However the studies had poor methodological quality and differed with respect to caries experience, intervention used, dietary sources of fluoride and the frequency with which varnish was applied. Caries incidence in test groups was as low as 0.30 and as high as 1.64 and the preventive fractions of the varnish had minimal value of 5% and maximal value of 63%.

Safety of varnish application

Duraphat varnish is amongst the most concentrated product available which contains almost double the quantity of fluoride present in other topical products like solutions and gels. However reports pertaining to any health hazard arising due to dental varnish have not been reported so far. Robers and Longhurst [78] stated that the mean amount of fluoride varnish used in preschool children had a lower limit of 0.7 and the upper limit of 14.5mg which were within the safety limits. Literature does not have any report of which mentions about toxicity due to use of dental varnish even from European countries where it is used frequently. Only possibility of side effect is contact dermatitis which is related to colophony component of dental varnish, though this is very rare.[79]

Role of *Streptococcus mutans* in ECC

Oral cavity hosts near about 700 species of bacteria, out of which half of them are not yet cultivated.[80] Today it is very well recognized that there are multiple factors responsible for the occurrence of dental caries amongst which micro organisms play a lead role in its initiation.

Among all the species which have been identified and cultivated, ECC is commonly associated with aciduric and acidogenic species known as species of mutans Streptococci (MS)- *S.mutans* and *S.sobrinus.*[81]

Taxonomy of *Streptococci* Amongst all bacterial species, the important one's in oral cavity is oral streptococci. Majority of them are alpha hemolytic on blood agar called as viridians group of streptococci. They are now clustered into four groups as shown in the table below [82]

Species of oral streptococci isolated from humans

Group	Species	
mutans-group*	*S. mutans*	serotypes *c,e, f, k*
	S. sobrinus	seroype *d, g*
	S. criceti	serotype *a*
	S. ratti	serotype *b*
salivarius-group	*S. salivarius*	
	S. vestibularis	
anginosus-group	*S. constellatus*	
	S. intermedius	
	S. anginosus	
mitis-group	*S. sanguinis*	
	S. gordonii	
	S. parasanguinis	
	S. oralis	
	S. mitis	
	S. cristatus	
	S. oligofermentans	
	S. sinensis	
	S. australis	
	S. peroris	
	S. infantis	

*mutans streptococci also include *S. ferus* (isolated from rats), *S. macacae* and *S. downei* (serotype *h*) (isolated from monkeys).

Reference (Marsh et al. 2009) [82]

Mutans group- it was first isolated by Clark in 1924.it was in 1960s that it was associated with dental caries. Mutans streptococci are recovered selectively from hard non shedding surfaces of mouth. They have been divided into seven species- *S.mutans* and *S.sobrinus* (human strains) and *S.rattus, S.cricetus,* and *S.ferus* (usually found in rodents) and *S macacae* and S.downei (isolated from monkeys.)[83] They are routinely obtained from carious sites. These group of organisms have site specific habitat in oral cavity. *S.salivarus* is commonly found on the tongue, *S.sanguis*, *S.mutans* are present on tooth surfaces and *S.mitis* are commonly found on buccal surfaces. All the 7 species of *mutans* group are physiologically very similar, particularly with their carbohydrate metabolism. Hence they have been labeled as mutans group.[83]

They are able to produce acids in large quantity, survive in acidic conditions and thus are critical in initiating and for further progressing the process of caries. *S. mutans* has c,e or f strains which possess the c, e, or f antigens and as the c serotype is responsible for almost all of the human isolates of mutans streptococci, it is appropriate that *S. mutans* be the specific epithet for the human type "mutans streptococcus." Most of human isolates of mutans possess d, g, and h carbohydrate antigens and are called S. sobrinus.[29]

Singla et al [84] conducted a study to compare *S.mutans* and *S.sobrinus* presence in plaque samples of caries active and caries free children. Polymerase Chain Reaction (PCR) method was used for diagnosis of micro organisms. They also quantified these micro organisms using Modified Sucrose – Bacitracin agar medium to correlate with caries findings in preschool children. *S.sobrinus* group was isolated significantly more from caries positive where as such pattern was not seen with *S.mutans* count. However positive correlation was seen among dmft index with PCR and culture findings.

Van Houte [81] and coworkers conducted another study among preschool children. Samples of plaque and saliva were collected from children suffering with nursing caries. More than 60% of the sample was positive for *S.mutans* from caries lesions and white spot lesions whereas the proportion of S.sanguis was low in comparison to *S.mutans*. Lactobacilli were detected in all the samples, however they helped in progression of the disease.

Aas et al [85] described in detail microbial species which are commonly linked with dental caries. They reported that *S.mutans* was absent in 10% of the samples collected from rampant caries children. They used molecular methods for

identification of microbial strains associated in both the types of human dentition which had decayed teeth. Plaque samples were collected and compared with caries free healthy controls. They concluded subjects who had *S.mutans* also harbored other species like *Propionibacterium, Atopobium* and species of *Propionibacterium.* The level of these micro organisms was significantly larger than level of *S.mutans*., Those subjects in whom *S.mutans* was absent harbored other species like *Bifidobacterium dentium,* low pH non *S.mutans* and *Lactobacillus* species. Initial lesions were commonly associated with species like *Actinomyces spp.* and *Streptpcocci* of *non-S. mutans* type, while those bacteria which could survive in acidic environment and produce acid were predominantly found during further progression of the disease. Bacterial colonies alter when dentition changes from milk teeth to permanent teeth. Bacterial species like *Veillonella, Atopobium Lactobacillus, Actinomyces spp Bifidobacterium,* and *Propionibacterium,* and few species of *non-S. mutans streptococci* species are most likely crucial for further advancement of the disease.

Another molecular study conducted on Canadian preschool children has corroborated the previous findings of the presence of *S.mutans* in the S-ECC children. Some species differed among the children, *Veillonella* and species of *Porphyromonas* were higher in SECC group, *Streptococcus gordoni* and *Streptococcus sanguinis* were higher in caries free children [86].

Another molecular study conducted by Ma et al. 2015 [87] on 40 preschool children, 20 of them had caries.PCR method was used to analyze plaque and saliva samples and they observed *S.mutans* count higher in caries group. Future research on ECC can be carried with biomarkers like *Streptococcus, Porphyromonas,* and *Actinomyces,* as they were positively associated with SECC.

Longitudinal investigations show that children with excessively higher level of *Mutans streptococci* do develop or at an increased risk of developing additional cavities over time than those without *Mutans streptococci* before their birthday. Relative risk of this association is more than 6 [88].

Even during phases of shedding of the primary teeth and eruption of secondary dentition also affects the type of oral microbiota. *Capnocytophaga, Corynebacterium, Campylobacter* were predominantly present in plaque sample of an individual while *Streptococcus, Granlicatella* were more prevalent in saliva. Earlier studies have similar results in subjects who had either only deciduous teeth or permanent teeth. It was reported by Xu et al. that *Actinobacteria* was observed abundantly in rather than suprgingival plaque in primary dentition. However, contrary to this trend is seen in permanent dentition of young adults in whom supragingival plaque contained more *Actinobacteria* than saliva.

Recent studies using molecular techniques have reaffirmed the abundance of *S. mutans* in the dental plaque of children with ECC. Longitudinal investigations show that children with excessively higher levels of MS have 6 times more chances to experience additional cavities over time than those without MS at first visit. In addition, children who are colonized with MS at younger age are at an increased risk to develop ECC than children who harbor the bacteria after the age of 6 years [88]. The virulence of MS is associated with their ability to produce acid and sustain acidic pH. The low pH results in demineralization of enamel.

Systematic review explored an association between mutans streptococci and dental caries among 5yr children which was conducted by Thenisch et al [89]. They screened around 981 articles from Medline, Embase, Cochrane and manual search of the reference lists. Out of these, only 9 studies which qualified criteria for inclusion

were further studied by the authors. The pooled risk analysis showed that result from plaque samples were more consistent and showed higher values in comparison to saliva sample values. The study concluded that if the child's plaque sample shows mutans *stretptococci*, then child is susceptible to dental caries. Authors also summarized various methodological flaws in the studies assessed like – there was no uniformity in the identification of *S.mutans* count in saliva and plaque. Different studies had used different sites for collection of plaque sample. Few studies collected stimulated and others studied unstimulated salivary sample. Degree to which samples were diluted, incubation procedures used varied among the studies all of which led to poor homogeneity of the studies. Thus lower level evidence was achieved in the systematic review.

Early Childhood Caries and Fluoride varnish

A systematic review was reported on the current evidence for the effectiveness of various preventive and curative modalities for ECC by Twetman and Dhar.[90] They searched the literature from 2000 to April 2014 and retrieved 329 articles which had reported on preventing and arresting carious lesions in early childhood caries. After screening, they were able to retrieve 19 articles which met their inclusion criteria. Study quality was appraised using GRADE assessment.(Grading of recommendations assessment, development and evaluation).The authors concluded that the strength of evidence for effectiveness of topical fluorides like tooth paste and varnishes was moderate, however other modalities like use of povidone iodine, chlorhexidine gel, restorative care had very low strength of evidence.

- Fluoride varnish has been tested by many studies and most of them have used oral health education along with fluoride varnish application. [91]

- Though many trials have been carried out to assess effectiveness and efficacy of fluoride varnish, most studies suffer from biases like use of fluoridated tooth-brushing by the study participants, study participants receiving fluoridated water, loss to follow-up of the subjects and few studies failed to include proper control, hence it was difficult to estimate true effect of fluoride on initial lesions.[92-95]

- A comprehensive systematic review on various antimicrobial agents and oral microbiota which are commonly associated with ECC was carried out by Yihoung et al [96].They carried a thorough literature review on various interventions which were tested to reduce *S.mutans* count in preschool children suffering with ECC like effect of fluoride applications, chlorhexidine varnish interventions, Povidone iodine treatment, full mouth comprehensive restoration, use of xylitol, effect of maternal xylitol trials, silver compounds and effect of ECC on oral microbes. Most of the studies reported reduction in *S.mutans* growth after application of the antimicrobial agents topically, but once the intervention ceased, *S.mutans* regrowth occurred and there was related incidence of ECC lesions. The authors however cautioned about the low level of evidence found in these studies and lack of well designed placebo controlled randomized controlled trial.

The first conference on ECC was held in 1997 in Bethesda which give detailed description about ECC right from its description on nomenclature to ways of defining it, diagnosing it, preventing and treating ECC.A follow up conference on ECC was held in 2014 at Baltimore, University of Maryland, School of dentistry which witnessed evidence base reviews on the epidemiology, prevention and disease

management of ECC. Proceedings of the conference were summarized by Garcia et al [97] .The conclusion was that fluoridated tooth paste self applied and fluoride varnish professionally applied are currently most effective for prevention of ECC. Motivational interviewing a form of counseling which is centered on patient is an effective way of reducing incidence of ECC. Integrating oral health with the medical health system was also suggested as an approach for reducing and preventing ECC.

LICORICE AND GENERAL HEALTH

As it is well known that tooth decay is an infectious multifactorial disease, thus use of antimicrobial agents against cavity causing bacteria should help in controlling the disease. But modern medicine has many side effects like antimicrobial resistance, altered taste; cost and availability call for alternative agents which are equally effective, efficacious, affordable, safe and sustainable. Recently, the focus of attention has shifted to research pertaining to natural products [98]. One such traditional plant which is grown and cultivated in Asian and Indian continent is Licorice.

Glycyrrhiza has around 30 species, and the classic main botanical sources of licorice root are *G.glabra.L* and *G.uralensis Fisch. Glycyrrhiza glabra* is grown in Mediterranean and in few regions of Asia. It was widely used as an expectorant by Egyptian, Chinese, Greek, Indian and Roman civilization. In modern medicine, Licorice is often added as a flavoring agent to mask bitterness of medicine.,

Glycyrrhiza uralensis is a perennial herbaceous plant native to the Urals, Siberia, and the steppes and semidesertic regions of East Asia. The root system is very similar to the glabra.

Details of Licorice

- Botanical name- *Glycyrrhiza glabra Linn*.
- Family – *Gabacceae*
- Classical name- Madhuyasti
- Sanksrit names- *Madhuyasti, Yastimadhu-Yastlitaka-madhuka, Madhuka.,*
- Regional names- Mulethi, Jethimadhu, Yastimadhu, Jetimadhu, Jathimadh, Atimadhuran, Yastimadhukam, Asluspus, Bekhanara and Liquorice.

Description The licorice shrub belongs to pea family. It grows best in subtropical climate which is rich in soil. It grows to about 4-5 feet tall. It has oval leaflets; flowers are white to dark pinkish in colour. It has a well developed underground taproot system and runners spread in all directions. For medicinal purpose the main taproot is harvested which is soft, fibrous and has a dark yellow colored inner part. *Glycyrrhiza* is a Greek word which has two words in it :- *Glycos* - sweet and *Rhiza* – root.

Active Constituents – Almost 50 percent total dry weight of licorice consists of water soluble, biologically active complex. This complex contains Flavonoids, Pectins, Simple sugars, Amino acids, Triterpene, Saponins, Flavonoids, polysaccharides, Pectins, simple sugars, , mineral salts and various other substances[99].

Pharmacokinetics –When licorice is taken orally, *glycyrrhetic* acid is quickly absorbed and through carrier molecules it is transported to the liver. Licorice is first broken down to glucuronide along with sulfate conjugates and these are then rehydrolyzed to produce glycyrrhetic acid.

Ayurvedic properties

- Rasa- Madhura
- Guna – Guru
- Veerya-Sheeta
- Vipaka- Madhura
- Doshaghnata- Vatapittashamaka.

Mechanism of action – Glycyrrhizin alongwith glycyrrhizic acid together have shown to possess antibacterial activity, anti inflammatory activity and antiviral activity especially against multiple RNA and DNA viruses. It is used for following general health.[100]

- **Chronic hepatitis** – It is standard treatment for chronic hepatitis for more than 60 years in Japan. A preparation of licorice Stronger NeoMinophagen C (SNMC), a glycyrrhizn preparation has been widely used with considerable success. SNMC has played significant role in suppressing inflammatory liver and as well as chronic hepatitis.[101]

- **Viral illness** – There is evidence to support that licorice inhibits growth and cellular changes of many DNA and RNA viruses. During this it does not affect multiplication or growth of normal cells.[101]

- Glycyrrhizin can irreversibly damage Herpes simplex virus and thus is effective in Herpes infection.

- **Hepatocellular carcinoma** – When Licorice is administered for hepatitis C, not only cures hepatitis, but is also effective in Hepatocellular carcinoma. Many trials are conducted which have concluded that IV glycyrrhizin not only

decreases ALT levels but also improves liver health which is evident at microscopic levels. Arase Y, cancer 1997.[102]

- **Aphthous ulcers** – Use of licorice mouth wash significantly reduces the average number of ulcers, reduction in pain sensation as well as eruption of new ulcers.[103]

- **Peptic ulcer disease**- Licorice has beneficial effects in peptic ulcers as a palliative therapy. It helps in healing of the ulcers in mucosal layer.

- **Infection caused due to Helicobacter pylori** is usually prevalent in patients suffering from peptic ulcer. The flavonoid components show promising anti H pylori activity against clarithromycin resistant strains.[104] Other therapeutic considerations include its use for reducing body fat by inhibiting 11-beta hydorxysteroid dehydrogenase in fat cells[105]

Licorice and oral health

Licorice and dental caries

Current research advocates that licorice extracts and its phytochemical ingredient have potential benefits in oral diseases. Licorice contains a number of biologically active compounds, to name a few are - glabridin, licoricidin, licorisoflavan, licochalcone A, and glycyrrhizin. They have many features like anti adherent property, anti inflammatory property and anti microbial property. The basic advantage of phytochemical products is that, it is structurally different from routinely used antibiotics and hence micro organisms do not show resistance against these products. However the results from invitro and in-vivo studies are conflicting in

nature. In-vitro studies have proven beneficial effects of licorice against oral pathogens, but the invivo studies reported till date, have not helped much in substantiating these results. The clinical trials suffered from draw backs like smaller sample size, failure to choose appropriate control and attrition bias. [106]

There are many factors responsible for occurrence of dental caries but oral pathogens which are producers of acids and which can sustain acidogenic environment are important precursor of the disease. Among the various pathogens responsible for caries, *S.mutans* along with *S.sobrinus* are frequently associated with dental caries more than any other organism followed by *Lactobacillus* species with actinomyces species .[107]

These bacteria are able to utilize exogenous sugars like sucrose, glucose, galactose and fructose and convert them to lactic acid. The final outcome of the bacterial metabolism is required for plaque formation and attachment of the organisms to the tooth surface. [108]

The caries preventive property of licorice is well known for more than 3 decades. The main component of licorice responsible for caries protective effect is glycyrrhizin, the diglucuronide of glycyrrhetinic acid. It gives sweet flavor to licorice, its saponin component which acts as an emollient and gives expectorating property.

Saponins are known to form gels which are stable in aqueous solution. They also have outstanding dispersing property. Thus the most useful product of a saponin would be topical application intra orally.[109]

An invitro study was conducted by Segal et al [110] who tested efficacy of licorice in terms of antibacterial activity and anti adherent property against *S.mutans*.

They tested glycyrrhizin in four different concentrations. They concluded that at higher concentration of 0.5-1% glycyrrhizin, *S.mutans* failed completely to adhere to glassware in spite of sucrose being present. At higher concentration of 10 and 5%, bacterial growth was inhibited but adhering property was completely abolished. This can help in preventing plaque formation which is a required for initiation of dental caries

Further research was carried on, in this aspect by Sela and coworkers[110] who attempted to find as to how glycyrrhizin acted on *S.mutans* and inhibited its adherent property. They concluded when Glycyrrhizin was present, the quantity of glucans which adhered to the glass reduced, the quantity of glucans which failed to adhere to glass increased and the total quantity of insoluble glucans both adhered and non adhered reduced. These results indicate that glycyhrizin affects the activity of extracellular enzyme glucosyl transferase the key enzyme of *S.mutans* involved in the production of glucans and dextrans.[110]

Another report of great interest was put forward by Gedalia [111] who tested the effect of glycyrrhizin which was mixed with fluoride and the uptake of fluoride by initial lesions. They concluded that when glycyrrhizin is added to fluoride, fluoride uptake by demineralized enamel increased. There rationale behind this mechanism was that, glycyrrhizin causes surface coating and deposition of fluoride in porous enamel structure. This reduces solubility of enamel.

However converse results were reported by, Deutchman et al[112] in their in vivo study. They failed to get any significant effect of glycyrrhizin on initial lesions with regard to mineral loss using 1% mouth rinse and lozenges. However they did put forward shortcomings of their study such as – the concentration of glycyrrhizin used

in their study was 1% which was low and which was further diluted by saliva. The participants received glycyrrhizin for a very short duration of time

A split mouth study was carried by Steinberg et al among 21 dental students. Glycyrrhizin solution was applied on one side. All oral hygiene practices were stopped and plaque accumulation was compared. They noted significant reduction in plaque on the side where glycyrrhizin was applied. The study lacked randomization, use of a suitable control and the sample was very low. Within the limitations of the study authors concluded that glycyrrhizin may help inhibiting plaque buildup.[113]

In another in vivo study, Glycyrrhizin gel reduced the acid production in the oral cavity. [114]Peters et al [115] conducted an in vivo study among preschool children who were asked to use licorice lollipop made with different concentrations. They assessed salivary *S.mutans* count which was statistically reduced in the test group.

A detailed study on licorice lollipop has been described by Hu et al [116].The authors procured large quantity of licorice and estimated its Minimal inhibitory concentration against standard strains of *S.mutans*, *S.sobrinus* and *L.casei*. MIC Against these strains was less than 40microgm/ml. Further lollipops were prepared, their stability and toxicity were studied and two pilot human clinical trials were conducted. Lollipops were consumed for 10 days with a dosage of two times per day and on data analysis significant reduction of cariogenic bacteria was observed.

Licorice and periodontal diseases Research in the past has indicated that licorice and its metabolites have favorable effects on the periodontal system both reversible conditions like gingivitis and irreversible conditions like Periodontitis. Cytokines, Interleukins are responsible for periodontal breakdown, licorice extract has licoricidin

and licroisoflavan A which have shown anti inflammatory properties against these immune responses. Thus it can help in osteoblastic activity and bone regeneration in advanced periodontal diseases. [117,118]

Licorice and Oral Candidiasis

Oral candidiasis a fungal infection commonly encountered in the oral cavity. It is caused by Candida species a common commensal, is harmless in normal individuals. However it may manifest its effect as an infectious lesion in few susceptible individuals like those suffering from diabetes, xerostomia, those on long term steroids, antibiotics and immune suppressants.[119] A bioactive component of licorice, G.glabra and glabridin was effective even in Candida strains resistant to amphotericin B.[120.121]

Recurrent apthous ulcers and licorice

Apthous ulcers can occur as minor, major and herpetiform. Children as well as adults are equally prone to these ulcers. The exact cause for these eruptions is very vast and includes systemic conditions, immune factors and viral agents as well as hypersensitivity to certain food and drugs [122] Licorice has shown to reduce pain, inflammation as well as reduce the duration for healing of the lesions. However conflicting result was published by Martin [123] in which no improvement was seen with pain and ulcer size. These reports indicate that in future rigorously conducted clinical trials should be planned to better ascertain the beneficial effects of licorice on ulcers.

Locus of control and its association with oral health It is well acknowledged fact that dental caries is multifactorial disease. Apart from interaction between micro

organisms and the environment, there are other attributes which can be considered to be of pivotal importance as far as etiology of caries is concerned. Social factor, cultural factors, knowledge and attitude of the parents can all be linked up in many ways with dental caries experience.[124] If we want to solve the problem of Childhood caries, it becomes imperative that we have a broader vision and incorporate multi sectoral approach for its prevention and treatment.[97] Psychological concepts are based on human behavior and attitudes.

One of the most accepted theory explaining behavioral pattern and its relation with health and disease is Locus of Control theory(LoC).[125] LoC is based on the theory of belief of an individual's abilities to control events occurring in his or her life. As per this theory, an individual belongs to any one among three personalities External LOC when one believes that health is dependent on professionals, Chance LOC believes on luck or fate and Internal LOC when one believes that his own behavior determines the health status.

Lencova et al [126] postulated cross sectional study where in the authors assessed the relationship between childhood caries experience with parental LoC. They examined 285 child parent pair. dmft index served as a proxy measure of caries experience and concluded that parents with better internal LoC had children with lesser caries experience.

A preventive trial on childhood caries reported by Albino et al [127], regarding American Indian tribal population. In this interventional study, 897 child parents were randomized to either of the two groups. Intervention group received 4 FV application, 5 Oral health promotion talks and 4 Health education talks for parents where as the usual group did not receive any of these interventions. However all children received

toothbrush and paste. During study period of 1 year, caries increment and improvement in knowledge domains were studied. Other socio demographic characteristics were also recorded. Authors concluded that parents with Internal LoC had children with fewer caries increment, where as Parent's with Chance or External LoC had significantly better improvement in oral health knowledge scores.

In another study reported by Chase et al [128,] 79 children who were treated for ECC were observed for 1 year. Parental psychosocial variables were measured against Relapse of treatment and follow up of treatment. Those parents who had internal LoC had higher probability to come for follow up visits; however there was no significantly difference seen among relapsed and non relapsed groups.

1.3 JUSTIFICATION OF THE STUDY:

The prevalence of ECC has an unequal trend. The prevalence seems to have plummeted in most of the developed countries but is increasing in many developing countries especially among the socially disadvantaged populations though the research in this field has progressed rapidly.[129]

Selection of age group Though many studies have been reported on caries status of school children, similar reports of preschool children are lacking given the fact that preschool children are not available easily for dental examination in conjunction with the practical difficulties encountered in handling this age group, literature is sparse especially in Indian scenario. ECC can be associated with infection, pain and early loss of milk teeth. Evidence so far suggests that children suffering from SECC have significantly stunted growth in terms of height and weight when compared to caries free children. [84] Hence by preventing ECC we can promote both oral and general health of a child.

Since the present interventional study was one year trial, hence we selected preschool children of 3-4 year old, who would still be within the purview of primary dentition by the time study ends.

Selection of fluoride varnish Fluoride varnish is accepted preventive tool for early childhood caries and a recommended method to apply topical fluoride to young children. [90]

Varnish can be easily applied, requires 1-4 mins for application [77]. It is the only topical fluoride indicated in the preschool children. Hence it was selected as control in the current study. In spite of these advantages it does have its drawbacks like it is an expensive preventive modality, requires a professional for its application, toxicity can occur if product not handled with care. Hence a need arises to find a

complementary product to fluoride.

Recently the focus has been on herbal products which are of value in dentistry [130]. We searched the literature of Ayurveda and came across a commonly used medicinal plant called Licorice- Glycyrrhizia glabra.

Selection of Licorice It was selected due to its following advantages-

- It has been used traditionally to relieve cough, sore throat and gastric problems.
- Drug of choice in children to enhance memory.
- Its sweetness is 50 times more than sucrose.
- Easily available and inexpensive.
- FDA lists licorice and its components as GRAS(Generally Regarded As Safe.)
- LD_{50} of Glycyrrhizin is 1.94 g /kg s.c. [131]

Use of indigenously prepared Licorice varnish Since varnish is the only topical fluoride which is indicated in preschool children, we prepared Licorice varnish as a test agent against fluoride varnish. Indigenously prepared Licorice varnish was similar to fluoride varnish in colour, consistency and appearance.

Use of Combination varnish Gedalia in 1986 [111] had reported that when glycyrrhizin was added to acidulated phosphate fluoride solution, uptake of fluoride and reduction in enamel solubility in an in-vitro study. He speculated that glycyrrhizin causes coating on the fluoride ions and gets deposited in the porous enamel structure. Thus combination varnish was selected to assess if Licorice could enhance the remineralising capacity of FV in an in vivo setting. Present study was initiated with an intention to find an alternative to fluoride varnish as well as to assess if anticariogenic properties of fluoride varnish could be enhanced.

1.4 AIM AND OBJECTIVES

Research Hypothesis: There is a difference in the effectiveness of Licorice Varnish, Combination Varnish and Fluoride Varnish in the remineralization of initial lesions and reduction of salivary *Streptococcus mutans* count.

Aim - To compare effectiveness of Licorice Varnish, Fluoride Varnish and Combination Varnishes on Early Childhood Caries.

Objectives -

- To assess effectiveness of Licorice Varnish, Fluoride Varnish and Combination Varnish on initial lesions of Early Childhood Caries.
- To assess effectiveness of Licorice Varnish, Fluoride Varnish and Combination Varnish on salivary *Streptococcus mutans* count in ECC.
- To assess existence of any association of parent's Locus of Control with caries status in their 3-4 year old preschool children

2. MATERIAL AND METHODS

Study aimed to compare three varnishes for their effectiveness on initial lesions of ECC in 3-4 year old children of Belagavi city. As per the protocol the trial was registered at Clinical Trial Registry of India.no. CTRI/2015/10/006305 before recruiting subjects. (Annexure I).

Study had two stages:-

- Invitro part was conducted from 12th June 2014 to 28th Dec 2014.
- Invivo part was conducted from 24th April 2015 to 10th October 2016.

In vitro study included extraction of licorice extract from Licorice root powder, preparation of extract, assessment of Minimal Inhibitory Concentration (MIC) of the extract against *S.mutans*. Further Licorice Varnish and Combination Varnishes were prepared and they were compared against Fluoride Varnish, commercially available (Bifluorid 12) for physical parameters and antibacterial activity. Safety aspect of the varnishes was assessed against fibroblast cell line.

Invivo part included randomized controlled field trial where the three varnishes were compared against each other for their effectiveness on initial lesions among preschool children of Belagavi city.

2.1 Pilot study – Pilot study was performed on 12 preschool children of Sidrameshwar anganwadi, Belagavi. The purpose of pilot study was to standardize the method for collection of saliva sample, identification of initial lesions using Nyvad's index and Diagnodent pen, application of dental varnish and standardization of the questionnaire.

Standardization of the method for collection of saliva sample

Drawing the pooled saliva using syringe was not practical neither was spitting of unstimulated saliva method. After undertaking all the trial and error procedures finally it was realized that drooling of the saliva directly into Eppendorf tube (which contained of 5 ml RTF) was most feasible.

Standardization for recording of relevant indices

The investigator underwent training for recording the relevant indices like Nyvad's index, Oral hygiene index simplified and for the recording of initial lesions using Diagnodent pen in Public Health Dentistry department under the supervision of the guide. Oral hygiene index (Annexure II) simplified was used to measure oral hygiene status of the child [132] Initial lesions were recorded using Nyvad's index [40](Annexure III) and Diagnodent pen[133] (Annexure IV).

After completing the training, investigator recorded both oral hygiene and initial lesions on 12 children. For assessing intra examiner reliability, the same children were again examined after 1 week by the principal investigator. Intra examiner kappa values were 0.84 for Nyvad's index and 0.88 for OHI S and 0.88 for Diagnodent pen respectively.

Standardization of Proforma: A self-designed proforma was prepared to collect information related to various parameters like socio demography, oral hygiene and parental aspect of Locus of Control (ANNEXURE V)

Details of the Proforma

Questionnaire- A self designed questionnaire was used, which had three parts – socio-demographic details, LOC questionnaire and oral hygiene practices. Socio demographic details included child's name, age, gender, parental name, parental occupation and income. Parental education, occupation and family income were used to determine Socioeconomic status as per Kuppuswamy's socio economic classification. [134] (ANNEXURE VI)

- Oral hygiene practice included details on aid used for maintaining oral hygiene, presence/absence of parental guidance, frequency of brushing, time of brushing, materials used and how often was tooth brush changed?

- Locus of control (LoC) of parents – LoC was explored using a LoC questionnaire items which were standardized and validated in a previous study. [126] Based on LoC theory, 13 questions which were relevant to LoC were selected. Out of these 13 questions, 1,2,5,7 and 11 expressed Internal LoC, 3R, 9R along with 13R represented External LoC where as 4R,6R,8R,10R and 12R represented beliefs in chance or bad luck. Five point (1- strongly agree to 5 strongly disagree) likert scale was used to measure each item. To maintain uniform gradient of scores and to ascertain that lower scores reflected more positive attitude, codes of negatively framed questions were reversed.

- Questionnaire was pilot tested on 12 participants to assess its comprehensiveness, validity and reliability. English questionnaire was translated to Kannada and Marathi by a person fluent in both the regional

languages. It was then back translated to English to conform that same meaning was conveyed. Few questions were modified and the questionnaire was finalized. This was given to 12 mothers and their responses were noted. Again after one month, the same questionnaire was given to same 12 mothers. To assess reliability of the data LoC items were subjected to test retest scores which was 0.84 confirming good reliability.

- **Scoring of the Clinical Parameters** – WHO Dentition status [135], Nyvad's index, Diagnodent scoring, OHIS score, Presence of malocclusion and microbiological count were recorded in recording proforma.(Annexure-VII). Details of pilot study on 12 subjects were collected and the applicability of the questionnaire assessed.

Standard operating procedures: Standard operating procedures were developed for the different equipment used during the course of this study (Annexure-IV)

In vitro study

- Digital weighing balance
- Laminar airflow unit
- Labtech bacteriological incubator
- Hot air Oven
- Vortex mixer

In vivo study

- Diagnodent pen
- Saliva collection
- Dental Varnish application

Study had 2 stages: First Stage (In vitro Experiment): Extract of the licorice was prepared by using both Cold maceration and Soxhlet method. Minimum Inhibitory Concentration (MIC) of both the extracts was determined using Broth dilution method and disc diffusion. Extract obtained from cold maceration was used for preparing Licorice Varnish and Combination Varnish. Both the varnishes were compared against Fluoride Varnish for physical parameters as well as for their antibacterial activity.

Second Stage (In vivo Experiment): In vivo experiment was undertaken to assess as well as compare the effectiveness of Fluoride Varnish, Licorice Varnish and Combination Varnish on initial lesions of preschool children of Belagavi city.

Chemicals and armamentarium required for in-vitro study

- Crude extract 99%
- Ethanol (Soxhlet extraction)
- Licorice root which was coarsely powdered
- Electronic Weighing machine
- Muslin cloth
- Soxhlet apparatus
- Conical flasks
- IKA Rotary evaporator
- Porcelain bowl

Phytochemical analysis

- Wagner's reagent
- Aqueous 5% ferric chloride solution
- Alcoholic 10% ferric chloride solution
- Sodium hydroxide solution

- 10% Ninhydrin solution
- 0.2% Acetic anhydride
- Concentrated sulphuric acid
- Molisch's reagent
- Glacial acetic acid
- Filter paper
- Distilled water

Minimum Inhibition Concentration

- Crude extract of Licorice root
- Standard strain of *Streptococcus mutans* ATCC 25175
- Electronic Weighing machine
- Microcentrifuge tubes
- Micropipette
- Aluminum foil
- Glass petri plates
- Laminar flow unit
- Anaerobic jar
- Labotech bacteriological incubator

Ingredients required for preparation of Licorice Varnish

- Licorice extract
- Ethyl acetate (IP grade)
- Pyroxylin (IP grade)
- Fumed silica (IP grade)
- Iso amyl propionate (IP grade)

2.3 Details of study procedure:

Collection of Licorice roots and preparation of licorice extract: Licorice roots were purchased from Pharmacy shop of KLE Ayurveda Belgaum, Karnataka. Recognized botanist at ICMR RMRC, Belgaum authenticated the licorice plant specimen.(Annexure VIII) Roots were thoroughly washed, cleaned and dried in shade for 3-4 days. Roots were cut and coarse powder was prepared by grounding them in a grinder.

Preparation of crude extract by Cold maceration and Soxhlet extraction-

Two different methods were used for preparation of licorice extract. Extracts were prepared in Department of Pharmaceutics and Pharmacognosy, KLE Society's College of Pharmacy, Belagavi under guidance of faculty of Pharmacognosy. Digital weighing balance was used to weigh licorice root powder.

Cold maceration - Licorice powder (100 gms) was mixed with 99% ethanol(500ml) in a conical flask. With the help of glass rod the mixture was dissolved thoroughly. Flask was kept with intermittent shaking for 72 hours. Muslin cloth and filter paper(Whatman No.1) were used to filter the mixture. The filtrate was passed through IKA Rotary evaporator which was maintained at 40°C, to get the concentrate which was refrigerated till further use.

Soxhlet method Crude extract was prepared of licorice root by hot solvent percolation method using Soxhlet extraction. The solvent was 99% ethanol. Apparatus for Soxhlet extraction consists of 3 parts:

- Flask
- Soxhlet Extractor
- Condenser

Licorice root powder (100gms) was placed in muslin cloth bag which was kept in the body of Soxhlet extractor and to this ethanol (500ml) which acts a solvent was added. The apparatus was then stabilized with clamps and stands which acted to support the Soxhlet apparatus, round bottom flask and condenser. Continuous water flow was maintained using a rubber tube which was connected to a tap water. Ethanol was heated slowly using the isomantle, and the solvent began to evaporate, evaporate moved continuously through the Soxhlet apparatus to condenser and condensate dripped back into extract present in the reservoir. When solvent touched the level of siphon, solvent poured back in flask and cycle repeated again. Entire procedure was run for 6 hours. Final extract was collected in round bottom flask. After completion of the process, IKA Rotary evaporator which was set at 40^{o}c was used for removal of ethanol with a resultant yield of 2-3ml. Extract was kept aside remaining ethanol evaporated completely .Quantity of extractable content was measured as mg/g of air dried in a digital weighing balance. The extract was stored at 4^{o}c in a refrigerator.

2.4 Phytochemical screening Extracts obtained from both the techniques were analysed for phytochemical contents following guidelines as outlined by Trease and Evans. [136] (Annexure IX)

Stages of In vitro study:

Chemicals required

- Mitis Salivarius HiVeg™ Agar Base
- Pottassium Tellurite 1% (1ml per vial)
- Sodium acetate anhydrous, A.R.
- Sodium sulphite, A.R.
- Phenol Red Sorbitol Broth
- Inulin, A.R.
- N-Acetyl-D-glucosamine D(+)-Trehalose dehydrate
- Autoclavable Petri Plates Clear, transparent and unbreakable, size : 90 mm diameter x 15 mm1
- Centrifuge Tubes, with Lid Moulded Graduation Vol.2ml
- Micro Centrifuge Tube - B Attached hinged cap, autoclavable, capacity 1.5 ml, air tight
- Brain Heart Infusion Agar (Special Infusion Agar)
- The tubes incubated for 24 hrs at 37^0C in bacteriological incubator and observed for turbidity. Turbidity was considered as an indicator of bacterial growth, and the minimal concentration which was free from turbidity was considered as relative MIC to ascertain the antimicrobial activity. Experiments were repeated three times and mean value was considered for both extracts. [137]
- **Licorice Varnish (LV) preparation** An expert with pharmacy background guided during the preparation of Licorice Varnish.

Ingredients belonging IP *(Indian Pharmacopeia*) grade products were procured to prepare varnish as shown below

- Iso amyl propionate (Sigma Aldrich) acts as a plasticizer.
- Ethyl acetate purchased from Sigma Aldrich acts as solvent
- Collodion solution, (Omatek Laboratories,Indore), acts as Lacquer
- Fumed silica (Aerosil 200 Pharma), gift sample from Evonik Industries, Germany acts as a Thickening agent
- Licorice extract indigenously prepared.

Manual of operation and Good Laboratory Practices (GLP) guidelines were prepared to maintain consistency and reliability of the procedure.

Licorice Varnish (LV) was prepared by adding licorice extract and ethyl acetate in a glass container which was kept in bath sonicator for almost 30 minutes. After the complete dissolution of extract, Collodion solution and Iso Amyl Propionate were added. The contents were vortexed and to this mixture fumed silica was added. Contents were mixed in a vortex for approximately 30 seconds following which, contents were transferred to an amber colored sterile bottle and labeled.

Fluoride Varnish (FV) - Commercially available Bifluorid12 varnish (VOCO Company, Germany. Lot no.1523310) served as a positive control.

Combination Varnish (CV) – This was initially prepared by mixing 80 % LV with 20 % FV but when it failed, other combinations were tested. Combination Varnish was prepared by mixing various concentrations of Licorice Varnish and Fluoride Varnish as described below-

- 50% LV + 50% FV
- 60% LV + 40% FV
- 75% LV + 25% FV
- 60% FV + 40% LV
- 75% FV + 25% LV

Evaluation of varnishes was done for Antimicrobial activity against *S.mutans* and inherent physical parameters of varnish.

Antimicrobial activity –

Disc diffusion method was used to assess antimicrobial susceptibility. BHI(Brain Heart Infusion) broth was used for cultivation o f *Streptococcus mutans* ATCC25175, after 18 hours, it was transferred to BHI agar which consisted of 5% sucrose.

Disc diffusion method-The direct colony suspension technique was used to prepare the inoculums. Inoculum of *S.mutans* was prepared by collecting the colonies which had grown on agar in a day's duration and the average number of micro organisms was measured with standard turbidity of 0.5 Mc Farland as basis which corresponds to $1.0x10^5$ CFU/ml. All the three varnishes were diluted in various concentrations of 100%, 50%, 25%, 12.5% and 6.25% using distilled water. A measured quantity, of twenty micro liter of varnish was placed on sterile filter paper (6.4mm diameter) which was kept in the agar plate. Following this, plates were incubated under anaerobic conditions at 37^oc for almost 48 hours. Varnishes, being alcoholic mixtures, had evaporated and result was unclear.

Broth dilution method-Broth dilution method was attempted next. As a procedural step varnish was mixed with BHI broth, but soon after preparing the mixture a precipitate occurred immediately which led to failure of this method. As varnish contains resins, a precipitate occurred when it was mixed with Broth. Routinely employed methods to assess antimicrobial activity were unsuccessful, which necessitated development of a new method to evaluate antibacterial activity of varnish.

Direct Contact Test (Novel method)- In this method, 0.5ml of varnish, BHI broth and *S.mutans* were mixed together and after 5 minutes Nichrome loop was used to spread mixture on the agar gel plate and incubated for 48 hours. Similar procedure was repeated with LV and CV and results obtained are depicted in Fig 14. As is evident clearly, CV in the ratio of 80% LV and 20% FV, failed to show antimicrobial activity in Fig 14a.

Three varnishes were evaluated for following physical parameters at KLE Dr. Prabhakar Kore BSRC, Belagavi.

- **Colour matching** – Colour of the indigenously prepared LV was matched with shade guide and the findings were noted down. To estimate shelf life of varnish, date of preparation was also noted. Test area had sufficient artificial daylight fluorescent illumination. The specimen was held 25cms away and was looked at perpendicularly. Colour matching was done between shade guide and specimen. The mean values of 6 specimen of the same shade which were observed within a time interval of 1 week were used to estimate intra examiner Kappa value.

- **Rate of evaporation** - A sterile glass slide was taken and its weight was noted down. Hundred micro liter of varnish was then dispensed on it, evenly distributed and placed on digital weighing scale. Stop watch was used to estimate time required for the slide to return to original weight.

- **Viscosity** – It was assessed using CAP 2000+ Viscometer, Brookfield. Two ml of the varnish was placed on viscometer plate and the test was run as per the manufacturer's instruction and values noted.

- **pH of the varnish** – pH of the varnish was established using pH strips

- **Film forming ability**– Human Tooth samples of 3 mm thickness were obtained using hard tissue microtome. Fifty micro liters of varnish was applied using the applicator tip. After the samples were dried, they were observed under Scanning Electron Microscope (SEM) to assess morphology of films formed due to application of varnish on extracted tooth surface . (JOELSEM, Model no. JSM- 6360LV,) which operated at 15 kV voltage.

- **Safety aspect** of the dental varnishes was assessed by carrying out Toxicity study on- Primary Gingival Fibroblasts.;Normal, Adult (HGF) ATCCPCS-201-018 .

- **Protocol for Cytotoxicity assessment** - To evaluate cell toxicity Primary Gingival Fibroblasts; Normal, Human, Adult (HGF) ATCC PCS-201-018 was used. Dulbecco's modified Eagle's medium (DMEM; Gibco Laboratories, Grand Is., NY, USA) was employed to culture cells. This media was supplemented with 10%FBS (Fetal Bovien Serum,Gibco, Invitrogen) Cat No - 10270106 ,Antibiotic –Antimycotic 100X solution (Thermofisher Scientific)-

Cat No-15240062.Approximately 5×10^3cells/well were seeded in micro plate with 96-wells. Plate was stored over night in controlled conditions with 37ºc temperature, 95% humidity and 5% CO_2.Each concentration of 300µl of samples was treated. Incubation period of the cells was extended by another 24 hours. Posphate buffer solution was used to wash the cells in the well and 80 µL of the methyl-thiazol-tetrazolium (MTT) staining solution was added. Further Plate was incubated at 37ºC. After 4h, 200 µL of di- methyl sulfoxide (DMSO) injected in each well for dissolution of formazan crystals, and recording of the absorbance was carried using 570 nm micro plate reader[138.]

Formula : Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100 graph Pad Prism Version5.1, we calculated IC 50 of compounds

Note – DMSO Concentration is less 1.5% in experiments Concentrations are in duplicates

2.5 In vivo study: The in vivo study includes Randomized Controlled field Trial conducted to assess and compare the effectiveness of LV, FV and CV on initial lesions of 3-4 year old preschool children residing in Belgaum city.

2.5.1. Permissions: Ethical clearance for the study was granted by Ph.D Human KLE University, Ethical Committee, Belgaum (Annexure-X). Permission was obtained from KLE Pharmacy College Belagavi to prepare crude extract. Permission was also obtained from Dr. Prabhakar Kore BSRC lab for determination of MIC of crude extract, preparation of the varnish, testing antibacterial activity of varnish and culture of salivary samples. Permissions were sought from BDCH, Davangere and Shivaji University Kolhapur for performing Scanning Electron Microscopy.

For the purpose of in vivo study permissions were obtained from women and child welfare department Anganwadi Officer (Annexure XI)and from principals of the selected preschools, parents of the study participants. The study protocol was explained to parents of the preschool children. They were also informed that participation was not compulsory and they had the liberty to opt out of anytime during the study period.

They were also assured that the details obtained from them will be maintained confidentially and will be used only for research purpose.

2.5.2. Study design-It was a randomized controlled field trial with concurrent parallel design with single blinding.

2.5.3. Sample size estimation: The sample size calculation is as follows[139]: Eta square (η^2) is used to estimate effect size. When eta squared cannot be estimated, researchers can predict whether effects are likely to be small, medium or large. For ANOVA situations the conventional estimates for small, medium and large effects would be values of (η^2) equal to .01, .06, and .14 respectively. This corresponds to sample size requirements of about 319, 53 or 22 subjects per group in a three group study, assuming an a of 0.05 and power of 0.80.Here we have considered Eta square to be small ie.0.05, $\alpha = 0.05$ and power of the study to be 80%.Thus the recommended sample was 62 in each group. (Annexure XII)

62 X 3= 186, assumed dropout rate is 5%. ie 186+9=195. Thus final sample in each group would be 65.

2.5.4. Selection of Subjects: Children aged 3-4 years studying in various preschools of Belagavi city formed the study population. List of all preschools was obtained from Women and Child Development Officer. Belgaum city was arbitrarily divided in zones of North, South, East and West.

Five preschools from each zone thus 20 preschools from Belgaum city were randomly selected and approached for permission. Principals of the selected preschools were explained about the study procedure. However permission was granted by only 14 schools. (Annexure XIII). The study sample of 195 subjects was selected from these 14 schools.

Children from selected schools were screened for the following study criteria

Inclusion Criteria:

- Children aged 3-4 years with minimum of one initial caries lesion as per Nyvad classification and Diagnodent score
- Parents willing to give consent.
- Child should have dmft of 3 to 6.

Exclusion Criteria:

- Children with any GIC restorations, pit and fissure sealants
- Developmental alterations like hypoplasia or flurosis.
- Use of orthodontic device or appliance.
- Being under medical treatment or taking medication in any form and
- Parents not willing to give consent.

2.5.5. Study procedure –Study comprised of two phases -Screening phase and Intervention phase.

- **Screening phase** –This phase consisted collection of data pertaining to parental perception concerning LOC and dental caries status among their young ones. Sensitization lecture on oral health was conducted during this phase. Caries prevalence of the preschool children was recorded and they were screened for inclusion criteria. On predetermined date; parents were called to attend an oral health educational talk of about 20-25 minutes delivered by principal investigator on "How best to prevent tooth decay in your young ones?" (Annexure XIV). Parents were sensitized regarding initiating oral hygiene at the earliest, importance of primary dentition, examination of tooth for detection of any carious lesions, proper feeding habits etc. Parents were also demonstrated Fones method of brushing and general principles to be followed for maintaining good oral health. They were also explained about the purpose and procedures of the study. After the talk, parents were invited for examination of their children. Consenting parents filled the proforma by providing socio demographic details, Locus of Control questionnaire, details regarding oral hygiene practices which was followed by child's examination.

Instruments and supplies Following instruments and supplies were used during screening procedure

- Plain mouth mirror
- CPI probes
- Explorers
- Diagnodent pen

- Cotton and gauze
- Tweezers
- Kidney tray
- Mouth mask and gloves
- Chemical Disinfecting solution – Savlon
- Hand towels
- Proforma

Armamentarium used for health education

- Laptop/LCD display
- Tooth model with brush
- Dental fluoride varnish

Armamentarium for saliva collection

- 2 ml Ependorf tubes with 0.5ml RTF
- Gauze,
- Gloves.

Armamentarium for varnish application

- Plane mouth mirrors
- Tweezers
- Kidney trays Containers (to segregate used and unused instruments)
- Gauze / cotton
- Disposable gloves and mouth masks
- Disposable plastic cups
- Sterile test tubes
- Fluoride Varnish

- Licorice Varnish
- Combination Varnish
- Korsolex disinfectant solution
- Varnish applicator brush
- Varnish dispensing well

Infection control – Autoclaved instruments were used throughout the study. Spot sterilization was done with chemical disinfectants. Diagnodent pen was sterilized after every examination using 70% Isopropanol.

2.5.6. Details of examination – Clinical examination of the child was done after obtaining parental consent. Examination was done in the preschool premises and child was accompanied by the parent.

Type III examination was followed to estimate caries prevalence which was measured using WHO dentition status 2013 [135]. The dmft was calculated from Dentition status. This was followed by search for initial lesions using Diagnodent pen and Nyvad's index. Investigator carried out all the examinations. A trained clerk recorded the findings.

Manual of operations pertaining to screening, collection of baseline data and for interventions were prepared prior to the initiation of the study, which were followed throughout the study. Recording of WHO Dentition status 2013 to calculate dmft using CPI probe and mouth mirror (ANNEXURE XV). This was followed by recording of initial lesion using Diagnodent pen.

Steps followed to record scores for initial lesions using Diagnodent pen

Child was made to sit comfortably on the chair.

- Teeth were cleaned with gauze and then air dried for 3-5 seconds.
- The entire tooth surface was scanned thrice and the single peak value was recorded.
- Peak values of the examination were recorded and were classified as per Lussi scale [140] which are as follows:-

Surface examined	Normal	Initial lesion	Restoration required
Occlusal	0-12	13-24	25 and above
Inter proximal	0-7	8-15	16 and above

- After the screening, parents of those children who fulfilled the inclusion criteria were informed about presence of initial lesions.
- Consenting parents were requested to sign a consent form and child was recruited into the study. (Annexure XVI)

Intervention phase – During the screening phase 407 children were examined, among whom 198 children fulfilled the inclusion criteria. Every subject who was selected was given a number in continuous sequence from 1 to 198 and random allocation was done to either one of the three groups - Group I (Licorice varnish), Fluoride Varnish (Group II) and Combination Varnish (Group III).

Random Allocation: The subjects were allocated randomly to one of the three groups using computer generated random number.

Concealment of Allocation: Concealment of allocation was done using sequentially numbered sealed opaque envelopes.

Blinding: Parents were unaware about the group to which their children belonged.

Children were blinded to the varnish group they belonged, as varnish was dispensed in identical amber colored bottles.

Clinical examination for invivo study

The intervention phase was performed in mobile dental van. Following steps were performed as per Standard Operating Procedures at baseline stage

- Recording of Oral Hygiene Score
- Recording of Diagnodent score
- Recording of Nyvad's index
- Collection of saliva sample
- Application of respective varnishes
- Post application instructions were given

Recording of Modification of Oral Hygiene Simplified index (Miglani et al. 1973)[132] Pattern of examination and scoring remain same as used by J.C.Greene and J.R.Vermillion in their Oral hygiene index simplified, except for the fact that following six surfaces were scored in primary dentition-

- Buccal surface of second upper primary molars
- Lingual surface of second lower primary molars
- Labial surface of upper right incisor
- Labial surface of lower left primary central incisor

Side of the sickle explorer was used for examining oral debris .The debris as well as calculus scores were recorded and mean of Debris and Calculus scores was added to arrive at oral hygiene score.

Recording of Diagnodent score for initial lesions. [133]

- Teeth were cleaned with gauze and then air dried for 3-5 seconds.
- The entire tooth surface was scanned thrice and the single peak value was recorded.
- Pictorial chart was used to draw site of the initial lesion. This chart would enable us to evaluate the same site for remineralisation after the period of 1 year.
- Calibration was repeated after every 10 students as per manufacturing instructions.
- Fissure Probe F was used for occlusal initial lesions and Proxa Probe A for smooth surfaces.
- Diagnodent tips were calibrated on a sound buccal surface/labial surface of teeth before the examination of the proximal / occlusal surface.

However some precautions are necessary prevent getting false positive results like the

- Tips should be aligned correctly on the test surfaces
- Prior to examination Tooth surface must be cleaned and dried without causing dehydration
- Entire surface should be carefully scanned with repeating beep pulses indicating good signal reception.

Recording of Nyvad's index [40] for visual inspection of initial lesions

- Cotton roll isolation was used.
- Compressed air was used for about 4-5 seconds to dry the teeth, followed by examination using mouth mirror and standard explorers.
- Activity was measured using 3 scores of Nyvad's scale

Score	Category	Criteria
0	Sound	Normal enamel translucency and texture
1	Active caries (intact surface)	Surface of enamel is whitish / yellowish opaque with loss of luster; generally covered with plaque. No clinically detectable loss of substance. Smooth surface – caries lesion typically located close to gingival margin.
3	Active caries (Cavity)	Enamel/ dentin cavity easily visible with naked eye; surface of cavity feel soft or leathery on gentle probing.
4	Inactive caries (intact surface)	Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the probe is moved gently across the surface. Smooth surface- caries lesion typically located at some distance from gingival margin.

Collection of saliva sample [141]

Unstimulated salivary sample was collected for estimation of salivary *Streptococcus mutans* count. Salivary sample was collected every time between 10-11.00 am to match with the circadian rhythm. Children were instructed to refrain drinking or eating except water, for 30 minutes prior to saliva collection. Children were made to sit comfortably for 2 minutes with mouth wide open. Unstimulated saliva was allowed to pool in the floor of the mouth. 0.5ml of saliva was then collected in 2 ml ependorf tube containing 0.5ml of Reducing Transport Fluid. Immediately after collection, sample was sent to laboratory for microbiological processing.

Application of respective varnishes [142]

- Each subject was instructed to rinse the mouth with plain water.
- Sterile cotton and gauze were used to clean the teeth.
- Varnish application was performed quadrant wise sequentially starting from the lower arches and then continued to the upper arch.
- Dropper was used to dispense 0.2 ml of varnish in to custom wells.
- Disposable brush was used to apply varnish to the teeth.
- It was air dried for 1 minute.
- Printed instructions were given as well as telephonic calls as a reminder were made to parents to reinforce the instructions to be followed which included –

- Child should not eat anything for 2 hours post varnish application,
- Avoid solid food for rest of the day and
- Refrain from brushing for next 24 hours.
- Parents were also instructed that their child would be able to appreciate varnish applied on the teeth. Once brushing is resumed, varnish would be removed.

Intermediate applications of the respective varnishes

Same procedure as mentioned above was followed for every applications of varnish which was carried out 4 times during baseline, 3rd month, 6th month and 9th month of study period.

Final phase- At end of one year Diagnodent scores, Nyvad's scores were reassessed.

Salivary sample was recollected for salivary estimation of *Streptococcus mutans.*

- Oral hygiene instructions along with demonstration of brushing technique was given to all the children.
- Children were gifted with a toothbrush and fluoridated toothpaste containing 450ppm sodium monofluorophosphate along with free referral card of the institute.

Laboratory procedure [82]

Sample was mixed together for 15 seconds in a cyclomixer. One loop(10µl) was inoculated on Mitis Salivarius Agar (MSA) medium and plates were incubated

for 48hrs at 37 ^{0} C in 5-10% CO_2 jar. After 48 hours, number of colonies of different types of Streptococcus *mutans* as well as their characteristics was studied. The typical colony morphology of *Streptococcus mutans*(0.5- 1.5mm raised convex opaque light blue colored colonies with rough margins, granular frosted glass appearance or a glistening bubble often accumulates on top of the colony when excess glucan is synthesized from sucrose) was observed. The numbers of colonies was counted with magnifying lens and after taking the dilution factor into consideration the *S.mutans* were expressed as number of CFU/ml of saliva. Semi quantification of the *S.mutans* number was product obtained between actual number of colony count and $1*10^2$ as sample was diluted 200 times. Ten microlitre of saliva was taken for culture ($1/100^{th}$ of a ml) and saliva was diluted in equal quantity of RTF, final dilution factor is 200.

Confirmation of the *Streptococcus mutans*(143) All colonies provisionally identified as S.mutans were confirmed by biochemical tests of Mannitol and Sorbitol fermentation test. Electronic colony counter manufactured by Deep Vision Company, Chennai, India was used to count colonies. All the microbiological procedures were performed by microbiology technician, blinded to culture plates of different groups and the group from which the salivary sample was collected. The CFU/ml of diluted saliva were measured based on the values obtained per 100 µl of diluted saliva.

Adverse events –No adverse events were encountered during study period. All the three varnishes were well accepted by the study participants.

Outcome measures

Primary outcome measure - Following were the primary outcome measures at end of one year

- Regression or progression of initial lesion
- Change in the Diagnodent score
- Change in the Nyvad's index
- Change in *Streptococcus mutans* count

Secondary outcome measure

- Study participants acceptability of the varnishes
- To assess if there is relation between ECC experience of children with their parental LOC.

3. DATA ANALYSIS PLAN

Data was entered in the excel sheet for further application of statistics and checked for entry. To minimize errors in data entry, 10% of the data was randomly rechecked with hard copy. SPSS version 20 (SPSS Inc, Chicago IL) was used for statistical analysis. For all the tests, when p value less than 0.05, it was considered statistically significant. Descriptive statistics served to summarize data.

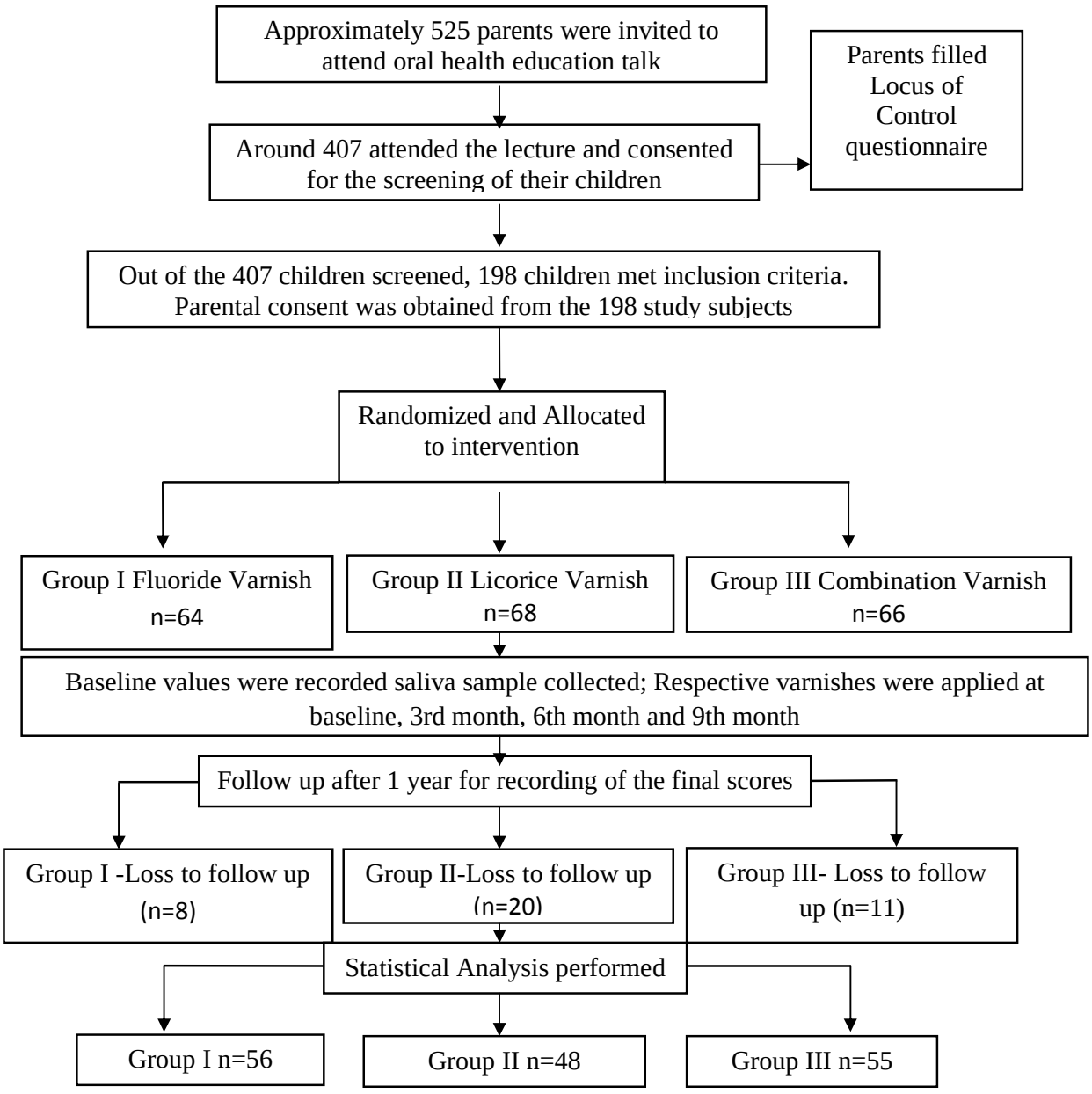
Following tests were used

- Chi square test - Categorical variables were compared using Chi Square Test.
- Mann Whitney U test –To compare the difference in baseline variables between Study and Control groups and also to compare difference in outcome variables between study and control groups.
- Kruskall Wallis test- For intergroup comparison of mean of baseline values and post intervention values between three groups.
- Wilcoxon matched pair test – It was used for comparison of paired data. It was used to find difference in the outcome variables within groups in comparison to baseline value. Intragroup comparison for Diagnodent scores, salivary *Streptococcus mutans,* OHIS score and Nyvad's index were calculated using this test.
- Predictive values like sensitivity, specificity and "Cut off" values for Area under ROC for Locus of Control were estimated using SPSS.
- Logistic regression analysis was used as a prediction model to identify factors which were significant for the successful outcome of the intervention. It was

used for adjusting the effects of various confounders on association between dependent and independent variables. The dependent variable was the outcome of intervention and all the independent variables like socio demographic factors, oral hygiene practices, location of the initial lesion and oral hygiene score were entered into the model. A backward stepwise logistic regression was used in the current study.

- Protocol analysis was performed on children who completed the 1 year follow up and received all the applications. Intention to treat analysis was conducted on those children who were lost during follow up and last observation carried forward (baseline value) was used in this analysis.

Flow diagram 1 of the study methodology

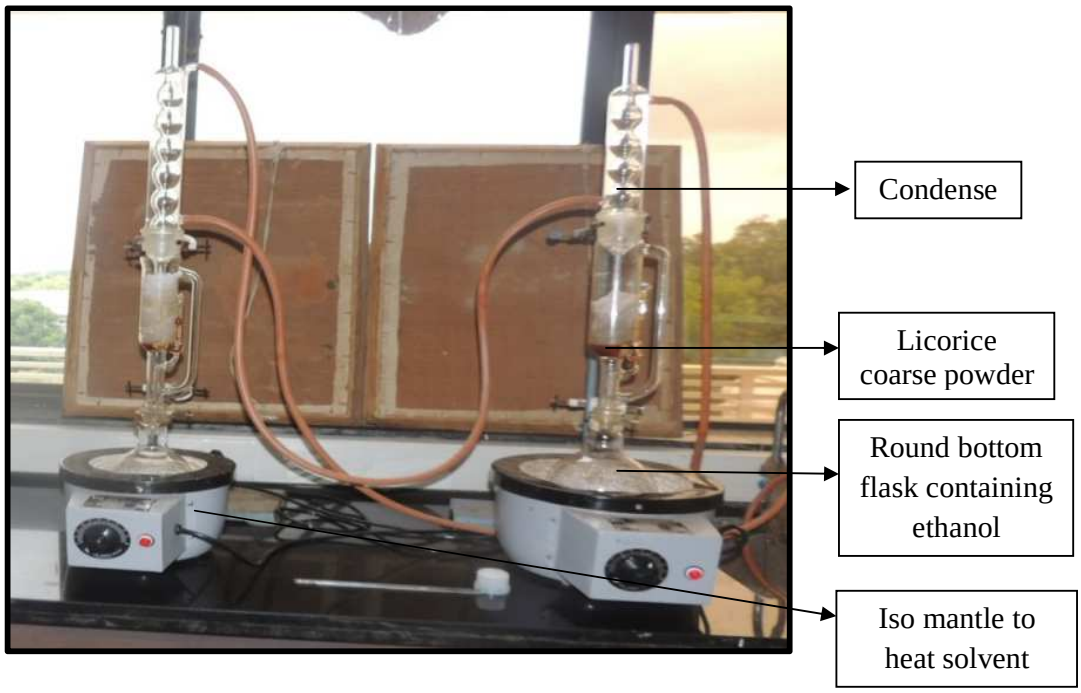


PHOTOGRAPHS

Photograph 1: Invitro study

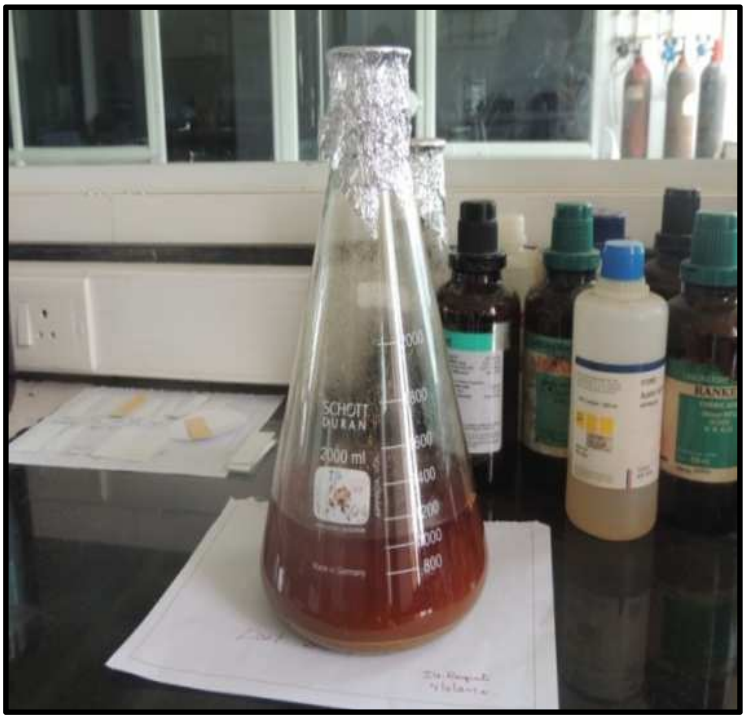
Photograph 2: Coarse licorice powder

Photograph 3: Preparation of the extract



Photograph no 4: Soxhlet Apparatus for Preparation of *Licorice root powder*

Crude extract

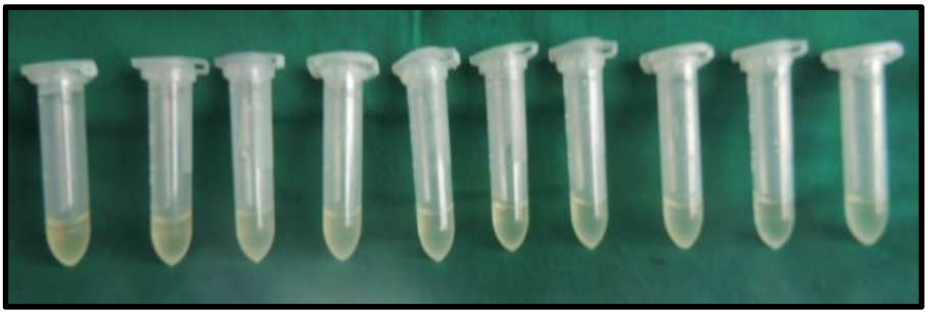


Photograph no 5: Cold maceration of licorice extract

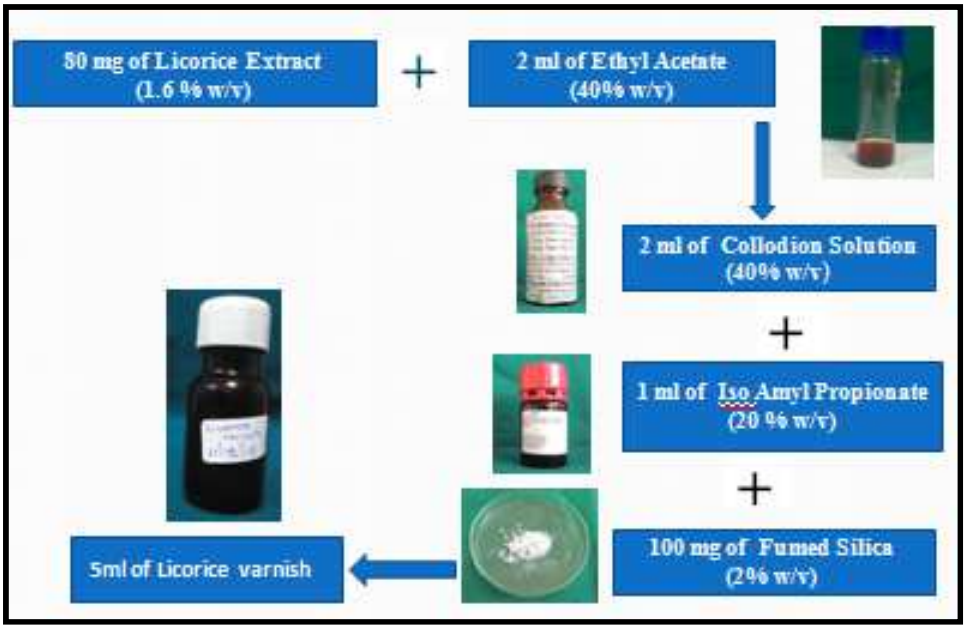
Photograph no 6: IKA Rotary evaporator to extract ethanol from crude extract

Photograph no 7: Licorice extract

Photograph no 8: MIC of Licorice extract (obtained from cold maceration) against *S.mutans*



Photograph no 9: MIC of Licorice extract (obtained from Soxhlet method) against *S.mutans*



Photograph no 10: Steps of Indigenous Licorice varnish preparation

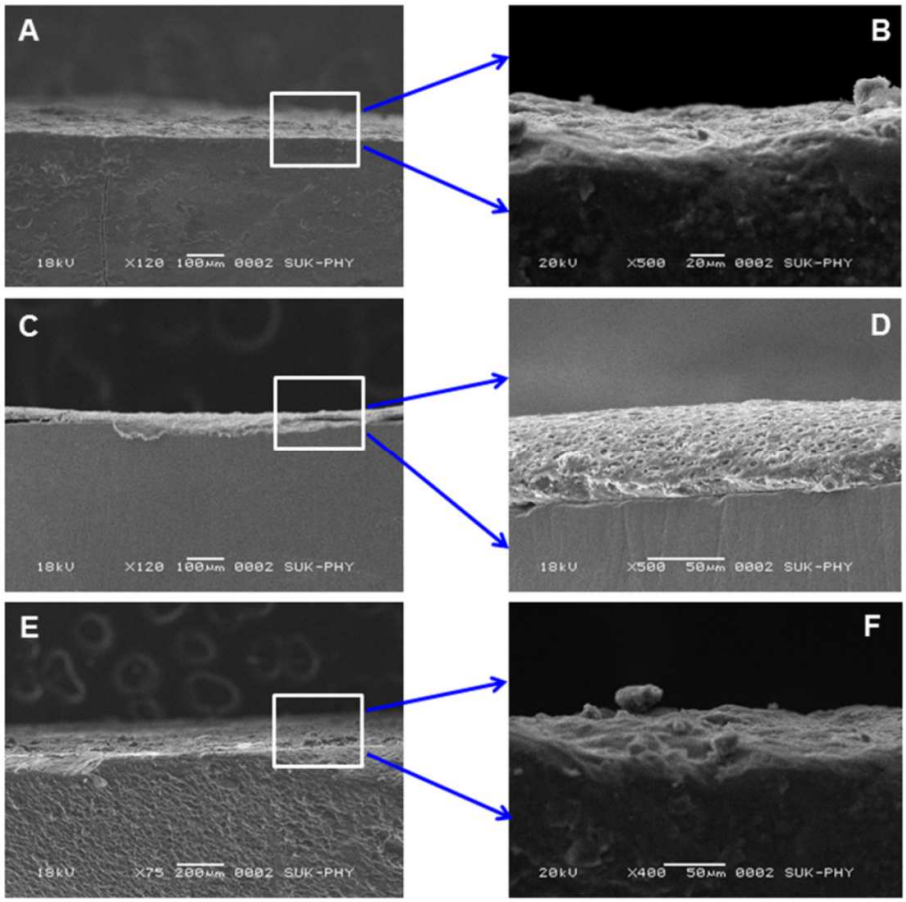
Hard tissue microtome

3 mm thickness slice of human enamel

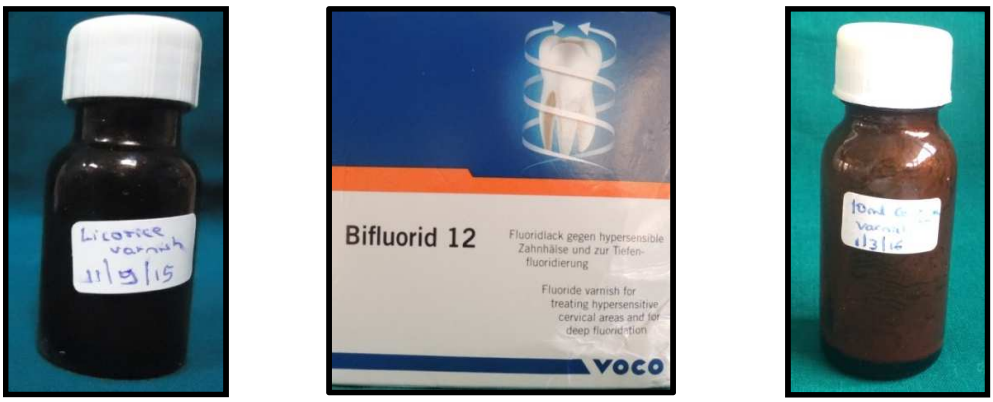
Gold coating application on the enamel specimens

JOEL Scanning Electron Microscope

Photograph no 11: Preparation of tooth sample for scanning electrom microscopy

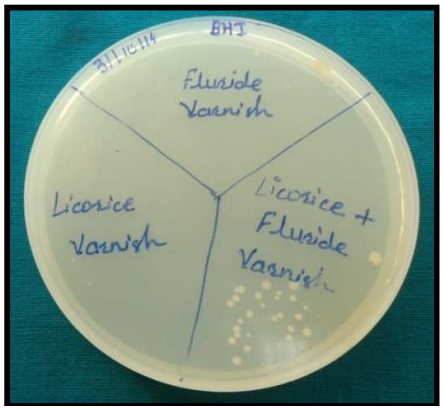


Photograph no 12: Morphological characterization of films formed on the tooth surface after application of varnishes.SEM images of I -Licorice varnish, II- Fluoride varnish, III- Combination varnish.(A,C,E side view; B,D,F expansion view).

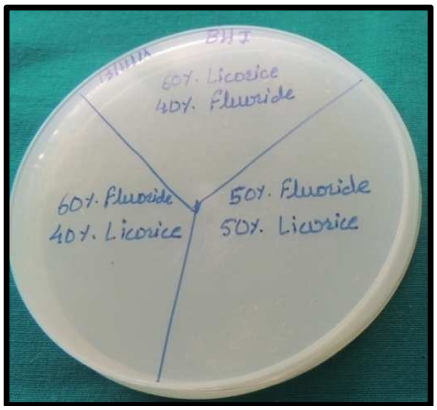


Licorice Varnish **Fluoride Varnish** **Combination Varnish**

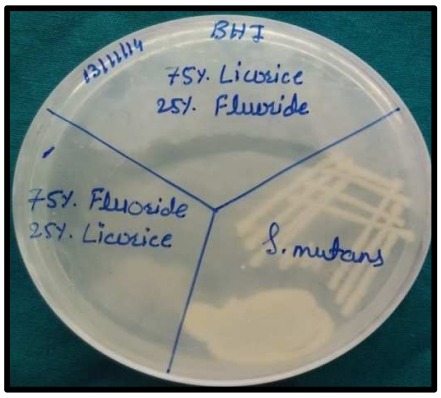
Photograph no 13: Three varnishes used in the study



a



b



c

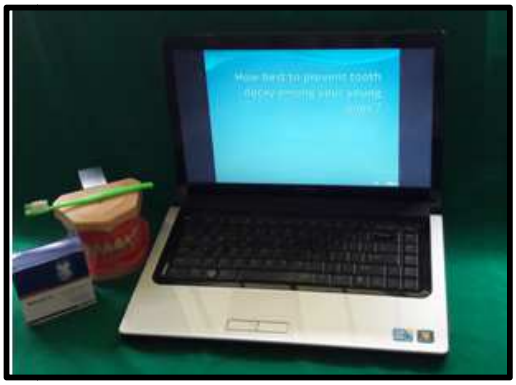
d

Photograph no 14: MIC of Fluoride varnish, Licorice varnish and various concentrations of combination varnishes by Direct method

Photograph no 15: Armamentarium used for microbiological investigation

Photograph no 16: Diagnodent kit

Photograph no 17: Armamentarium used for Invivo study



Photograph no 18: Armamentarium used for health education

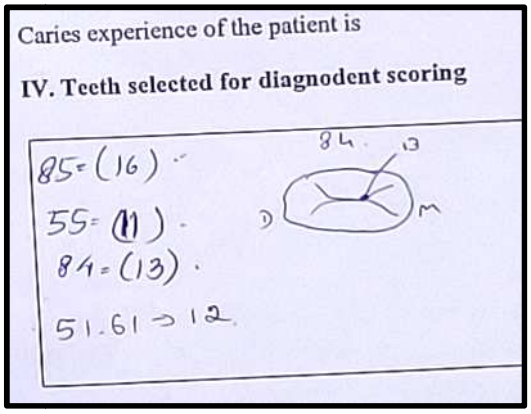
Photograph no 19: Parents being educated about "How best to prevent tooth decay in your young ones?"

Photograph no 20: Parents filling the LOC questionnaire

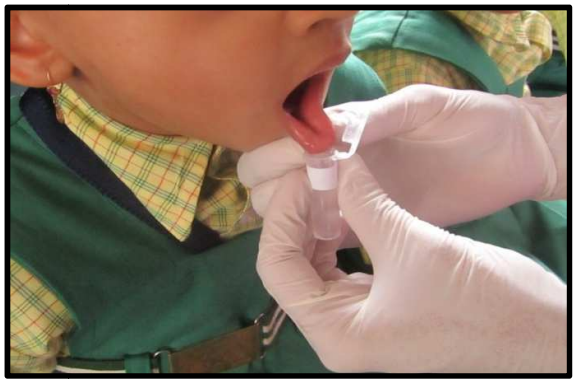
Photograph no 21: Parents signing the consent form

Photograph no 22: Recording of OHIS score

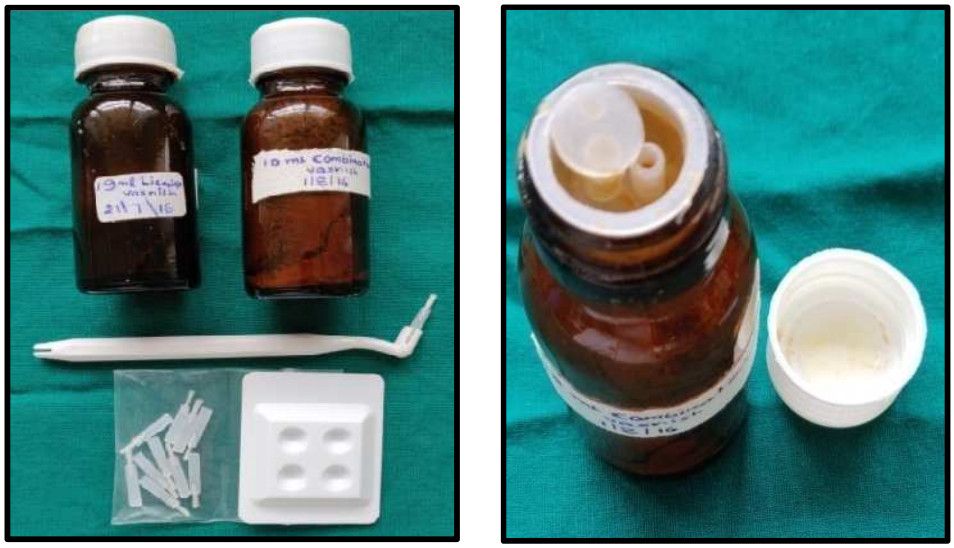
Photograph no 23: Recording of Diagnodent score



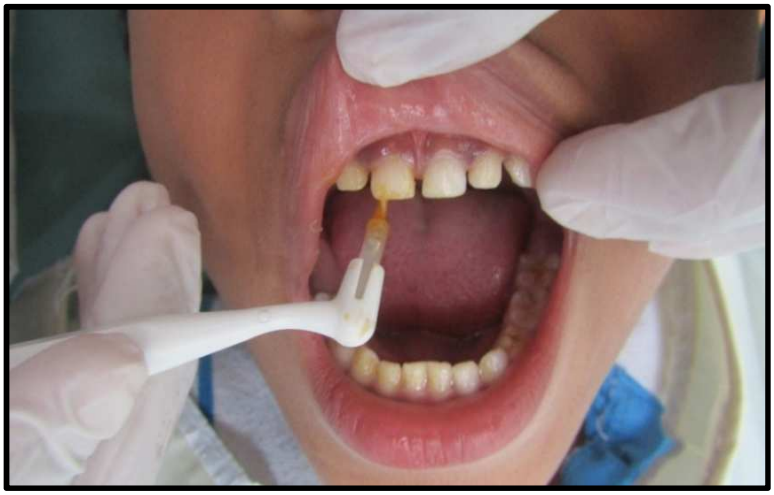
Photograph no 24: Pictorial chart for recording site of the lesion for future comparisons



Photograph no 25: Salivary sample collection using drooling method



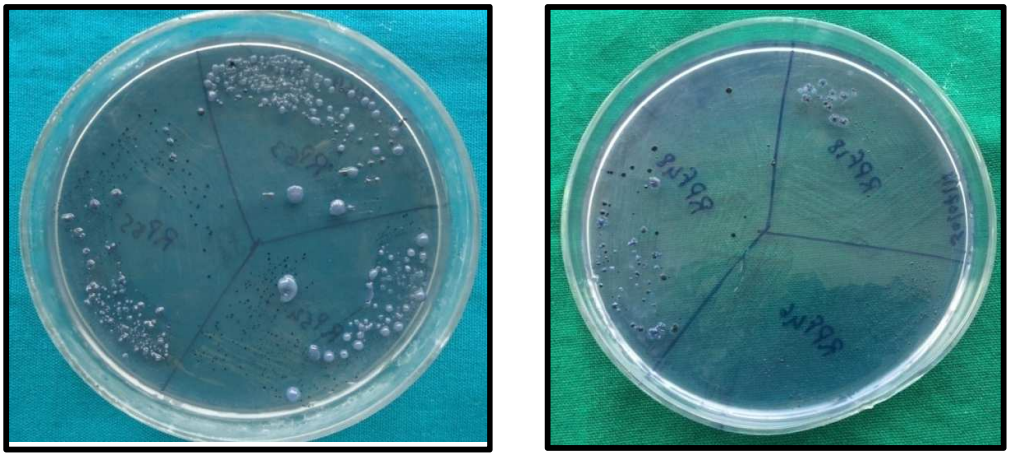
Photograph no 26: Armamentarium used for varnish application



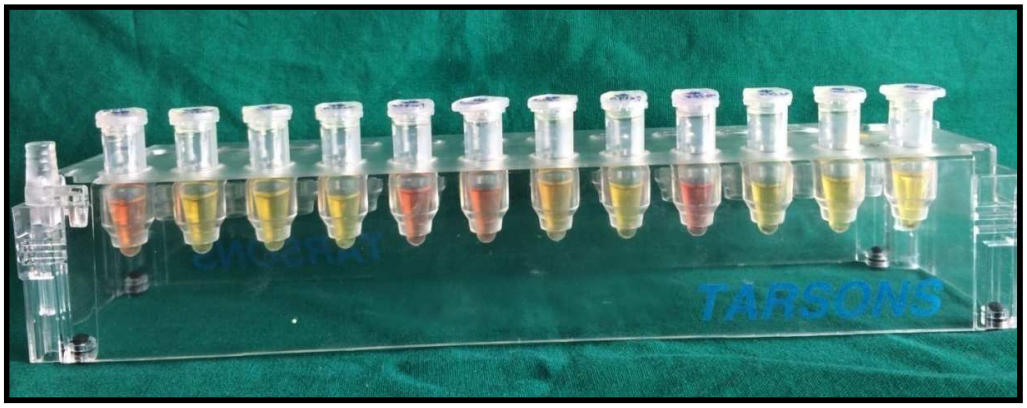
Photograph no 27: Varnish application

Photograph no 28: Recording of Diagnodent score post intervention after 1 year

Photograph no 29: Demonstration of brushing technique



Photograph no 30: Comparision of salivary *Streptococcus mutans* CFU/ml at baseline and post intervention



Photograph no 31: Sorbitol fermentation confirmatory test for *S.mutans*

4. RESULTS

- **Results of the In vitro study**

The present assessed effectiveness of three varnishes on ECC in preschool children of Belagavi city. The study was done in 2 stages- Invitro and Invivo. In vivo was further divided into Screening phase and Intervention phase.

Table 1 shows Mean value of MIC (triplicate experiment) of Licorice extract obtained through two methods against *Streptococcus mutans.* The experiment was repeated thrice and cold maceration extract had mean MIC value of 1.8mg/ml±0.145 and Soxhlet method had mean MIC value of 3.80 mg/ml±0.097 .When unpaired T test was applied and difference found was significant with a p value of 0.0001 with 4 degree of freedom and t value of -19.180

Table 2 shows Phytochemical screening of extracts. Phytochemical evaluation of the licorice extract showed that the following phytochemical constituents were present in both the extracts: Sterols, triterpenoids, alkaloids, tannins, Saponins, reducing sugars and Anthroquinones. Alkaloids, proteins and Aminoacids. Carbohydrates, Fats and oils, Glycosides, Flavanoids, Saponins, Triterpenes Sterols, Phenols, Tannins.

Table 3 shows the result of MIC of licorice extract and the three varnishes against *Streptococcus mutans.* Licorice extract showed antibacterial activity against standard strain of *Streptococcus mutans* with a MIC value of 2mg/ml. Both the Licorice varnish and Combination varnish had antibacterial activity when tested against *S.mutans.* Combination varnish was tested in five different combinations: -

80% Licorice Varnish + 20% Fluoride Varnish which failed to show antibacterial activity. Other combinations tested were 50% Licorice Varnish + 50% Fluoride Varnish, 60% Licorice Varnish + 40% Fluoride Varnish, 75% Licorice Varnish + 25% Fluoride Varnish and 60% Fluoride Varnish + 40% Licorice Varnish and all these combinations showed antibacterial activity to *Streptococcus mutans.*

Table 4 shows comparison of various physical parameters of the three varnishes. Fluoride varnish had a rate of evaporation of 150 seconds, with pH of 4, viscosity of 48Pa's, shelf life of 2 years and costs about Rs 4500/bottle. Indigenously prepared Licorice varnish had a rate of evaporation of 156 seconds, with pH of 4.5, viscosity of 52Pa's, shelf life of 4 months and costs about Rs 700 /bottle. Combination varnish had a rate of evaporation of 160 seconds, with pH of 4.5, viscosity of 49 Pa's, shelf life of 4 months and costs about Rs 2200 /bottle.

Diagram 1.Toxicity study on- Primary Gingival Fibroblasts;Normal, Adult(HGF) ATCCPCS-201-018

Toxicity study on- Primary Gingival Fibroblasts;Normal,Adult(HGF) ATCCPCS-201-018 revealed 80% cell viability with Fluoride Varnish , 86% with Licorice varnish and 87% with Combination varnish respectively.

RESULTS OF THE INVIVO STUDY –

SCREENING PHASE

Table 5 .Prevalence of ECC according to age. Out of the total 407 children examined, 119 were 3 year old and 288 were 4 years old. Among 3 year old 36(30.3%) were caries free whereas 83(69.7%) suffered from. Among 4 year old, 73(25.3%) had no caries whereas 215(74.7%) suffered from caries.

Thus among the total screened children, 109 (26.78%) had no caries whereas 298(73.21%) suffered from caries.

Table 6 Prevalence of ECC according to gender. Out of the total 407 children examined, 204 were males and 203 were females. Among males 50(24.5%) were caries free whereas 154(75.5%) suffered from caries. Among females, 59(29.1%) had no caries whereas 144(70.9%) suffered from caries.

Thus among the total screened children, 109 (26.78%) had no caries whereas 298(73.21%) suffered from caries.

Table 7 Prevalence of Initial lesions according to age. Out of the total 407 children examined, 119 were 3 year old and 288 were 4 years old. Among 3 year old initial lesion was absent in 63 (52.95%) and present in 56 (47.05%). Among 4 year old, initial lesion was absent in 146 (50.7%) and present in 142 (49.30).Thus among the total screened children, initial lesion was absent in 209 (51.355%) and present in 198 (48.64%).

Table 8 Prevalence of Initial lesions according to gender. Out of the total 407 children examined, 204 were males and 203 were females. Among males, initial

lesion was absent in 50 (24.5%) and present in 154 (75.5%). Among females, initial lesion was absent in 59(29.1%) and present in 144(70.9%). Thus among the total screened children, initial lesion was absent in 109 (51.35%) and present in 298 (48.64%) children.

Table 9 ECC's association with various demographic and oral hygiene factors. A total of 407 children were examined among whom, 119 were 3 year old and 288 were 4 year old. Among 3 year old, 83(69.7%) had caries whereas 215(74.7%) among 4 year old had caries. Difference found was not significant with respect to age. (p=0.31) Of the total children, 204 were males and 203 were females. Among males, 154(75.5%) and 144(70.9%) of females had caries. Difference found was not significant(p=0.30). Of the total children, 70 belonged to upper class among whom 46(65.7%) had caries, 192 were of middle class, 146(76.0%) had caries, 118 were of lower middle class whereas 83(70.3%) among them had caries where as 27 were of lower class among whom 23(85.2%) had caries.

Caries prevalence among various socio economic classes was not statistically significant.(p=0.15) Among the total children 389 used toothbrushes to brush and 287(73.8%) had caries, whereas 18 used finger to clean teeth among whom 11(61.1%) had caries. Difference found was not significant (p=0.28). Of the total children, 152 brushed teeth themselves among them, 115(75.7%) had caries, where as 255 had parental assistance for brushing and among them 183(71.8%) suffered from caries. Difference found was not significant (p=0.39) Among the total sample 229 brushed once daily among them 162(70.7%) had caries, whereas 178 brushed twice daily and 136(76.4%) of them suffered from caries and difference found was not significant 20) When asked about frequency in changing toothbrush, 297 replied that they changed

brush once every 3 months and 217(73.1%) had caries , 74 changed every 6 months and 57(77.0%) had caries whereas 36 of them changed brush in more than 6 months and 24(66.7%) had caries. Difference found was not significant for caries prevalence. (p=0.51)

Table 10 shows Distribution of Parental responses to "Locus of Control" questionnaire on 5point likert scale. When asked "As a family, we are confident that we can reduce the chances of our child developing decay." 272 (66.8%) of the parents agreed to it, 116 (28.5%) did not know and 19 (4.7) disagreed to the statement. Mean LoC score was 1.85 (1.03) When asked "As parents, responsibility lies with us to take measures and prevent our child from developing decay 283 (69.5) of the parents agreed to it, 120 (29.5) did not know and 4 (1.0) disagreed to the statement. Mean LoC score was 1.67 (0.93) When asked "Dentist is responsible to prevent our child from developing decay" 283 (69.5) of the parents agreed to it, 150 (36.9%) did not know and 141 (34.6%) disagreed to the statement. Mean LoC score was 3.18 (1.21%). When asked "No matter how best we try , our child will develop decay." 173 (42.5%) of the parents agreed to it, 141 (34.6%) did not know and 93 (22.9%) disagreed to the statement. Mean LoC score was 2.86 (1.08%).

When asked "We can prevent development of decay in our children by restricting sugary foods between meals." 178 (43.7%) of the parents agreed to it, 179 (44.0%) did not know and 50 (12.3%) disagreed to the statement. Mean LoC score was 2.45 (1.49%). When asked "It just happens that child develops decay." 147 (36.1%) of the parents agreed to it, 156 (38.3%) did not know and 104 (25.6%) disagreed to the statement. Mean LoC score was 2.95 (1.05%) When asked "Brushing twice daily, can help in preventing our child developing decay in the future." 262

(64.4%) of the parents agreed to it, 121 (29.7%) did not know and 24 (5.9%) disagreed to the statement. Mean LoC score was 1.92 (1.06%) When asked "If our child develops decay, it is by chance." 196 (48.2) of the parents agreed to it, 125 (30.7%) did not know and 86 (21.1%) disagreed to the statement. Mean LoC score was 2.77 (1.07%).

When asked "It will hardly make any difference to child's decayed tooth even if we assisted him/her to brush every day.." 153 (37.6%) of the parents agreed to it, 133 (32.7%) did not know and 121 (29.7%) disagreed to the statement. Mean LoC score was 3.06 (1.16%). When asked "Some have soft teeth naturally." 107 (26.3%) of the parents agreed to it, 188 (46.2%) did not know and 112 (27.5%) disagreed to the statement.

Mean LoC score was 3.14 (1.06%). When asked "As a family, we plan to control the frequency of sugary foods consumed by our child with or between meals." 213 (52.3%) of the parents agreed to it, 168 (41.3%)did not know and 26 (6.4%) disagreed to the statement. Mean LoC score was 2.19 (1.04%). When asked "It is because of bad luck that our child develops decay." 227 (55.8%) of the parents agreed to it, 110 (27.0%) did not know and 70 (17.2%) disagreed to the statement. Mean LoC score was 2.61 (1.10%). When asked "best person to prevent development of decay in child is the dentist." 57 (14.0%) of the parents agreed to it, 163 (40.0) did not know and 187 (45.9%) disagreed to the statement. Mean LoC score was 3.58 (1.16%).

Table 11 shows cut off values for the LoC scores as estimated from Receiver Operating Curve. The cut off value for locus of control score was 29.5, lower the score parent is likely to have more internal LoC. The sensitivity value was 0.826 and specificity was 0.284.Area under the curve was 0.55 and the p value was 0.13 which

was statistically non significant.

Table 12:- Association of dental caries experience with Locus of control scores below and above cut off point. When parental LoC scores were arranged, 83 of the parents had LoC score with less than or equal to 29.5 and 52(62.7%) of their children had caries.324 of the parents had LoC score more than 29.5 and 246 (75.9%) of their children had caries .Significant difference was found with caries prevalence and LoC scores (p=0.02) when Fischer test was applied.

Lesser score means that parents had good LoC, and it was statistically significant and the Odds ratio was calculated as 1.88. Parents with internal LoC had 1.8 times higher chance to raise caries free children than parents with external LoC.

Table 13 Association of LoC scores in quintiles with caries experience. When LoC scores were into five quintiles, 63 of the parents had LoC score less than 28.5 and 40(63.5%) had caries. 93 of the parents had LoC score ranging from 29 – 32.5and 69(74.2%) had caries. 69 of the parents had LoC score ranging from 33 – 35.5and 50(72.5%) had caries. 83 of the parents had LoC score ranging from 36 – 38.5 and 65(78.3%)had caries. 99 of the parents had LoC score greater than 39 and 74(74.7%) had caries. The prevalence of caries across LoC quintiles was statistically non significant.(p=0.14).

Lesser score means that parents had internal LoC and it was statistically significant. Kids whose Parents had internal LoC had 1.8 times more chance to be caries free then parents who had E LoC.

Table 14 Multiple logistic regression model which depicts effect of multiple risk factors on the caries experience.

In the logistic regression model, dental caries experience was dependent variable with age, gender, socioeconomic status and dichotomized Locus of control score as independent variable. Among all the variables, LoC score showed significant association with caries experience.

RESULTS OF THE INVIVO STUDY INTERVENTIONAL PHASE

Table 15 (Diagram 1)Study subjects age distribution .Among 198 study subjects, 56 (28.3%) and 142(71.7%) were 3 and 4 year old respectively. mean age being 3.72 ± 0.45.

Table 16. (Diagram 2) Study subjects Gender distribution. Among 198 study subjects, 103 (52%) and 95 (48%) were male and females respectively.

Table 17 shows study subject's distribution according to father's education. Among the total 198 parents, 8 (4%) had middle school education, 43 (21.7%) had High school education, 54 (27.3%) had PUC education, 74 (37.4%) had Graduate education, 19 (9.6%) had profession education.

Table 18 shows Study subject's distribution according to father's occupation. Among the total 198 parents, 4 (2%) had Unskilled occupation, 13 (6.6%) had Semi skilled occupation, 21 (10.6%) had skilled occupation, 113 (57.1%) had Clerical occupation, 34 (17.7%) had Semi profession occupation and 13 (6.5%) had profession occupation.

Table 19 shows Study subject's distribution according to mother's education. Among the total 198 parents, 6 (3%) were illiterate, 1 (0.5%) primary education, 3(1.5%) had middle school education, 51 (25.8%) had High school education, 51 (25.8%) had PUC education, 79 (39.9%) had Graduate education, 7 (9.6%) had

Profession education.

Table 20 shows Subject's distribution according to mother's Occupation. Among the total 198 parents, 170 (85.9%) were homemakers, 2 (1%) had Semi skilled occupation, 1 (0.5%) had skilled occupation, 21 (10.6%) had Clerical occupation, 2 (1%) had Semi profession occupation and 2 (1%) had profession occupation.

Table 21 Study subjects distribution according to family income. Among the total 198 parents, 4 (2.02%)had family income less than Rupees 1520, 7 (3.54%)had family income ranging from Rupees 1521-4555, 21 (10.61%) had family income ranging from Rupees 4556-7593, 45 (22.73%) had family income ranging from Rupees 7594-11361, 6 (3.03%) had had family income ranging from Rupees 11362-15187 and 41 (20.71%) had family income ranging from Rupees 30375 or more.

Table 22 Study subject's distribution according to Kuppuswamy's socio economic classification. Out of 198 subjects, 31(15.7%) belonged to upper most class, 89 (44.9%) were of Upper middle class, 78 (39.4%) belonged to Lower class.

Table 23.Study subjects distribution according to oral hygiene aid with age, gender and socio economic classification among study subjects.

All the 56 subjects of 3 year old 136 (95.8%) of 4 year old used brush as oral hygiene aid. No significant difference found between age groups and oral hygiene aid (p=0.295).Five subjects and 1 subject of 4 year old used Finger and datum respectively. Among males, 101 (98.1) used brush, 91 (95.8) of females used toothbrush as oral hygiene aid. No significant difference found between gender and oral hygiene aid.(p=0.0.497) All 31 subjects of upper most class used toothbrush as oral hygiene aid where as 88 (98.9%) of upper middle class and 73 (93%) of lower

class used toothbrush. No significant difference found between SES and oral hygiene aid.(p=0.262) . Note –Lower Middle Class was clubbed with Upper Middle Class whereas Upper Lower Class was added to Lower Class.

Table 24. Study subject's distribution according to self brushing /parental assistance for brushing with age, gender and socio economic classification. Among 3 year old, 43 (76.8%) subjects and in 4 year old 72 (50.7%) subjects teeth were brushed by parents which was statistically significant.(p=0.001) Among gender, 60 (58.3%) and 55 (57.9%) of girls teeth were brushed by parents and and difference found was not significant. (p=.537) In upper most class 22 (71.0%), middle class 58 (65.2%) and in lower class 35 (44.9%) had parental assistance for brushing which was statistically significant.(p=0.008). Note –Lower Middle Class was clubbed with Upper Middle Class whereas Upper Lower Class was added to Lower Class.

Table 25. Study subject's distribution according to according to frequency of brushing with age, gender and socio economic classification. Among 3 year old, 31 (55.4%) and among 4 year old 67 (47.2%) brushed once daily, however this factor is found to be no significant found.(p=0.441) Among boys, 48 (46.6%) and among girls 50 (52.6%) brushed once daily however difference found is not significant. (p=0.574) Among social class, 13 (41.9) of the upper class, 46 (51.7) of middle class, 36 (46.2) of lower class brushed 2 times daily and difference found is not significant.(p=0.333)

Table 26. Study subject's distribution according to material used for brushing with age, gender and socio economic classification. Almost all 56 (100.0) of 3 year old and 140 (98.6) of 4 year old used toothbrush with paste,this factor is not found to be significant (p=0 .513) When gender comparison was used 102 (99.0) of boys and 94 (98.9) of girls used brush with paste and difference is not found to be significant.

(p=0 .731) When SES was compared 31 (100.0) of upper most class, 88 (98.9%) of upper middle class and 77 (98.7) of lower class used toothpaste with brush and this factor is not found to be significant (p=0 .825)

Table 27 Subject's distribution according to frequency of changing toothbrush with age, gender and socio economic classification. When age wise comparison was done, 40(71.4%) of 3 year old and 111(78.2%) changed brush every 3 months, this factor is not found to be significant (p=0 .480) When gender wise comparison was done,76(73.8%) of boys and 75(78.9%) of girls changed brush every 3 months this factor is not found to be significant (p=0 .528) When SES was compared, 26(83.9)of upper class,61(68.5%) of upper middle class and 64(82.1%) of lower class changed brush every 3 months this factor is not found to be significant (p=0 .113)

Table 28. (Diagram 3)Study subject's distribution among varnish groups. There were 64(32.3%), 68(34.3%) and 66(33.3%) of subjects in Fluoride, Licorice and Combination group respectively.

Table 29(Diagram 4) Study subject's distribution according to caries severity at baseline. The caries experience ranged from 1-3 dmft (mild caries) was 7 (31.8%) in Fluoride Varnish group, 57 (32.4%) in Licorice varnish and 62 (35.2%) combination varnish. The caries experience ranged from 4-6 dmft (moderate caries) was 57(32.4%) in Fluoride Varnish group, 11 (50.0%) in Licorice varnish and 4 (18.2%) Combination varnish, this factor is not found to be significant (p=0.176)

Table 30 Study subjects distribution as per mean baseline Diagnodent scores. Among 54 children who had mean Diagnodent scores ranging from 0 - 2. 50, 24 (44.4%) belonged to Fluoride, 19 (35.18) and 11 (20.03%) belonged to Licorice and

Combination group respectively. Among 109 children who had mean Diagnodent scores ranging from 2.51-2.99, 30 (27.5%) belonged to Fluoride, 36 (33.0%) and 43 (39.4%) belonged to Licorice and Combination group respectively. Among 3children who had mean Diagnodent scores ranging from 3.00-3.50, one subject belonged to each group. Among 32 children who had mean Diagnodent scores ranging from 3.51-4.00, 9 (28.1%) belonged to Fluoride, 12 (37.5%) and 11 (34.4%) belonged to Licorice and Combination group respectively. This factor is not found to be significant (p=0.143).

Table 31 (Diagram 5) Shows study subject's distribution according to Diagnodent score grouping at baseline and post intervention.

At baseline none of the subjects belonged to Diagnodent score group of 0-4 whereas Post intervention 32 subjects belonged to this group. Diagnodent score group of 4.01-10.00 had 28 subjects at baseline which changed to 68 subjects post intervention Diagnodent score group of 10.01-18 had 132 subjects at baseline which changed to 21 subjects post intervention Diagnodent score group of 18.01 and above had 38 subjects at baseline which remained same post intervention.

Table 32. (Diagram 6) Distribution of study subjects according to Diagnodent score grouping at baseline and post intervention among three varnish groups. In fluoride varnish group at baseline, none of the subjects belonged to 0-4.00 group, 6 subjects belonged to 4.01-10.00group, 46 belonged to 10.01-18.00 and 12 belonged to 18.01 and above. After post intervention 8 subjects belonged to 0-4.00 group, 20 subjects belonged to 4.01-10.00group, 10 belonged to 10.01-18.00 and 18 belonged to 18.01 and above.

In Licorice varnish group at baseline , none of the subjects belonged to 0-4.00 group, 13 subjects belonged to 4.01-10.00group, 10 belonged to 10.01-18.00 and 45 belonged to 18.01 and above. After post intervention 15 subjects belonged to 0-4.00 group, 23 subjects belonged to 4.01-10.00group, 5 belonged to 10.01-18.00 and 5 belonged to 18.01 and above.

In Combination varnish group at baseline , none of the subjects belonged to 0-4.00 group,9 subjects belonged to 4.01-10.00group, 46 belonged to 10.01-18.00 and 11 belonged to 18.01 and above. After post intervention 9 subjects belonged to 0-4.00 group, 25 subjects belonged to 4.01-10.00group, 6 belonged to 10.01-18.00 and 15 belonged to 18.01 and above.

Table 33. Descriptive statistics of pre and post intervention Diagnodent scores 198 subjects formed sample and the Diagnodent score at 25^{th}, 50^{th} and 75^{th} percentile were 12.00, 15.00 and 18.00 respectively. Post intervention there were 159 subjects and the Diagnodent score at 25^{th}, 50^{th} and 75^{th} percentile was 6.00, 9.00 and 22.00 respectively

Table 34. (Diagram 7) Shows distribution of the initial lesions in pre and post intervention in maxillary arch in the three varnish groups. At baseline there were 64 subjects in FV group with 0.87 as mean of IL in upper quadrant which reduced to 56 subjects and mean of IL to 0.32 post intervention and significant difference was found.($Z=3.96$; $p<0.001$) LV group had 68 subjects in Licorice varnish with 1.27 as mean of IL in upper quadrant which reduced to 48 subjects and mean of IL to 0.19 post intervention and significant difference was found ($Z=8.58$; $p<0.001$. There were 66 subjects in CV group with 1.25 as mean of IL in upper quadrant which changed to 55 subjects and mean of IL to 0.38 post intervention and this factor was found to be

significant (Z=6.23; p<0.001). Overall at baseline there were 198 subjects with 1.14 as mean number of initial lesions in upper quadrant which reduced to 55 subjects and mean of IL to 0.38 post intervention and this factor was found to be significant. (Z=6.23; p<0.001)

Table 35. (Diagram 8) Distribution of the initial lesions (IL) in pre and post intervention in mandibular arch in three varnish groups. Altogether there were 64 subjects in FV group with 0.81 as mean of IL was in mandibular arch which changed to 56 subjects and mean of IL to 0.32 post intervention and difference found was significant.(Z=3.50879; p<0.001) Altogether there were 68 subjects in Licorice varnish group with 0.85 as mean of IL in lower quadrant which changed to 48 subjects and mean of IL to 0.08 post intervention and difference found was significant (Z=8.58; p<0.001). There were 66 subjects in Combination varnish group at baseline with 0.87 as mean of IL in lower quadrant which changed to 55 subjects and mean of IL to 0.48 post interventions and this was statistically significant (Z=6.23; p<0.001). Overall at baseline there were 198 subjects with 0.76 as mean of IL in mandibular arch which changed to 55 subjects with 0.18 as mean of IL in post intervention and difference found was significant.(Z=6.23; p<0.001)

Table 36. (Diagram 9) Distribution of the initial lesions (IL) in pre and post intervention in anterior arch in three varnish groups. There were 64 subjects in Fluoride Varnish group with 0.87 as mean of IL in anterior quadrant which changed to 56 subjects with 0.32 as mean of IL to post intervention difference found was significant (Z=3.48; p<0.001) .There were 68 subjects in Licorice varnish group at baseline with 1.79 mean of IL in anterior quadrant which changed to 48 subjects with 0.21 mean of IL to post intervention and difference found was significant.(Z=10.56;

p<0.001) There were 66 subjects in Combination varnish group with 1.83 mean of IL in anterior quadrant which changed to 55 subjects with 0.47 mean of IL to post intervention. (Z=8.08; p<0.001) and difference found was significant. Overall at baseline phase there were 198 subjects the 1.51 mean of IL was in anterior quadrant which changed to 55 subjects with 0.34 as mean of IL to post intervention.(Z=8.08; p<0.001) and difference found was significant.

Table 37. (Diagram 10) Distribution of the initial lesions in pre and post intervention in posterior arch in three varnish groups. There were 64 subjects at baseline in FV group with 0.81 mean of IL in posterior arch which changed to 0.32 post intervention and difference found was significant.(Z=3.48; p<0.001). At baseline there were 68 subjects in Licorice varnish group with 0.33 mean of IL in posterior quadrant which changed to 0.21 post intervention difference found was significant (Z=10.56; p<0.001) There were 66 subjects in Combination varnish group with 0.06 mean of IL in posterior arch which reduced to 0.05 post intervention difference found was not significant.(Z=0.11; p>0.05) Overall there were 198 subjects at baseline with 0.39 mean of IL was in posterior arch which changed to 0.15 post intervention difference found was significant.(Z=3.62; p<0.001) There was a drop out of 39 subjects.

Table 38. (Diagram 11) Distribution of the IL in pre and post intervention on smooth surface lesions in three varnish groups. There were 64 subjects in FV group with 0.92 mean of IL was on smooth surface which changed to 0.33 post intervention and this factor was found to be significant (Z=3.48; p<0.001). At baseline there were 68 subjects in Licorice varnish with 1.83 mean of IL on smooth surface which changed to 0.19 post intervention and this factor was found to be

significant.(Z=10.56; p<0.001 There were 66 subjects in Combination varnish group with 1.84 mean of IL on smooth surface which changed to 0.45 post intervention and this factor was found to be significant(Z=8.08; p<0.001). Overall there were 198 subjects at baseline with 1.54 mean of IL on smooth surface lesions which changed to 0.33 post intervention and this factor was found to be significant. (Z=8.08; p<0.001) There was a drop out of 39 subjects.

Table 39. (Diagram 12) Distribution of the IL in pre and post intervention on pit and fissures in three varnish groups. There were 64 subjects in FV group with 0.76 mean of IL on pit and fissures which changed to 0.30 post intervention and this factor was found to be significant (Z=3.07; p<0.001). There were 68 subjects in Licorice varnish group with 0.27 mean of IL was in pit and fissures which changed to 0.06 post intervention which was statistically significant (Z=10.56; p<0.001). There were 66 subjects in Combination varnish group with 0.04 mean of IL on pit and fissures which changed to 0.036 post intervention and this factor was found to be significant.(Z=8.08; p<0.001). Overall there were 198 subjects at baseline with 0.35 mean of IL was on pit and fissures which changed to 0.13 post intervention and this factor was found to be significant found.(Z=3.31; p<0.001) There was a drop out of 39 subjects.

Table 40. Comparison of Diagnodent scores among baseline and post intervention among varnish groups. There were 64 subjects in FV group at baseline with a mean Diagnodent score of 16.14±4.29 and median score of 15.50 which changed to 56 subjects with a mean Diagnodent score of 25.55±27.43 and 12 as a median score post intervention. There were 68 subjects in Licorice varnish group at baseline with a mean Diagnodent score of 14.60 ± 4.05 and median score of 14.00 which changed to 48 subjects with a mean Diagnodent score of 11.73 ± 17.58 and 7 as

median score post intervention. There were 66 subjects in Combination varnish group at baseline with a mean Diagnodent score of 14.49± 3.88 and median score of 14.00 which changed to 55 subjects with a mean Diagnodent score of 21.85 ± 28.50 and 9 as median score post intervention. Difference was not significant at baseline when test of Kruskall Wallis was applied. However difference found was significant when Kruskall Wallis test which was applied for post intervention data. When Mann Whitney test was applied for post intervention Diagnodent scores, difference found was significant between Fluoride Varnish and Licorice varnish group. When Wilcosxon Sign rank test was applied, a significant difference found with Licorice varnish group for before and after Diagnodent scores.

Table 41. (Diagram 13) Pre and post intervention Median Diagnodent scores across varnish groups. In Fluoride Varnish group, baseline scores were 3, 4 and 6 in I Quartile, II Quartile and III Quartile respectively. At post intervention the quartile score changed to 2,2.5 and 4 in I Quartile, II Quartile and III Quartile respectively. In licorice varnish group, baseline scores were 3, 3 and 3 in I Quartile, II Quartile and III Quartile respectively. At post intervention the quartile score changed to 2,1 and 2 in I Quartile, II Quartile and III Quartile respectively. In combination varnish group, baseline scores were 3, 3 and 3 in I Quartile, II Quartile and III Quartile respectively. At post intervention the quartile score changed to 2, 2 and 4 in I Quartile, II Quartile and III Quartile respectively.

Table 42. (Diagram 14) Comparison of Nyvad's score between varnish groups in the post intervention phase. In FV group, out of 56 subjects, initial lesion had changed to inactive caries in 34(60.7%) where as it remained active in 22 (39.30%) subjects. In the licorice varnish group, out of 48 subjects, initial lesion had changed to

inactive caries in 42 (87.50%) where as it remained active in 6 (12.50%) subjects. In the combination varnish group, out of 55 subjects, initial lesion had changed to inactive caries in 39 (70.90%) where as it remained active in 16 (29.10%) subjects. Difference in conversion from initial lesion to inactive lesion among three varnish groups was statistically significant. $p<0.001$.

Table 43. (Diagram 15) Caries incidence among varnish groups. Out of 56 subjects in Fluoride Varnish group, 29(51.80%) had no new caries incidence, 20 (35.70%) had 1-3 new caries lesion and 7 (12.50%) had 4-10 caries incidence. Out of 48 subjects in Licorice varnish group, 30 (62.50%) had no new caries incidence, 16 (33.33%) had 1-3 new caries lesion and 2 (4.16%) had 4-10 caries incidence. Out of 55 subjects in Combination varnish group, 39 (70.90%) had no new caries incidence, 15 (27.30%) had 1-3 new caries lesion and 1 (1.80%) had 4-10 caries incidence. A significant difference in caries incidence found among varnish groups. Chi square value- 7.8;df 4; $p=0.100$

Table 44(Diagram 16).Shows Distribution of Salivary *Streptococcus mutans* CFU/ml (log_{10} scores) at baseline and post intervention in the three varnish groups. There were 64 subjects in FV group with 3.20 ± .50 mean of *Streptoccous mutans* cfu/ml (log10 scores) which reduced to 3.10 ±0.40 post intervention and this difference was non significant.(Z=1.215). At baseline there were 68 subjects in Licorice varnish with 3.29 ± 0.80 mean of *Streptococcus mutans* cfu/ml (log10 scores) which reduced to 3.06 ± 0.35 post intervention and this difference was significant. (Z=2.097; $p<0.05$). There were 66 subjects in Combination varnish group with 3.11 ± 0.87 mean of *Streptococcus mutans* cfu/ml (log10 scores) which reduced to 2.81± .48 post intervention and this difference was significant. (Z=2.386; $p<0.05$).

Overall there were 198 subjects at baseline with 3.22 mean of *Streptococcus mutans* cfu/ml (log10 scores) which reduced to 2.99 ± 0.41 post intervention and this difference was significant. ($Z=3.450$; $p<0.001$).There was a drop out of 39 subjects.

Table 45. Distribution of Post intervention oral hygiene score in varnish groups in comparison to baseline scores. Oral hygiene scores at baseline in FV group is as follows- 31 subjects belonged to 0 score, 16 belonged to score1, 7 belonged to score 2 and 2 belonged to score 3.After intervention 32, 15, 7 and 2 belonged to score 0, 1, 2 and 3 respectively. However difference found was significant between scores of baseline and post intervention. Oral hygiene score at baseline in Licorice varnish group is as follows- 17 subjects belonged to score 0,4 had OHI score of 1, 12 had score of 2 and 12 had score of 3.Post intervention , 21 subjects had score of 0, 8 had OHIS score of 1,10 had score of 2 and 9 had OHIS score of 3.The difference in scores of OHIS between pre and post intervention differed from each other significantly. Oral hygiene score at baseline in Combination varnish group is as follows- 23 subjects belonged to score 0,while 15 had OHI score of 1, another 9 had score of 2 and 7 had score of 3.Post intervention , 29 subjects had score of 0, while 12 had OHIS score of 1,another 13 had score of 2 and 1 had OHIS score of 3. Difference in scores of OHIS between pre and post intervention differed from each other significantly.

Table 46 Study subjects of varnish groups who were referred to dentist for comprehensive treatment. Altogether 65 subjects were referred to dentist for comprehensive treatment among whom 17(26.20) belonged to Fluoride Varnish group, 31(47.70) and 17(26.20) belonged to Licorice and combination varnish group respectively and this factor found was of no significance.

Table 47. Study subjects distribution among varnish groups according to their visit to dentist. Out of the total 198 subjects, 65 subjects were referred to dentist for comprehensive treatment. Only 10 subjects actually visited dentist among whom 7 belonged to Licorice varnish group and 3 belonged to Combination varnish group. None of the subjects from Fluoride Varnish group visited the dentist and factor was found to be significant.

Table 48 Study subjects liking for taste of varnishes. Among 64 subjects in Fluoride Varnish group, 4 subjects did not like the taste of Fluoride Varnish, among 68 subjects of Licorice varnish group, 5 disliked the taste and among 66 subjects in Combination varnish group, 2 subjects disliked the taste. However difference found was non significanct.

Table 49(Diagram 17) Shows Study subjects distribution according to outcome of the intervention. Out of the total 56 subjects in Fluoride Varnish group, remineralization was not seen in 18(32.10%) subjects whereas remineralization was observed in 38 (67.90%)subjects. In Licorice varnish group, remineralization was absent in 4(8.33%) subjects and present in 44(91.60%) subjects and whereas remineralization was absent in 9(16.40%) subjects and present in 46(83.60%) subjects of combination varnish group. A significant difference was found with remineralization potential among 3 groups. $P<0.01$

Table 50 Shows subjects distribution according to remineralization of initial lesion (IL) post intervention. Out of the total 159 subjects, intervention had failed to remineralize any of the incipient lesions in 31 subjects. Among these 31 subjects, 18 belonged to Fluoride Varnish group, 4 belonged to Licorice varnish group and 9 belonged to Combination varnish group. At least 1 initial lesion was remineralized in

51 subjects, among whom 21 belonged to Fluoride Varnish group, 12 to Licorice varnish group and 18 to Combination varnish group. At least 2 initial lesions were remineralized in 44 subjects, among whom 11 belonged to Fluoride Varnish group, 14 to Licorice varnish group and 19 to Combination varnish group. At least 3 initial lesion was remineralized in 17 subjects, among whom 2 belonged to Fluoride Varnish group,9 to Licorice varnish group and 6 to Combination varnish group. At least 4 initial lesion was remineralized in 14 subjects, among whom 4 belonged to Fluoride Varnish group,8 to Licorice varnish group and 2 to Combination varnish group. At least 5 initial lesion was remineralized in only 1 subjects, who to Combination varnish group. Altogether 6 initial lesions were remineralized in only 1 subject who belonged to Licorice varnish group. Significant difference was found.

Table 51. Frequency distribution and results from logistic regression models of the variables in the study associated with success of the intervention (n=159). The Bivariate analysis of dichotomous were completed using $Wald^2$ from logistic regression. The dependent variable was the outcome of intervention and all the independent variables were entered into the model. A backward stepwise logistic regression was performed to remove the variables that were not statistically significant. The final model contained only those variables which were statistically significant. The level of significance for all tests was set at 0.05. Oral hygiene played a significant role for remineralising initial lesions and the intervention had 5.9 times more chances of succeeding in those who had good oral hygiene. Location of the tooth in anterior region was significant factor for success of intervention and anterior region had 7.6 times more chances to succeed. Type of varnish also made significant impact on the success of intervention. Fluoride Varnish group was 0.2 times worse than combination varnish which was significant; Licorice varnish was 2.5 times better

than combination varnish, however significant difference was not found.

Table 52. (Diagram 18, 19, 20) Comparison of Pre- post intervention Diagnodent scores across varnish groups using per protocol analysis and intention to treat analysis. As per protocol analysis in Fluoride Varnish group, post intervention mean Diagnodent score was 25.55 ± 27.43 with a median value of 12.When Intention to treat was applied in FV group for post intervention Diagnodent scores, mean Diagnodent score was 24.09 ± 25.965 with a median value of 12.5 was found. The effect size changed from 4.5 in per protocol analysis to 2.5 in ITT.

As per protocol analysis in Licorice varnish group, post intervention mean Diagnodent score was 11.73 ± 17.58 with a median value of 7.When Intention to treat was applied in LV group for post intervention Diagnodent scores, mean Diagnodent score was 12.75 ± 15.01 and a median value of 9 was found. The effect size changed from 6 in per protocol analysis to 5 in ITT.

As per protocol analysis in Combination varnish group, post intervention mean Diagnodent score was 21.85 ± 28.50 with a median value of 9. When Intention to treat was applied in CV group for post intervention Diagnodent scores, mean Diagnodent score was 20.64 ± 26.16 and a median value of 9.5 was found. The effect size changed from 5 in per protocol analysis to 4 in ITT.

When per protocol analysis and ITT were performed at baseline, difference found was not significant among groups, when Kruskall Wallis was applied.

However significant difference observed for test of Kruskall Wallis which was applied for post intervention data in both the analysis. When Mann Whitney test was applied for post intervention Diagnodent scores, significant difference was observed

between Fluoride Varnish and Licorice varnish group, in both the analysis. When Wilcosxon Sign rank test was applied, significant difference was noted with Licorice varnish group for before and after Diagnodent scores in both, per protocol and ITT analysis. Thus conclusions of per protocol analysis and ITT analysis are similar.

Table 1: Mean value of MIC (triplicate experiment) of Licorice extract obtained through two methods against *Streptococcus mutans*

Cold maceration	Soxhlet method
1.95 mg/ml	3.90 mg/ml
1.78 mg/ml	3.71 mg/ml
1.66mg/ml	3.78mg/ml
Mean value 1.8mg/ml±**0.145**	**Mean value** 3.80 mg/ml±**0.097**

T test applied T value -19.18, df=4, $p<0.001$

Table 2. Phytochemical screening of the extracts

Phytochemical test	**Cold maceration**	**Soxhlet method**
Test for sterols and tritepenoids • Salkowski test	+	+
Test for flavinoids • Alkaline reagent test	+	+
Test for Alkaloids • Hagers Test, • Mayers Test, • Dragendorrf ‘s Test	+	+
Test for Tannin • Lead Acetate Test • Ferric Chloride Test	+	+
Test for Saponins • Froth Test	+	+
Test for Reducing sugars • Benedicts Test	+	+
Test for Anthroquinones • Borntrager’sTest	+	+

Table 3. Result of MIC of licorice extract and the three varnishes against *Streptococcus mutans*

Test group	**Antibacterial activity against *S. mutans***
Licorice extract	Positive - **2.0mg/ml**
Licorice varnish	Positive
Fluoride Varnish	Positive
Combination varnish	
80% Licorice Varnish + 20% Fluoride Varnish	Negative
50% Licorice Varnish + 50% Fluoride Varnish	Positive
60% Licorice Varnish + 40% Fluoride Varnish	Positive
75% Licorice Varnish + 25% Fluoride Varnish	Positive
60% Fluoride Varnish + 40% Licorice Varnish	Positive

Table 4. Physical parameters of the three varnishes being compared

Varnish	**Rate of evaporation**	**pH**	**Viscosity**	**Shelf life**	**Cost**
Fluoride Varnish	150 seconds	4	48 Pa·s	Stable for 2 years	Rs 4500 per bottle †
Licorice varnish	156 seconds	4. 5	52 Pa·s	Shelf life 4 months	Rs 700 per bottle; 6-7 times cheaper ‡
Combination varnish	160 seconds	4.5	49 Pa·s	Shelf life 4 months	Rs 2200 per bottle

†Bifluorid 12 Voco product ; Pa·s - pascal seconds ‡Indigenously prepared

RESULTS OF THE INVIVO STUDY – SCREENING PHASE

Table 5 .Prevalence of ECC according to age

Age	Caries free	Caries present	Total
3 Yrs	36(30.3%)	83(69.7%)	119
4 Yrs	73(25.3%)	215(74.7%)	288
Total	109 (26.78%)	298(73.21%)	407

Table 6 Prevalence of ECC according to gender

Gender	Caries free	Caries present	Total
Male	50(24.5%)	154(75.5%)	204
Female	59(29.1%)	144(70.9%)	203
Total	109 (26.78%)	298 (73.21%)	407

Table 7 Prevalence of Initial lesions according to age

AGE	Initial lesions absent	Initial lesions present	Total
3 Yrs	63 (52.95%)	56 (47.05%)	119
4 Yrs	146 (50.7%)	142 (49.30%)	288
Total	209 (51.355%)	198 (48.64%)	407

Table 8 Prevalence of Initial lesions according to gender

Gender	Initial lesions absent	Initial lesions present	Total
Male	50(24.5%)	154(75.5%)	204
Female	59(29.1%)	144(70.9%)	203
Total	109 (51.35%)	298(48.64%)	407

Table 9 Association of Caries prevalence (ECC) with various demographic and oral hygiene factors.

Factors		Caries experience		Total	Chi square test	
		0	>1		Chi square value	p-value
Age In years	3	36(30.3%)	83(69.7%)	119	1.03	0.31(NS)
	4	73(25.3%)	215(74.7%)	288		
Gender	Male	50(24.5%)	154(75.5%)	204	1.08	0.30(NS)
	Female	59(29.1%)	144(70.9%)	203		
Kuppuswamy scale	Upper Class	24(34.3%)	46(65.7%)	70	5.26	0.15(NS)
	Middle	46(24.0%)	146(76.0%)	192		
	Lower middle	35(29.7%)	83(70.3%)	118		
	Lower class	4(14.8%)	23(85.2%)	27		
Clean teeth with the aid of	Tooth brush	102(26.2%)	287(73.8%)	389	1.03	0.28(NS)[#]
	Finger	7(38.9%)	11(61.1%)	18		
Teeth are Cleaned by	Self	37(24.3%)	115(75.7%)	152	0.74	0.39(NS)
	Parents	72(28.2%)	183(71.8%)	255		
How often brushing performed?	Once daily	67(29.3%)	162(70.7%)	229	1.64	0.20(NS)
	Twice daily	42(23.6%)	136(76.4%)	178		
How frequently is the tooth Brush changed?	1-3months	80(26.9%)	217(73.1%)	297	1.34	0.51(NS)
	3-6 months	17(23.0%)	57(77.0%)	74		
	More than 6 months	12(33.3%)	24(66.7%)	36		

*p<0.05 statistically significant, p>0.05 non significant, NS [#]Fisher's exact test

Table 10:- Distribution of Parental responses to "Locus of Control" questionnaire on likert scale with 5 points.

	Agree (1- 2)	Do Not Know (3)	Disagree (4-5)	Mean (SD)
1.As a family, we are confident that we can reduce the chances of our child getting tooth decay.	272 (66.8%)	116 (28.5%)	19 (4.7%)	1.85 (1.03)
2.As parents, it is our responsibility to prevent our child getting tooth decay.	283 (69.5%)	120 (29.5%)	4 (1.0%)	1.67 (0.93)
3R.It is the responsibility of the dentist to prevent our child getting tooth decay.	116 (28.5%)	150 (36.9%)	141 (34.6%)	3.18 (1.21)
4R.No matter what we do, our child is likely to get tooth decay.	173 (42.5%)	141 (34.6%)	93 (22.9%)	2.86 (1.08)
5.We can prevent tooth decay in our child by reducing sugary foods and drinks between meals.	178 (43.7%)	179 (44.0%)	50 (12.3%)	2.45 (1.49)
6R.It just happens that children get tooth decay.	147 (36.1%)	156 (38.3%)	104 (25.6%)	2.95 (1.05)
7.If we brush our child's teeth twice a day, we can prevent our child getting tooth decay in the future.	262 (64.4%)	121 (29.7%)	24 (5.9%)	1.92 (1.06)
8R.If our child gets tooth decay, it is by chance.	196 (48.2)	125 (30.7%)	86 (21.1%)	2.77 (1.07)
9R.It would not make any difference to our child getting tooth decay, if we helped him/her brush every day.	153 (37.6%)	133 (32.7%)	121 (29.7%)	3.06 (1.16)
10R.Some people just naturally have soft teeth.	107 (26.3%)	188 (46.2%)	112 (27.5%)	3.14 (1.06)
11.As a family, we intend controlling how often our child has sugary foods or drinks between meals.	213 (52.3%)	168 (41.3%)	26 (6.4%)	2.19 (1.04)
12R.It is just bad luck if our child gets tooth decay.	227 (55.8%)	110 (27.0%)	70 (17.2%)	2.61 (1.10)
13R.The dentist is the best person to prevent tooth decay in our child	57 (14.0%)	163 (40.0)	187 (45.9%)	3.58 (1.16)

Table 11:- Cut off values for the LoC **scores as estimated from Receiver Operating Curve.**

Cut off value for Locus of control score	Sensitivity	Specificity
29.5	**0.826**	**0.284**

Area under the curve	Std. Error	p-value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.55	0.03	0.13	0.48	0.61

Table 12 Shows Association of dental caries experience with Locus of control scores below and above cut off point.

		Caries experience		Total	Chi square test		Odds ratio (95% CI)
		0	>1		Chi square value	p-value	
Total	<=29.5	31(37.3%)	52(62.7%)	83	5.94	0.02*	1.88(1.13 – 3.14)
	>29.5	78(24.1%)	246(75.9%)	324			

*p<0.05 statistically significant, p>0.05 non significant, NS

#Fisher's exact test

Table 13:-Association of LoC **scores in quintiles with caries experience** LoC **scores**

LoC scores		Caries experience		Total	Trend chi square test		Odds ratio (95% CI)
		0	>1		Chi square value	p-value	
Total (quintile)	<28.5	23(36.5%)	40(63.5%)	63	2.16	0.14(NS)	-
	29 – 32.5	24(25.8%)	69(74.2%)	93			1.65(0.83-3.30)
	33 – 35.5	19(27.5%)	50(72.5%)	69			1.51(0.73-3.16)
	36 – 38.5	18(21.7%)	65(78.3%)	83			2.07(0.99-4.32)
	>39	25(25.3%)	74(74.7%)	99			1.70(0.86- 3.38)

*p<0.05 statistically significant, p>0.05 non significant, NS

#Fisher's exact test

Table 14 :- Effect of multiple risk factors on the caries experience as per multiple logistic regression model.

Risk factors	B	S.E.	Wald	df	p-value	Odds ratio	95% C.I.for odds ratio	
							Lower	Upper
Age(1)	0.24	0.25	0.92	1	0.34(NS)	1.27	0.78	2.07
Gender(1)	-0.27	0.23	1.36	1	0.24(NS)	0.77	0.49	1.20
Kuppuscale			4.24	3	0.24(NS)			
Kuppuscale(1)	0.46	0.31	2.23	1	0.14(NS)	1.58	0.87	2.89
Kuppuscale(2)	0.12	0.33	0.14	1	0.71(NS)	1.13	0.59	2.16
Kuppuscale(3)	0.92	0.61	2.28	1	0.13(NS)	2.52	0.76	8.34
Total dico(1)	0.58	0.27	4.66	1	0.03*	1.78	1.06	3.00
Constant	0.23	0.38	0.39	1	0.53(NS)	1.26		

Variable(s): Age, Gender, Kuppuscale, Total LoC score adjusted.

*p<0.05 statistically significant, p>0.05 non significant, NS

#Fisher's exact test

RESULTS OF THE INVIVO STUDY –INTERVENTIONAL PHASE

Table 15. Age wise distribution of the study subjects.

Age	Frequency	Percent	Mean±SD
3 years	56	28.3	3.72 ± 0.45
4 years	142	71.7	
Total	198	100	

Table 16. Gender wise distribution of the study subjects.

Gender	Frequency	Percent
Male	103	52
Female	95	48
Total	198	100

Table 17. Study subject's distribution according to father's education

Father's education	Frequency	Percent
Middle school	8	4
High school	43	21.7
PUC	54	27.3
Graduate	74	37.4
Profession	19	9.6
Total	198	100

Table 18. Study subject's distribution according to father's occupation

Father's occupation	Frequency	Percent
Unskilled	4	2
Semi skilled	13	6.6
Skilled worker	21	10.6
Clerical	113	57.1
Semi profession	34	17.7
Profession	13	6.5
Total	198	100

Table 19. Study subject's distribution according to mother's education

Mother's education	Frequency	Percent
Illiterate	6	3
Primary	1	0.5
Middle school	3	1.5
High school	51	25.8
PUC	51	25.8
Graduate	79	39.9
Profession	7	3.5
Total	198	100

Table 20. Subject's distribution according to mother's occupation

Mother's occupation	Frequency	Percent
Unemployed/house wife	170	85.9
Semi skilled	2	1
Skilled worker	1	0.5
Clerical	21	10.6
Semi profession	2	1
Profession	2	1
Total	198	100

Table 21 Shows distribution of the study subject's according to family income.

Monthly Family income	Frequency	Percent
<1,520 Rupees	4	2.02
1,521-4,555 Rupees	7	3.54
4,556-7,593 Rupees	21	10.61
7,594-11,361 Rupees	45	22.73
11,362-15,187 Rupees	6	3.03
15,188-30,374 Rupees	74	37.37
30,375+ Rupees	41	20.71
Total	198	100

Table 22 Study subject's distribution according to Kuppuswamy's socio economic classification

Kuppuswamy's socio economic classification	Frequency	Percent
Upper most class	31	15.7
Upper middle class	89	44.9
Lower class	78	39.4
Total	198	100

Note –Lower Middle Class is clubbed with Upper Middle Class and Upper Lower Class is clubbed with Lower Class.

Table 23.Association of oral hygiene aid with Age, Gender and Socio Economic classification among study subjects.

Age	Finger	Brush	Datum	Total	p value
3 Yrs	0 (0.0)	56 (100.0)	0 (0.0)	56 (100.0)	0.29(NS)
4 Yrs	5 (3.5)	136 (95.8)	1 (0.7)	142 (100.0)	
Total	5 (2.5)	192 (97.0)	1 (0.5)	198 (100.0)	
Gender					
Male	2 (1.9)	101 (98.1)	0 (0.0)	103 (100.0)	0.49(NS)
Female	3 (3.2)	91 (95.8)	1 (1.1)	95 (100.0)	
Total	5(2.5)	192 (97.0)	1 (.5)	198 (100.0)	
Socio economic classification					
Upper most class	0 (0.0)	31 (100.0)	0 (0.0)	31(100.0)	0.26(NS)
Upper middle class	1 (1.1)	88 (98.9)	0 (0.0)	89 (100.0)	
Lower class	4(5.1)	73 (93.6)	1 (1.3)	78 (100.0)	
Total	5 (2.52)	192(96.96)	1(0.50)	198 (100)	

Note –Lower Middle Class is clubbed with Upper Middle Class and Upper Lower Class is clubbed with Lower Class.
Chi square test applied

Table 24. Study subject's distribution according to self brushing /parental assistance for brushing with Age, Gender and Socio Economic classification.

Age	By child	Parents	Total	p value
3 Yrs	13 (23.2)	43 (76.8)	56 (100.0)	0.001*
4 Yrs	70 (49.3)	72 (50.7)	142(100.0)	
Total	83 (41.9)	115 (58.1)	198(100.0)	
Gender				
Male	43 (41.7)	60 (58.3)	103 (100.0)	0.53(NS)
Female	40 (42.1)	55 (57.9)	95 (100.0)	
Total	83 (41.9)	115 (58.1)	198 (100.0)	
Socio economic classification				
Upper most class	9 (29.0)	22 (71.0)	31 (100.0)	0.008*
Upper middle class	(31 34.8)	58 (65.2)	89 (100.0)	
Lower class	43 (55.1)	35 (44.9)	78 (100.0)	
Total	83 (41.9)	115 (58.1)	198 (100.0)	

*p<0.05 statistically significant;Chi square test applied

Table 25. Study subject's distribution according to according to frequency of brushing with Age, Gender and Socio economic classification.

Age	Once (%)	Twice(%)	After every meal (%)	Don't clean everyday (%)	Total (%)	p value
3 Yrs	31 (55.4)	25 (44.6)	0 (0.0)	0 (0.0)	56 (100.0)	0.44(NS)
4 Yrs	67 (47.2)	70 (49.3)	4 (2.8)	1 (0.7)	142 (100.0)	
Total	98 (49.5)	95 (48.0)	4 (2.0)	1 (0.5)	198 (100.0)	
Gender						0.57(NS)
Male	48 (46.6)	53 (51.5)	2 (1.9)	0 (0.0)	103 (100.0)	
Female	50 (52.6)	42 (44.2)	2 (2.1)	1 (1.1)	95 (100.0)	
Total	98 (49.5)	95 (48.0)	4 (2.0)	1 (0.5)	198 (100.0)	
Socio economic classification						0.33(NS)
Upper most class	16 (51.6)	13 (41.9)	2 (6.5)	0 (0.0)	31 (100.0)	
Upper middle class	42 (47.2)	46 (51.7)	0 (0.0)	1 (1.1)	89 (100.0)	
Lower class	40 (51.3)	36 (46.2)	2 (2.6)	0 (0.0)	78 (100.0)	
Total	98 (49.5)	95 (48.0)	4 (2.0)	1 (0.5)	198 (100.0)	

Note –Lower Middle Class is clubbed with Upper Middle Class and Upper Lower Class is clubbed with Lower Class.

Chi square test applied

Table 26. Study subject's distribution according to material used for brushing with Age, Gender and Socio economic classification

Age	Tooth paste and brush	Tooth paste and finger	Total	p value
3 Yrs	56 (100.0)	0 (0.0)	56 (100.0)	Fisher exact test 0.51 (NS)
4 Yrs	140 (98.6)	2 (1.4)	142 (100.0)	
Total	196 (99.0)	2 (1.0)	198 (100.0)	
Gender				0.73(NS)
Male	102 (99.0)	1 (1.0)	103 (100.0)	
Female	94 (98.9)	1 (1.1)	95 (100.0)	
Total	196 (99.0)	2 (1.0)	198 (100.0)	
Socio economic classification				0.82 (NS)
Upper most class	31 (100.0)	0 (0.0)	31 (100.0)	
Upper middle class	88 (98.9%	1 (1.1)	89 (100.0)	
Lower class	77 (98.7)	1 (1.3)	78 (100.0)	
Total	196 (99.0)	2 (1.0)	198 (100.0)	

Note –Lower Middle Class is clubbed with Upper Middle Class and Upper Lower Class is clubbed with Lower Class.

Chi square test applied

Table 27 Subject's distribution according to frequency of changing toothbrush with Age, Gender and Socio economic classification.

Age	1-3months	3-6months	6 Months or more	Total	p value
3 Yrs	40 (71.4)	11 (19.6)	5 (8.9)	56 (100.0)	0.48(NS)
4 Yrs	111 (78.2)	24 (16.9)	7 (4.9)	142 (100.0)	
Total	151 (76.3)	35 (17.7)	12 (6.1)	198 (100.0)	
Gender					0.52(NS)
Male	76 (73.8)	19 (18.4)	8 (7.8)	103 (100.0)	
Female	75 (78.9)	16 (16.8)	4 (4.2)	95 (100.0)	
Total	151 (76.3)	35 (17.7)	12 (6.1)	198 (100.0)	
Socio Economic Classification					0.113(NS)
Upper most class	26 (83.9)	5 (16.1)	0 (0.0)	31 (100.0)	
Upper middle class	61 (68.5)	19 (21.3)	9 (10.1)	89 (100.0)	
Lower class	64 (82.1)	11 (14.1)	3 (3.8)	78 (100.0)	
Total	151 (76.3)	35 (17.7)	12 (6.1)	198 (100.0)	

Note –Lower Middle Class is clubbed with Upper Middle Class and Upper Lower Class is clubbed with Lower Class.

Chi square test applied

Table 28. Study subject's distribution in the varnish groups.

	Frequency	Percent
Fluoride Varnish	64	32.3
Licorice varnish	68	34.3
Combination varnish	66	33.3
Total	198	100

Table 29 Study subject's distribution according to caries severity at baseline in the three varnish groups

Caries experience	Fluoride Varnish	Licorice varnish	Combination varnish	Total
1-3 dmft(mild caries)	57 (32.4%)	57 (32.4%)	62 (35.2%)	176 (100.0%)
4-6 dmft (moderate caries)	7 (31.8%)	11 (50.0%)	4 (18.2%)	22 (100.0%)
Total	64 (32.3%)	68 (34.3%)	66 (33.3%)	198 (100.0%)

Table 30 Shows study subject's distribution as per mean baseline Diagnodent scores

Mean of Baseline Diagnodent score	Fluoride Varnish	Licorice varnish	Combination varnish	Total	p value
0 - 2. 50	24 (44.4%)	19 (35.18	11 (20.03%)	54 (100.0%)	0.14(NS)
2.51-2.99	30 (27.5%)	36 (33.0%)	43 (39.4%)	109 (100.0%)	
3.00-3.50	1 (33.3%)	1 (33.3%)	1 (33.3%)	3 (100.0%)	
3.51-4.00	9 (28.1%)	12 (37.5%)	11 (34.4%)	32 (100.0%)	
Total	64 (32.3%)	68 (34.3%)	66 (33.3%)	198 (100.0%)	

Chi square test applied

Table 31 Shows study subject's distribution according to Diagnodent score grouping at baseline and post intervention

Diagnodent score group	Frequency of Study subjects at Baseline	Frequency of Study subjects Post intervention
0-4	0	32(20.12%)
4.01-10.00	28 (14.14%)	68 (42.76%)
10.01-18	132 (66.69%)	21(13.20%)
18.01 and above	38 (19.19%)	38(23.89%)
Total	198	159

Table 32. Distribution of study subjects according to Diagnodent score grouping at baseline and post intervention among three varnish groups.

Varnish group	Diagnodent score Group	0-4.00	4.01 to 10	10.01-18.00	18.01 and above
Fluoride varnish	Baseline score	0	6	46	12
	Post intervention score	8	20	10	18
Licorice varnish	Baseline score	0	13	10	45
	Post intervention score	15	23	5	5
Combination varnish	Baseline score	0	9	46	11
	Post intervention score	9	25	6	15

Table 33. Descriptive statistics of pre and post intervention Diagnodent scores.

Diagnodent scores	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25^{th}	50^{th} (Median)	75^{th}
Diagnodent score at baseline	198	15.03	4.15	7.00	33.00	12.00	15.00	18.00
Final Diagnodent score	159	20.10	25.77	1.00	99.00	6.00	9.00	22.00

Table 34. Shows distribution of the initial lesions in pre and post intervention in maxillary arch in the three varnish groups.

		Initial lesions in Maxillary arch at baseline	Initial lesions in Maxillary arch after 1 year	Z value
Fluoride Varnish	N	64	56	
	Mean***	0.87	0.32	3.95
	Std. Deviation	0.93	0.57	
Licorice varnish	N	68	48	
	Mean+++	1.2	0.19	8.58
	Std. Deviation	0.89	0.44	
Combination varnish	N	66	55	
	Mean---	1.25	0.38	6.23
	Std. Deviation	0.86	0.68	
Total	N	198	159	
	Mean	1.14	0.30	10.50
	Std. Deviation	0.91	0.58	

Note:***;Z=3.96,+++;z=8.58,---;z=6.23, All $p<0.001$

Table 35. Distribution of the initial lesions in pre and post intervention in lower quadrant in three varnish groups.

		Initial lesions at baseline in Mandibular arch	Initial lesions at the end of one year in Mandibular arch	Z value
	N	64	56	
Fluoride Varnish	Mean***	0.81	0.32	3.50
	Std. Deviation	0.95	0.54	
	N	68	48	
Licorice varnish	Mean+++	0.85	0.08	5.70
	Std. Deviation	1.02	0.35	
	N	66	55	
Combination varnish	Mean---	0.63	0.14	3.90
	Std. Deviation	0.87	0.48	
Total	N	198	159	
	Mean@@@	0.76	0.18	7.42
	Std. Deviation	0.95	0.48	

Note:***;Z=6.34, +++;z=8.95, ---;z=9.98, @@@;Z=7.42;p<0.001

Table 36. Distribution of the initial lesions in pre and post intervention in anterior arch in three varnish groups.

		Initial lesions at baseline in Anterior arch	Initial lesions at the end of one year in Anterior arch	Z value
	N	64	56	
Fluoride Varnish	Mean***	0.87	0.32	3.48
	Std. Deviation	1.01	0.71	
	N	68	48	
Licorice varnish	Mean+++	1.79	0.21	10.56
	Std. Deviation	1.072	0.50	
	N	66	55	
Combination varnish	Mean---	1.83	0.47	8.08
	Std. Deviation	1.01	0.83	
Total	N	198	159	
	Mean$^{@@@}$	1.51	0.34	11.95
	Std. Deviation	1.12	0.71	

Note:***;Z=3.48, +++;z=10.56, ---;z=8.08, $^{@@@}$z=11.95;p<0.001

Table 37. Distribution of the initial lesions in pre and post intervention in posterior quadrant in three varnish groups.

		Initial lesions at baseline in Posterior arch	Initial lesions at the end of 1 year in Posterior arch	Z value
	N	64	56	
Fluoride Varnish	Mean***	0.81	0.32	3.25
	Std. Deviation	1.03	0.57	
	N	68	48	
Licorice varnish	Mean+++	0.33	0.06	2.58
	Std. Deviation	0.78	0.32	
	N	66	55	
Combination varnish	Mean	0.06	0.05	0.11
	Std. Deviation	0.29	0.29	
Total	N	198	159	
	Mean	0.39	0.15	3.62
	Std. Deviation	0.82	0.43	

Note:***;Z=3.25, +++;z=2.58 All $p<0.001$

Table 38. Distribution of the initial lesions in pre and post intervention on smooth surface lesions in three varnish groups.

		Initial lesions at baseline in Smooth surface	Initial lesions post intervention in Smooth surface	Z value
Fluoride Varnish	N	64	56	
	Mean***	0.92	0.33	Z=3.48,
	Std. Deviation	1.07	0.72	
Licorice varnish	N	68	48	
	Mean+++	1.83	0.19	+++;z=10.56
	Std. Deviation	1.05	0.49	
Combination varnish	N	66	55	
	Mean---	1.84	0.45	---;z=8.08
	Std. Deviation	1.01	0.71	
Total	N	198	159	
	Mean	1.54	0.33	
	Std. Deviation	1.12	0.66	

Note:***;Z=3.48, +++;z=10.56,---;z=8.08, All $p<0.001$

Table 39. Distribution of the initial lesions in pre and post intervention on pit and fissures in three varnish groups.

		Initial lesions at baseline in Pit and fissure	Initial lesions at the end of 1 year in pit and fissure	Z value
Fluoride Varnish	N	64	56	
	Mean***	0.76	0.30	
	Std. Deviation	1.03	0.56	3.07
Licorice varnish	N	68	48	
	Mean+++	0.27	0.06	
	Std. Deviation	0.72	0.32	2.14
Combination varnish	N	66	55	
	Mean---	0.04	0.03	
	Std. Deviation	0.27	0.26	0.18
Total	N	198	159	
	Mean###	0.35	0.13	
	Std. Deviation	0.79	0.42	3.31

Note:***;Z=3.078, +++;z=2.14,---;z=0.18, ###;z=3.31. All p<0.001

Table 40.Comparision of Diagnodent scores among baseline and post intervention among varnish groups

Pre-Diagnodent score	**Fluoride Varnish**	**Licorice varnish**	**Combination varnish (NS)**
n	64	68	66
Mean	16.14	14.60***	14.49
Std. Deviation	4.29	4.05	3.88
Median	15.50	14.00	14.00
Post-Diagnodent score			
n	56	48	55
Mean	25.55	11.73***	21.85
Std. Deviation	27.43	17.58	28.50
Median	12.00	7.00	9.00
Improvement ###			
n	56	48	55
Mean	9.4107++	2.8750++	7.363
Std. Deviation	27.37	16.07	28.47
Median	4.5	6.0	5.0

Note - ###Kruskall – Walis test value =16.6 (df=2); ($p<0.001$) was significant in post intervention phase across the three varnish groups.

++;Z =2.84;$p<0.01$ Mann Whitney U test was significant between Fluoride and Licorice varnish group in post intervention phase.

***;$p<0.001$for using Wilcoxson sign rank test value was significant between Licorice group in pre and post intervention phase.

Table 41. Pre and post intervention Median Diagnodent scores across varnish groups

Varnish groups	Diagnodent scores	I Quartile (Q1)	Median (Q2)	III Quartile (Q3)
Fluoride Varnish	Baseline scores	3.00	4.00	6.00
	Post intervention scores	2.00	2.50	4.00
Licorice varnish	Baseline scores	3.00	3.00	3.00
	Post intervention scores	1.00	2.00	2.00
Combination varnish	Baseline scores	3.00	3.00	3.00
	Post intervention scores	2.00	2.00	4.00

Table 42. Comparison of Nyvad's score between varnish groups in the post intervention phase.

Post intervention Nyvad's score	Fluoride Varnish (%)	Licorice varnish (%)	Combination varnish (%)	Total (%)
Active caries (Score 3-cavity)	22 (39.30)	6 (12.50)	16 (29.10)	44 (27.70)
Inactive caries (Score 4)	34 (60.70)	42 (87.50)	39 (70.90)	115 (72.30)
Total	56 (100.00)	48 (100.00)	55 (100.00)	159 (100.00)

Chi square value 9.349, $p<0.001$, df 2.

Table 43. Incidence of dental caries among varnish groups

Incidence	Fluoride Varnish	Licorice varnish	Combination varnish	Total
0 incidence	29 (51.80)	30 (62.50)	39 (70.90)	98 (61.40)
1-3 incident caries lesions	20 (35.70)	16 (33.33)	15 (27.30)	51 (32.30)
4-10 incident caries lesions	7 (12.50)	2 (4.16)	1 (1.80)	10 (6.30)
Total	56 (100.00)	48 (100.00)	55 (100.00)	159 (100.00)

Chi square value- 7.8;df 4; p=0.100

Table 44. Shows Distribution of Salivary *Streptococcus mutans* CFU/ml (log_{10} scores) at baseline and post intervention in the three varnish groups

		***S.mutans* score at Baseline**	***S.mutans* score at Post intervention**	**Z value**
	N	64	56	
Fluoride Varnish	Mean	3.20	3.10	1.21 (NS)
	Std. Deviation	0.50	0.40	
	N	68	48	
Licorice varnish	Mean+++	3.29	3.06	2.09
	Std. Deviation	0.80	0.35	
	N	66	55	
Combination varnish	Mean	3.11	2.81	2.38
	Std. Deviation	0.87	0.48	
Total	N	198	159	
	Mean	3.20	2.99	3.45
	Std. Deviation	0.75	0.41	

note : z= 1.21 (NS) , +++;z=2.09, ---;z=2.38, both $p<0.05$

Table 45. Distribution of Post intervention oral hygiene score in varnish groups in comparison to baseline scores

Oral Hygiene Score At The End of the study							
Fluoride Varnish							
Oral Hygiene Score at the baseline		0 (%)	1(%)	2(%)	3(%)	Total (%)	Improvement (/100 n)
	0	31 (100)	0 (0.00	0 (0.)	0 (0.0)	31(100.0)	0
	1	1 (6.20)	15 (93.8)	0 (0)	0 (0.0)	16 (100.0)	1(6.25) NS
	2	0 (0.00)	0 (0.00	7 (100)	0 (0.0)	7 (100.0)	0
	3	0 (0.00)	0 (0.00	0 (0.00	2 (100)	2 (100)	0
	Total	32(60.70)	15 (24.6	7 (11.5	2 (3.30)	56 (100)	1(1.63)
Licorice varnish							
Oral Hygiene Score at the baseline	0	17(100)	0 (0.00)	0 (0.0)	0 (0.0)	17(100.0)	0
	1	0 (0.00)	4 (100.0)	0 (0.00	0 (0.0)	4 (100.0)	0
	2	4 (28.60)	2 (14.3)	5 (50.0)	1 (7.1)	12 (100.0)	6 (42.86) **
	3	0 (0.00)	2 (12.5)	5 (31.2)	8 (56.2)	12 (100.0)	7 (43.75)**
	Total	21(42.60)	8 (16.7)	10 (22.2)	9 (18.5)	48(100.0)	13(24.07)**
Combination Varnish							
Oral Hygiene Score at baseline	0	23 (96.00)	1 (4.00)	0(0.00)	0(0.00)	23(100.0)	0
	1	3 (20.00)	10 (66.70)	2(13.3)	0(0.00)	15(100.0)	3 (20.00)**
	2	2 (22.20)	0 (0.00)	7(77.8)	0(0.00)	9(100.0)	2(22.22)**
	3	1 (14.30)	1 (14.30)	4(57.1)	1(14.30)	7(100.0)	6(85.71)**
	Total	29(53.60)	12 (21.40)	13(23.20)	1(1.80)	55(100.0)	11(19.64)**

Note - ** - p value<0.001

Table 46 Study subjects of varnish groups who were referred to dentist for comprehensive treatment

Referred to dentist	Fluoride Varnish (%)	Licorice varnish (%)	Combination varnish (%)	Total (%)
Not referred to dentist	47(35.30)	37 (27.80)	49 (36.80)	133 (100.00)
Referred to dentist	17 (26.20)	31 (47.70)	17 (26.20)	65 (100.00)
Total	64 (32.30)	68 (34.30)	66 (33.30)	198 (100.00)

p value – 0.39 Nonsignificant, df 2.

Table 47. Study subjects distribution among varnish groups according to their visit to dentist

Visited dentist	Fluoride Varnish(%)	Licorice varnish(%)	Combination varnish(%)	Total (%)
Did not visit dentist	64 (34.00)	61 (32.40)	63 (33.50)	188 (100.00)
Visited dentist	0	7	3	10
	0.00%	70.00%	30.00%	100.00%
Total	64	68	66	198
	32.30%	34.30%	33.30%	100.00%

Chi square value 7.388, df 2, $p<0.025$

Table 48. Study subjects liking for taste of varnishes

Taste perception	Fluoride Varnish(%)	Licorice varnish(%)	Combination varnish(%)	Total (%)
Liked taste	60 (93.70)	63 (92.6.00)	64 (96.60)	187 (96.46)
Disliked taste	4 (6.30)	5 (7.40)	2 (3.40)	11 (5.5)
Total	64 (100.00)	68 (100.00)	66 (100.00)	198(100)

Chi square value 5.301, df 2, p>0.05

Table 49. Distribution of Study subjects according to outcome of the intervention

Success rate	Fluoride Varnish(%)	Licorice varnish(%)	Combination varnish(%)	Total (%)
None of the Initial Lesion remineralized	18 (32.10)	4 (8.33)	9 (16.40)	31 (19.49)
At least one of the Initial Lesion remineralized	38 (67.90)	44 (91.60)	46 (83.60)	128 (80.50)
Total	56 (100.00)	48 (100.00)	55 (100.00)	159 (100.00)

Chi square =11.4 (df=2); p=0.01**

Table 50 Subjects according to the remineralization of the Initial Lesion (IL) post intervention

Number of IL remineralized	Fluoride Varnish (%)	Licorice varnish (%)	Combination varnish (%)	Total (%)
None of the IL remineralized	18 (32.10)	4 (8.33)	9 (16.40)	31 (19.49)
1 IL remineralized	21 (37.50)	12 (25.00)	18 (32.70)	51 (32.07)
2 IL remineralized	11 (19.60)	14 (29.16)	19 (34.50)	44 (27.67)
3 IL remineralized	2 (3.60)	9 (18.75)	6 (10.90)	17 (10.69)
4 IL remineralized	4 (7.10)	8 (16.66)	2 (3.60)	14 (8.80)
5 IL remineralized	0 (0.00)	0 (0.00)	1 (1.80)	1 (0.62)
6 IL remineralized	0 (0.00)	1 (2.08)	0 (0.00)	1 (0.62)
Success rate	38 (67.90)	44 (91.60)	46 (83.60)	128 (80.50)
Total	56 (100.00)	48 (100.00)	55 (100.00)	159 (100.00)

Chi square=28.09 (df=12) $p<0.01^{**}$

Table 51. Frequency distribution and results from logistic regression models of the variables in the study associated with success of the intervention (n=159)

	Frequency	**B**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp (B)**	**95% C.I. for EXP(B)**	
OHIS score 0 OHIS (1)	82 77	1.77	0.55	10.18	1	0.001	5.90	1.984	17.578
Anterior teeth remineralized (1) Not remineralized	121 38	2.04	0.49	17.16	1	0.001	7.69	2.93	20.185
Combination Varnish	55			13.85	2	0.001			
Fluoride Varnish	56	-1.60	0.53	9.18	1	0.002	0.201	0.071	0.56
Licorice varnish	48	0.93	0.67	1.89	1	0.168	2.537	0.674	9.54

Table 52. Comparison of Pre and post intervention Diagnodent scores across varnish groups using per protocol analysis and intention to treat analysis.

Pre-Diagnodent score	Fluoride Varnish Baseline scores		Licorice varnish Baseline scores		Combination varnish Baseline scores	
	Per protocol analysis	Intention to treat analysis	Per protocol analysis	Intention to treat analysis	Per protocol analysis	Intention to treat analysis
n	64	64	68	68	66	66
Mean	16.14	16.14	14.60***	14.60***	14.49	14.49
Std. Deviation	4.29	4.29	4.05	4.05	3.88	3.88
Median	15.50	15.50	14.00	14.00	14.00	14.00
Post-Intervention Diagnodent scores						
n	56	64	48	68	55	66
Mean	25.55	24.09	11.73***	12.75	21.85	20.64
Std. Deviation	27.43	25.965	17.58	15.019	28.50	26.161
Median	12.00	12.5	7.00	9	9.00	9.5
Improvement ###						
n	56	64	48	68	55	66
Mean	9.41++	-8.23	2.87++	2.0	7.36	-6.13
Std. Deviation	27.37	25.77	16.07	13.52	28.47	26.103
Median (Effect size)	4.50	2.5	6.00	5	5.00	4

Note - ###Kruskall – Walis test value =9.799, (df=2);($p<0.05$) was significant in post intervention phase across the three varnish groups.

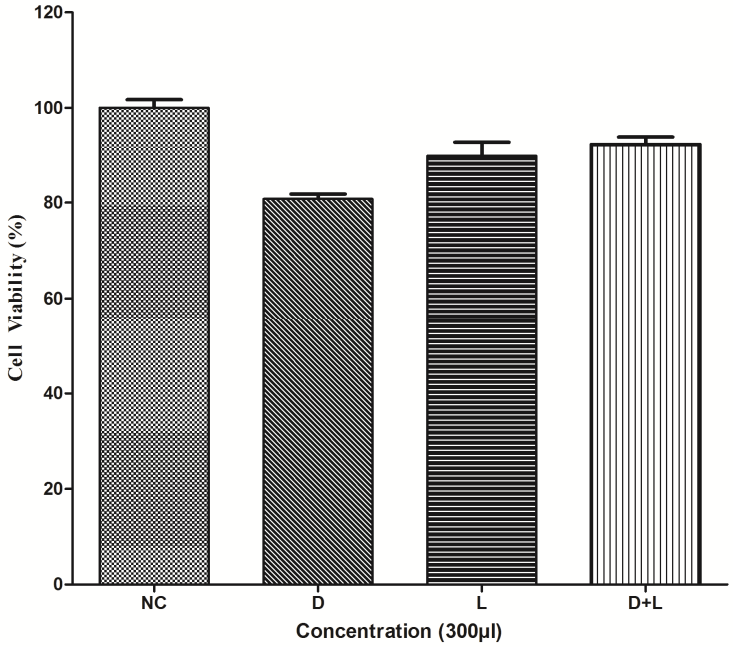
***;$p<0.001$for using Wilcoxson sign rank test value was significant between Licorice group in pre and post intervention phase.

+++;$Z = 3.23$;$p<0.01$ Mann Whitney U test was significant between Fluoride and Licorice varnish group in post intervention phase.

In vitro study result

Diagram 1.Toxicity study on- Primary Gingival Fibroblasts;Normal,Adult(HGF)

ATCCPCS-201-018



NC- Control,
D- Fluoride varnish,
L- Licorice varnish,
D+L- Combination varnish

In vivo study result

Diagram 1. Distribution of study subjects according to age

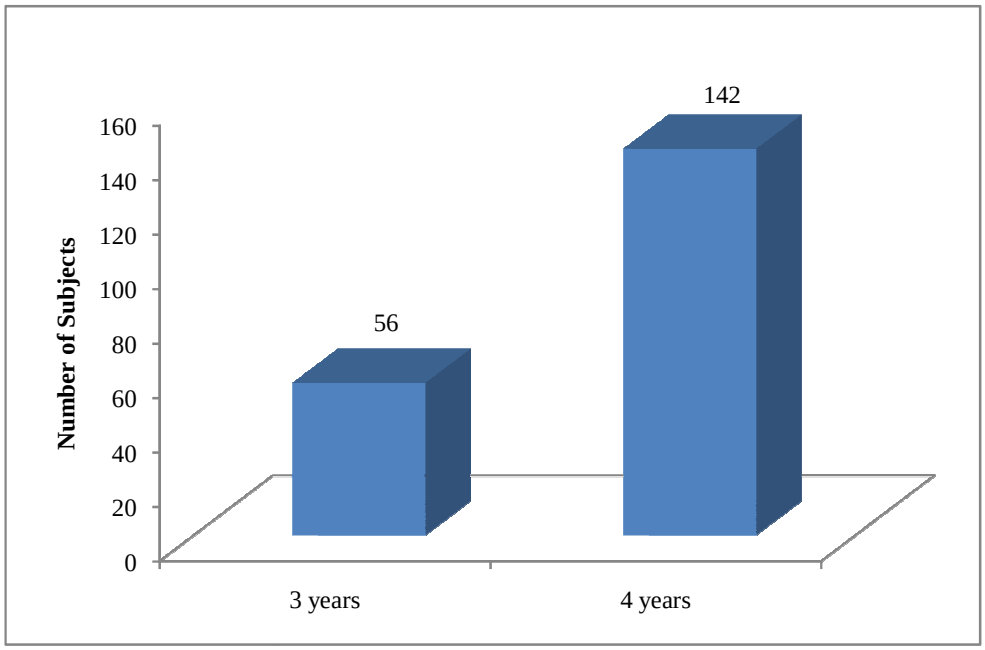


Diagram 2. Distribution of study subjects according to gender

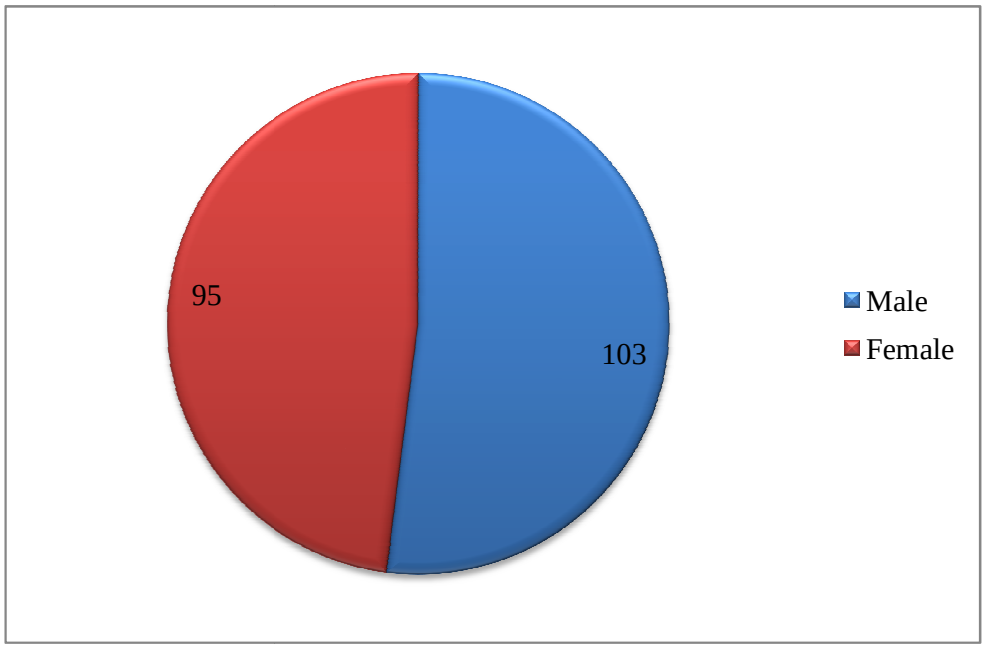


Diagram 3. Distribution of study subjects in varnish groups

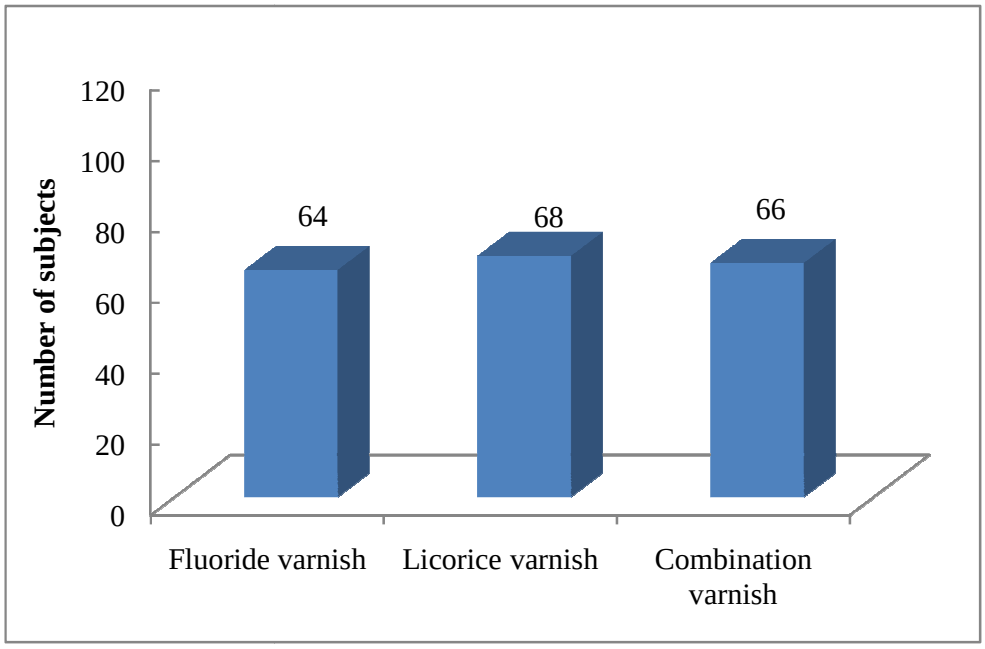


Diagram 4. Distribution of the study subject's in the three groups according to caries severity at baseline

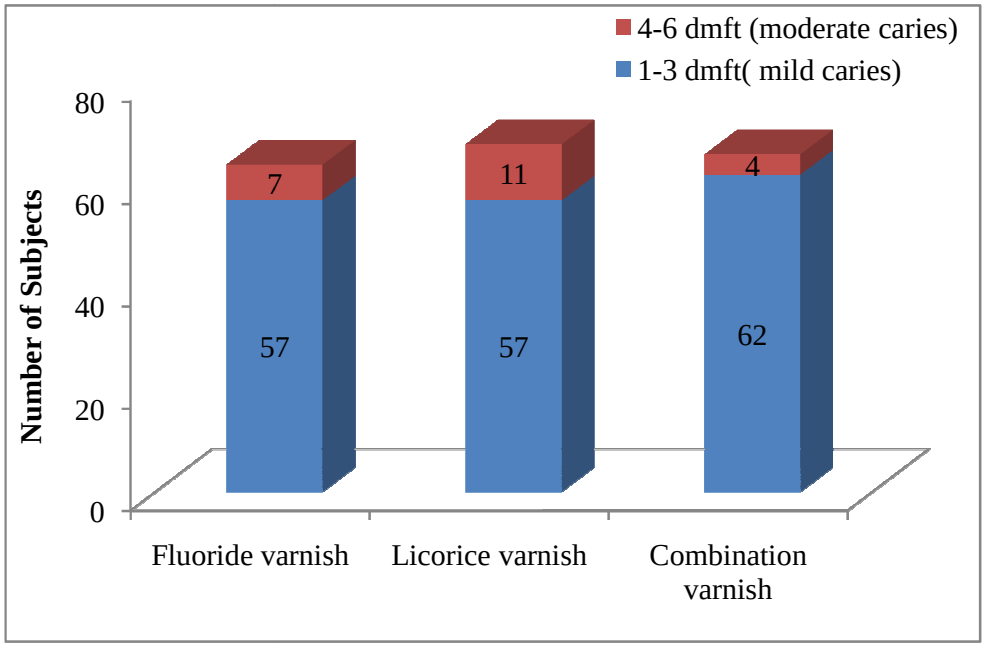


Diagram 5 Distribution of study subjects according to Diagnodent score grouping at baseline and post intervention

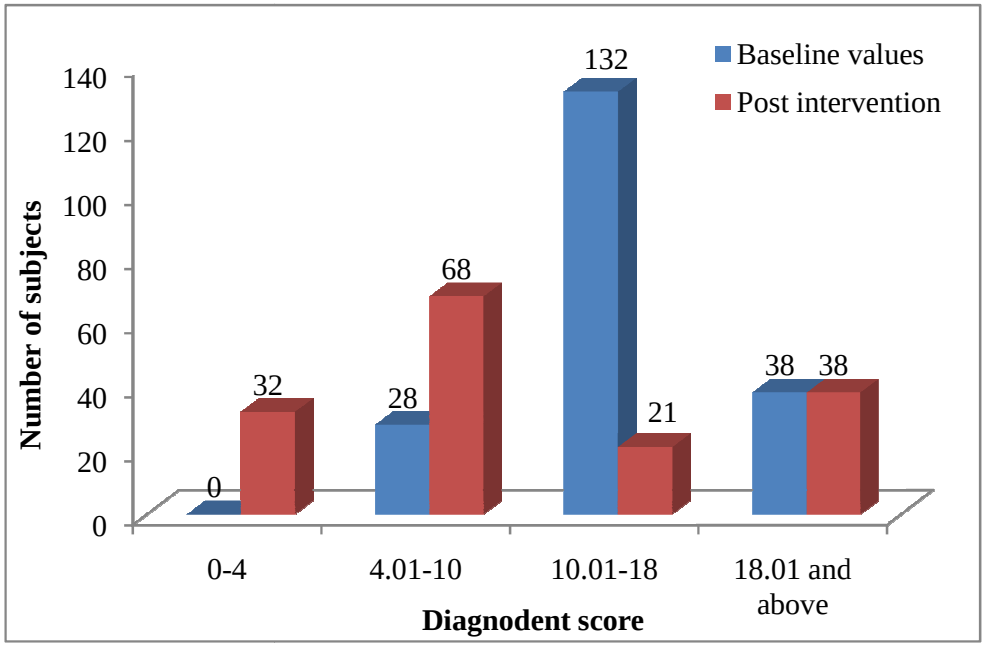


Diagram 6. Distribution of study subjects according to Diagnodent score grouping at baseline and post intervention among three varnish groups

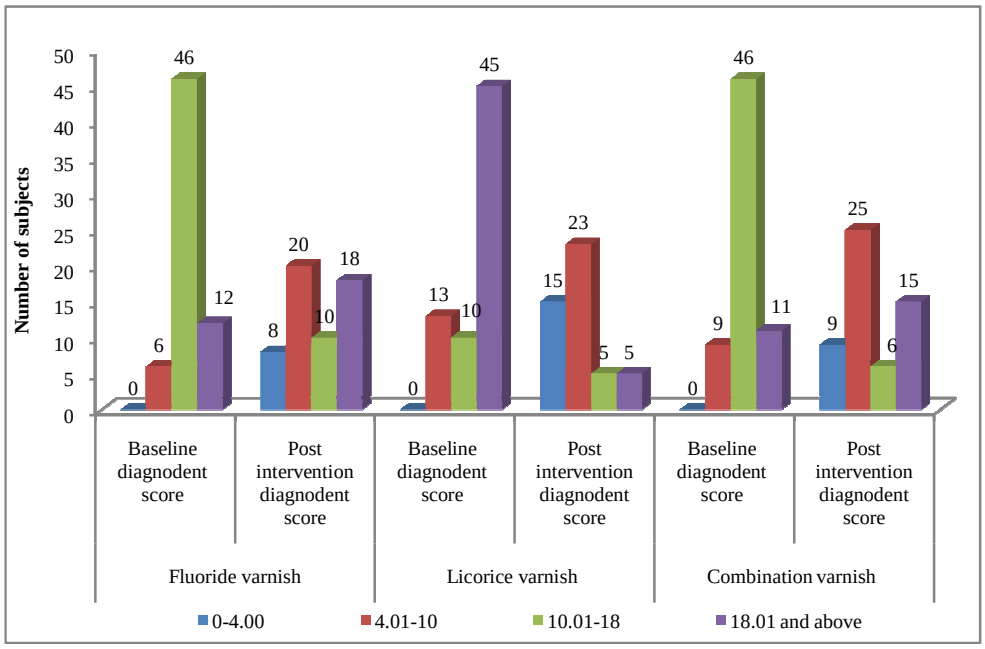


Diagram 7. Pre and post intervention Median Diagnodent scores across varnish groups

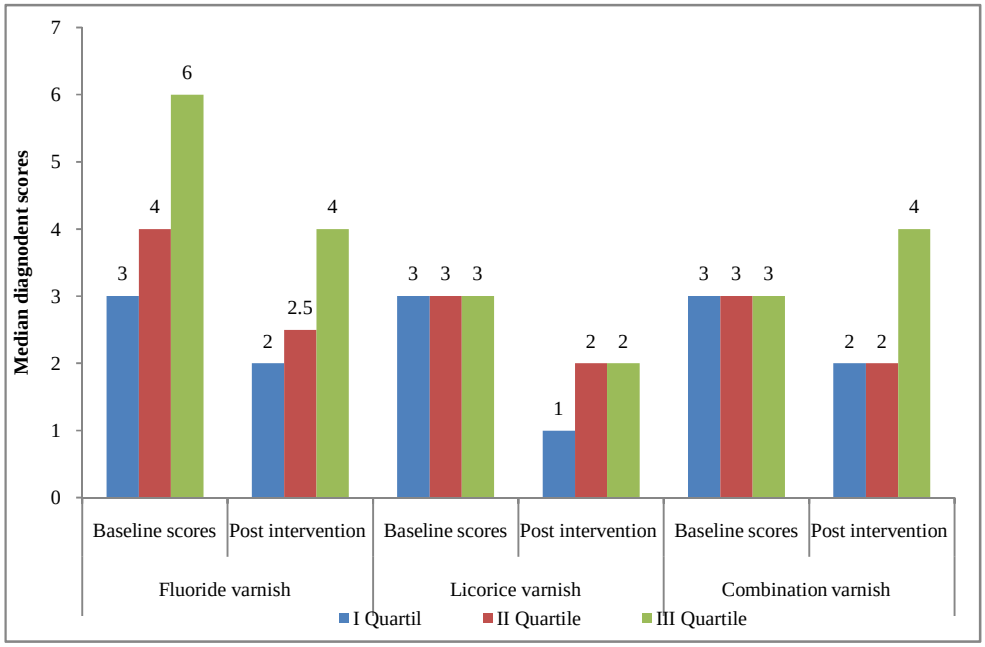


Diagram 8. Comparison of Nyvad's score between varnish groups in the post intervention phase.

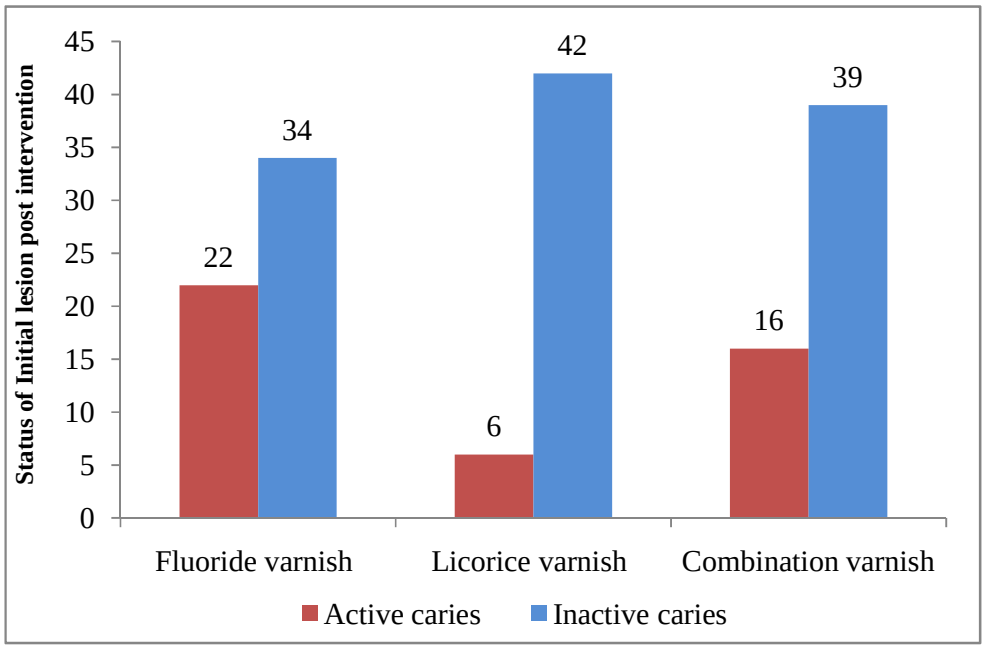


Diagram 9.Distribution of the mean Diagnodent value of initial lesions in pre and post intervention phases in maxillary arch in the three varnish groups

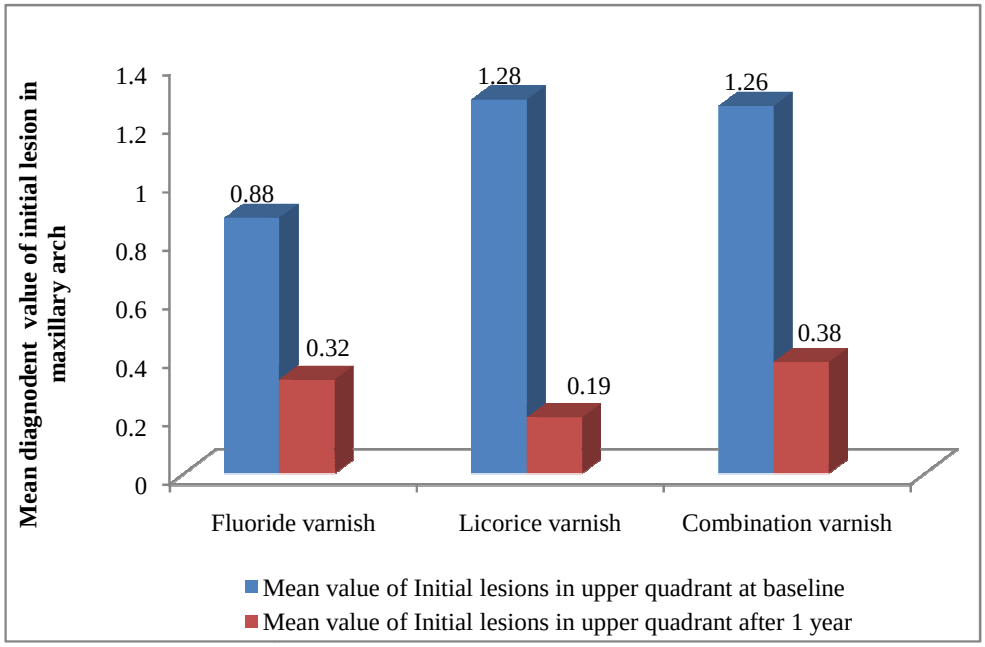


Diagram 10. Distribution of the mean Diagnodent value of initial lesions in mandibular arch three varnish groups.

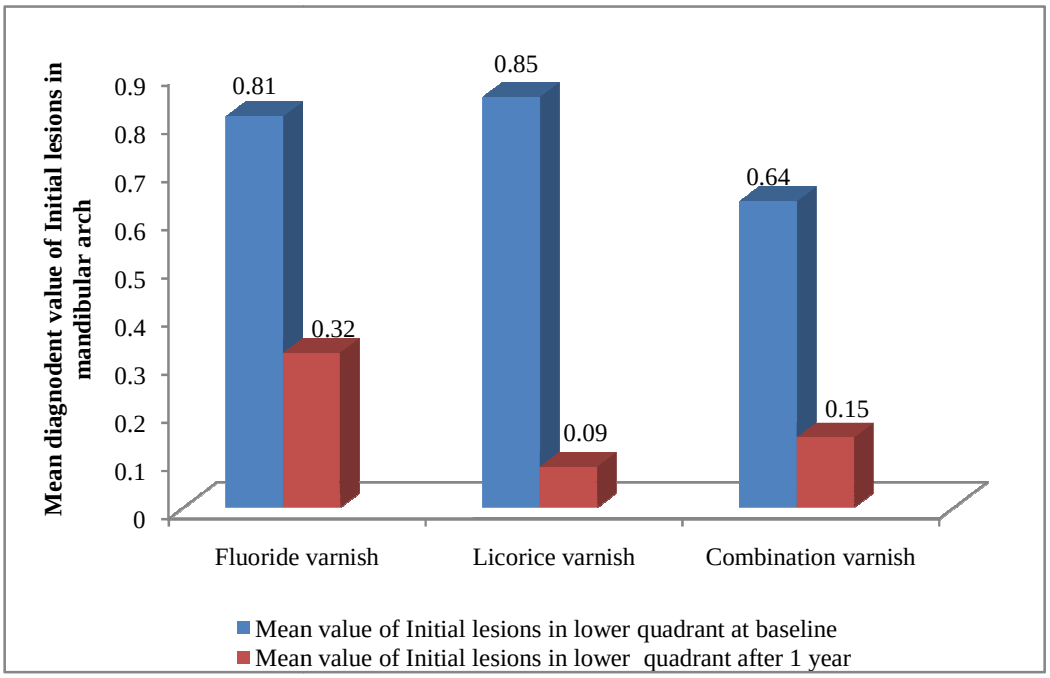


Diagram11. Distribution of the mean Diagnodent value of initial lesions in anterior arch in three varnish groups.

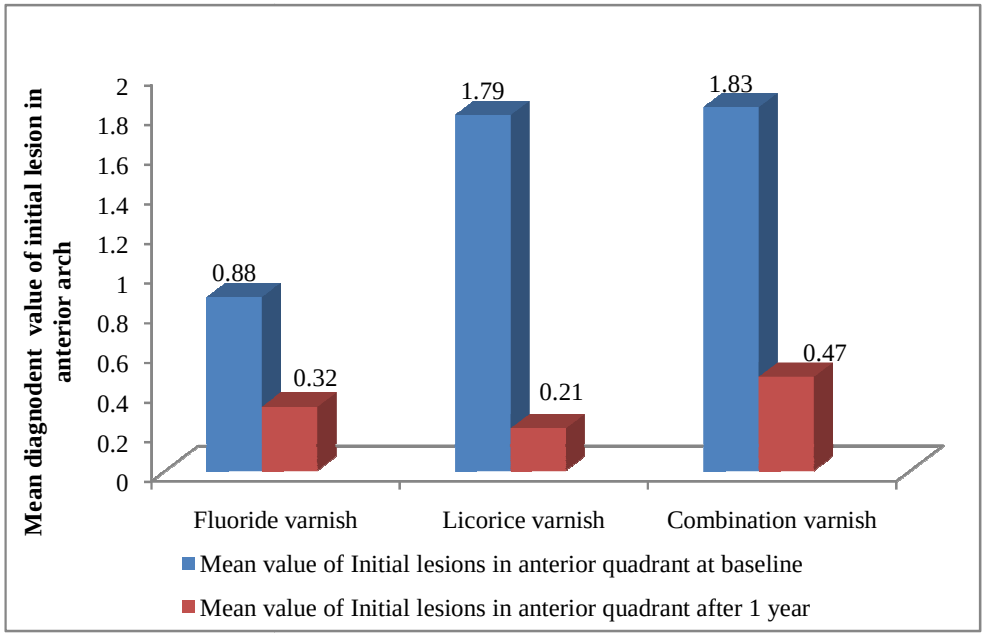


Diagram 12. Distribution of the mean Diagnodent value of initial lesions in posterior arch in three varnish groups.

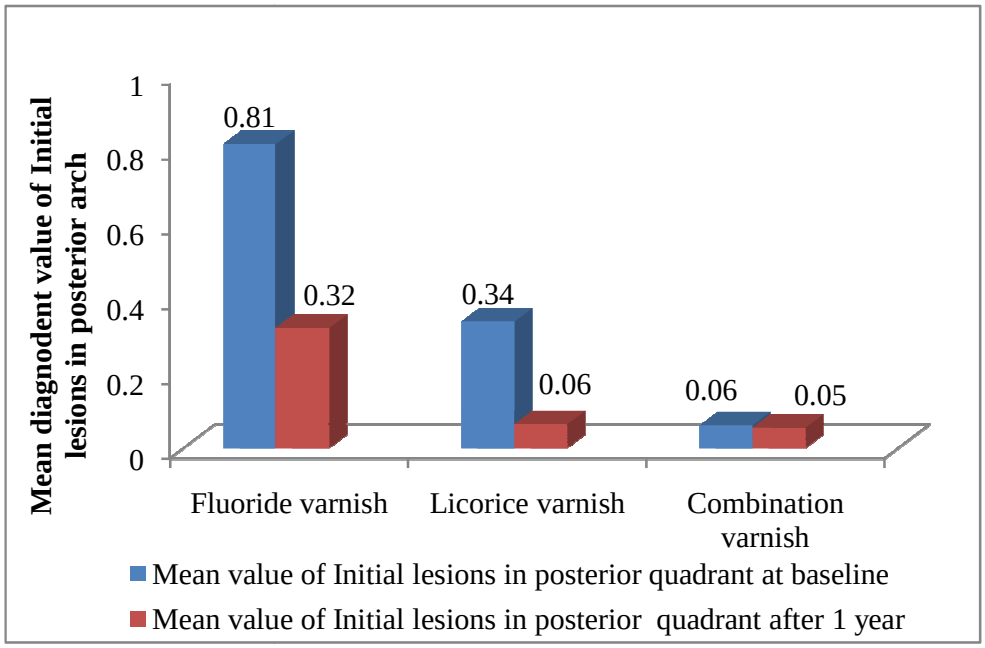


Diagram 13. Distribution of the mean Diagnodent value of initial lesions in pre and post intervention on smooth surface lesions in three varnish groups

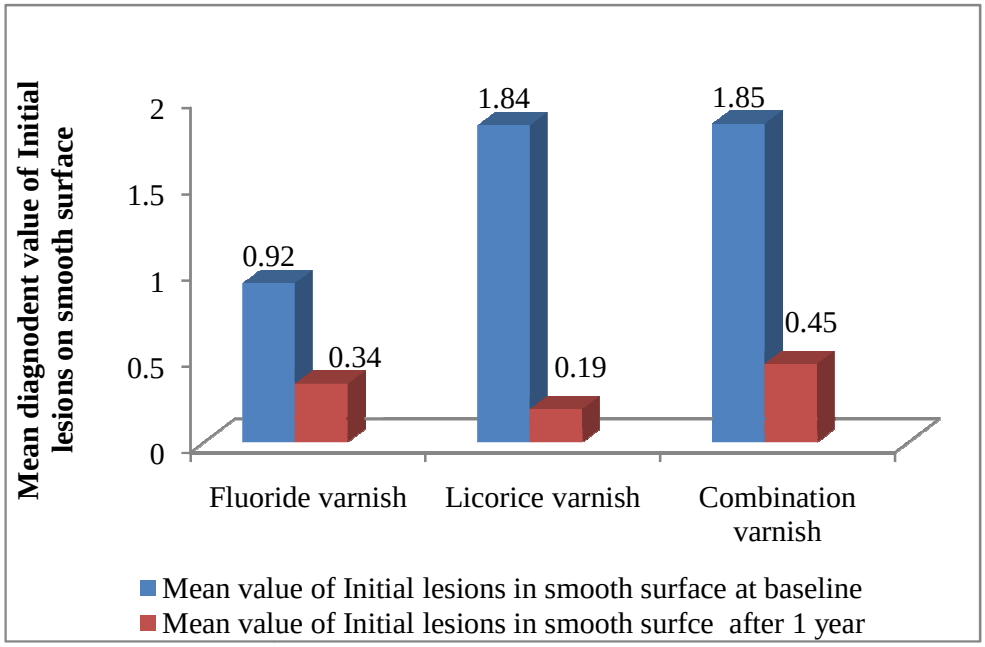


Diagram 14. Distribution of the mean Diagnodent value of initial lesions in pre and post intervention in pit and fissures in three varnish groups

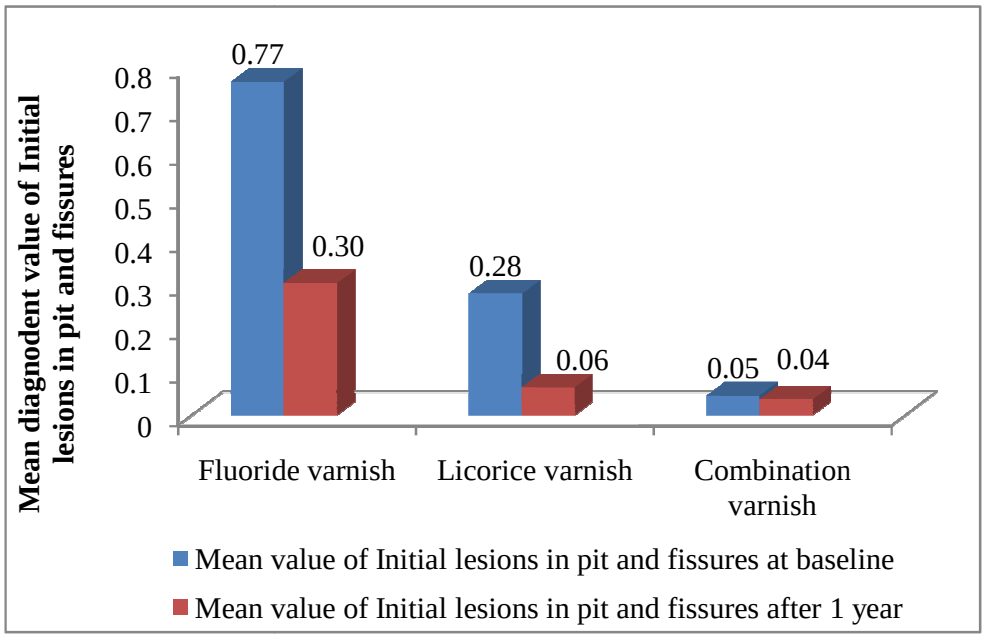


Diagram 15.Incidence of dental caries among varnish groups

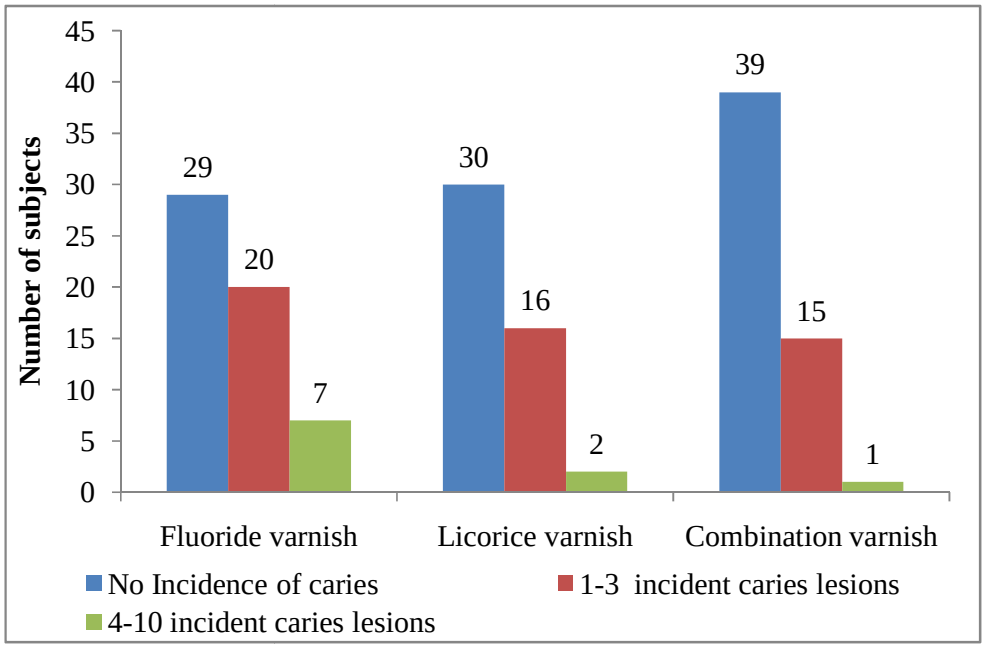


Diagram 16.Distribution of the salivary *Streptococcus mutans* count in pre and post intervention phases in three varnish groups.

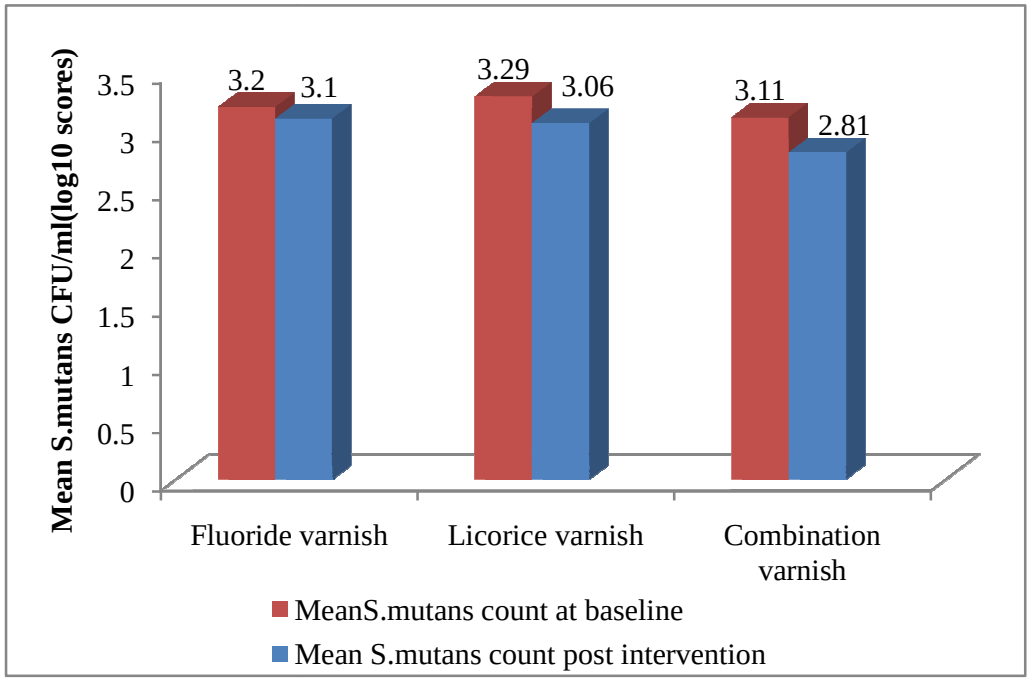


Diagram 17. Distribution of Study subjects according to outcome of the intervention

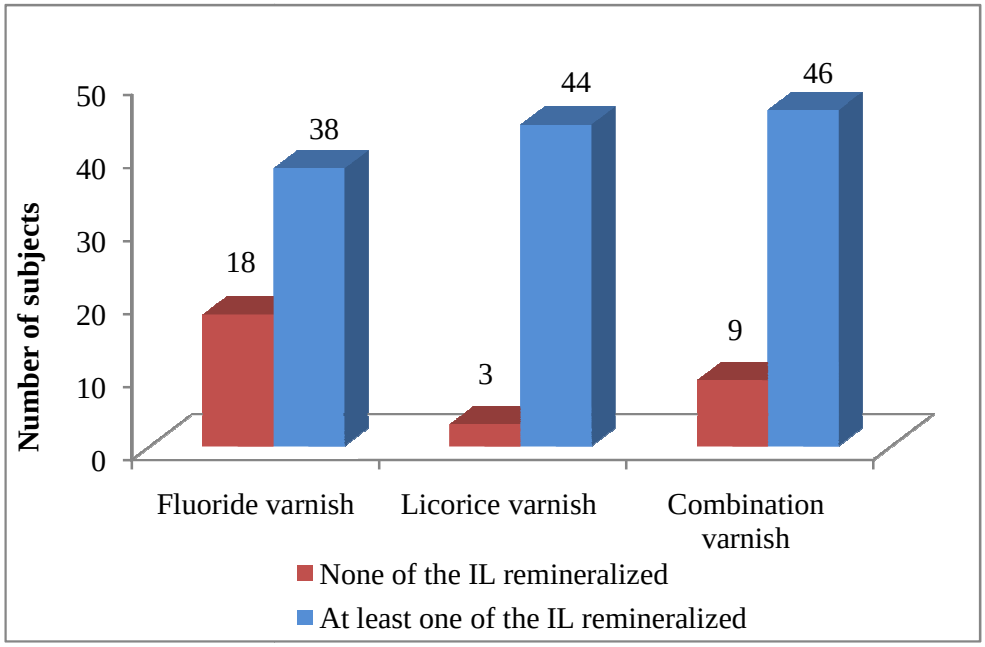


Diagram 18. Box plot illustrating Baseline Diagnodent scores of three varnish groups

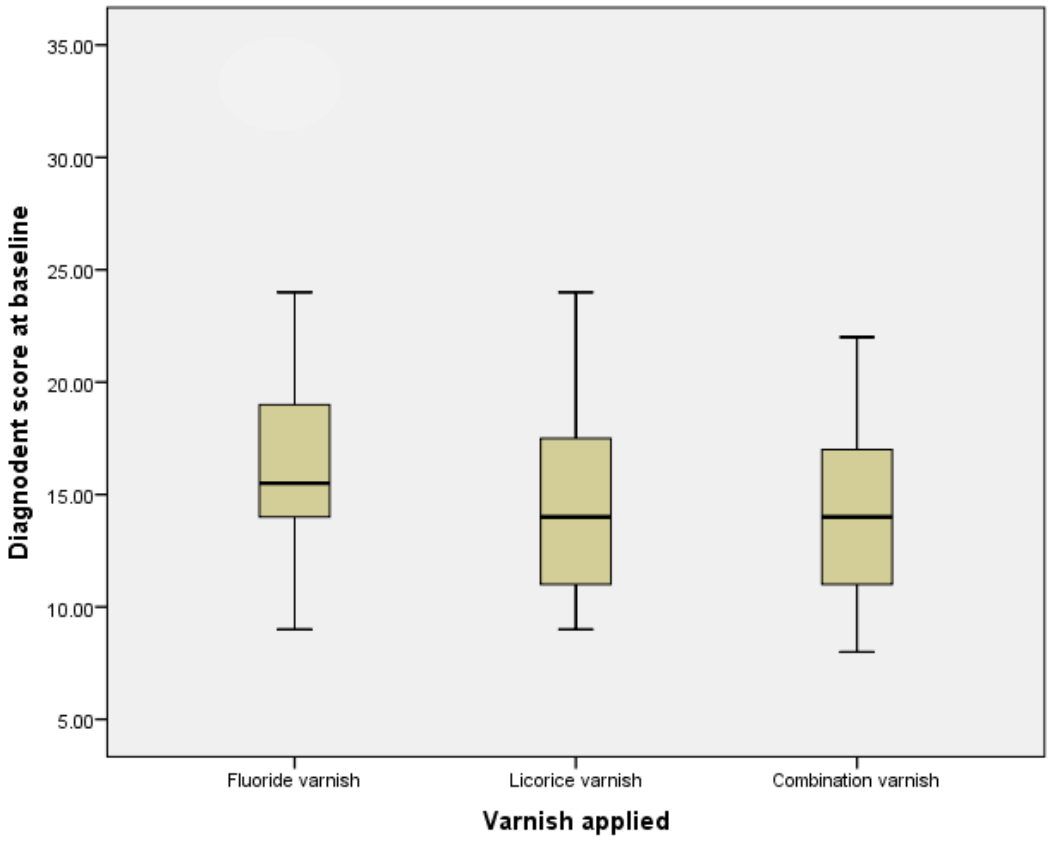
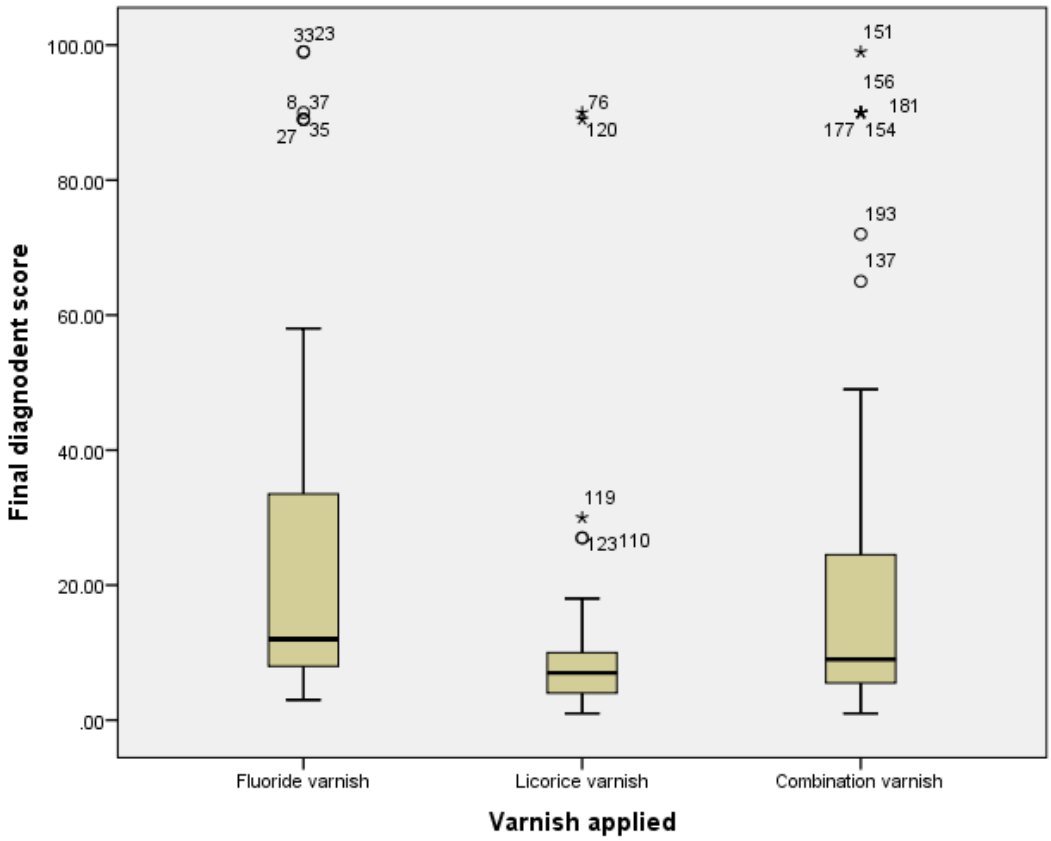
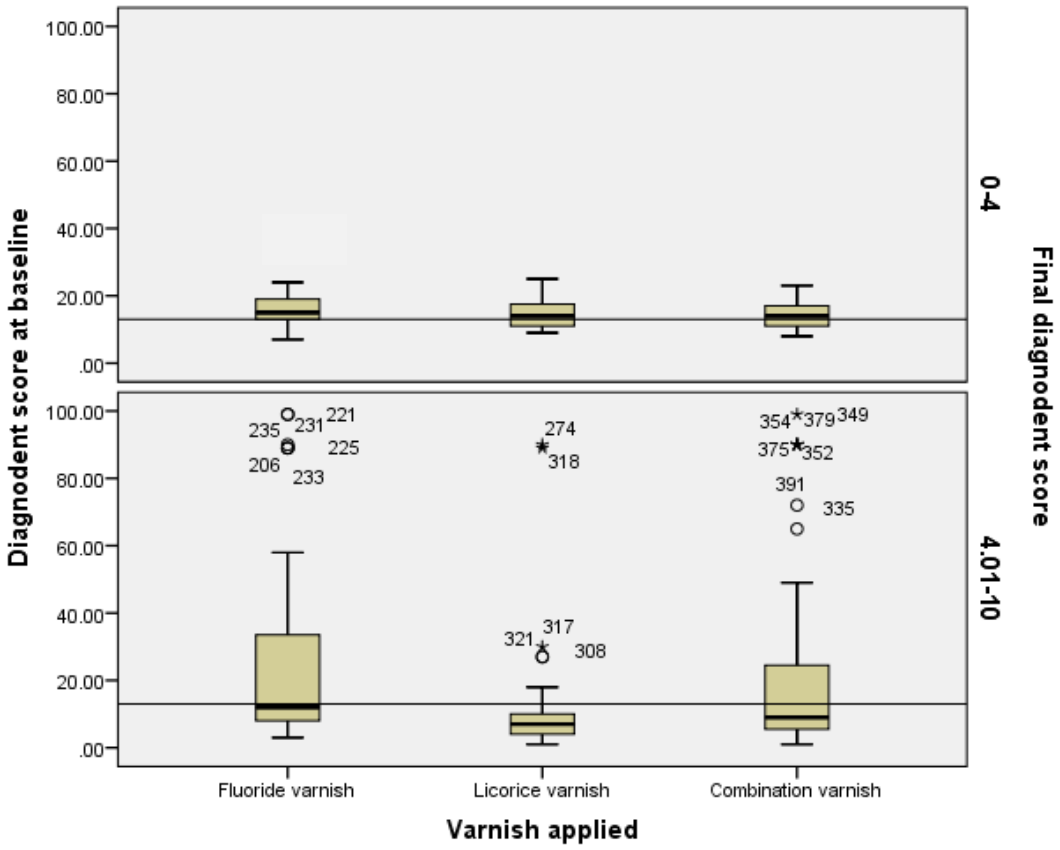


Diagram 19. Box plot illustrating Final Diagnodent scores of three varnish groups



(*, ° - Outliers)

Diagram 20. Box plot showing comparison of Baseline Diagnodent scores and Final Diagnodent score of three varnish groups



(*, ° - Outliers)

5. DISCUSSION

Current study attempted to assess effectiveness of three varnishes –FV, LV and CV on initial lesions of preschool children of Belagavi city. Study design was randomized trial which used a positive control, conducted in field setting. "Early childhood caries" is the term coined for older terms like Nursing caries, Bottle caries, Rampant caries and baby bottle syndrome.[144]. In early 80's caries in children was linked with feeding bottle and dietary habits. When it was recognized that caries of younger children is multifactorial in nature and has oral, dietary, social components responsible for its occurrence, it was named "Early childhood caries" and defined "as any decay seen in children below 6 years of age" at a conference held from 18th to 19th Oct, in 1997 at Bethesda, in USA. This conference helped in establishing the definition of ECC so that there would be uniformity of studies addressing ECC. [4] After more than 15 years, a second conference on ECC was held in 2014 at Baltimore, University of Maryland, School of dentistry which witnessed evidence based reviews on epidemiology, prevention and disease management of ECC.[97] The recent evidence has supported the notion that ECC, can be prevented if appropriate measures are taken. With ECC affecting almost 50 percent of the Indian preschool children,[9] and given the perspective that the treatment being very expensive, so all efforts must be focused on its prevention.

Fluoride is a recognized caries inhibiting agent but it does suffer from some limitations. The present research was planned with two purposes - Overcoming the drawbacks of fluoride by testing its substitute- Licorice, being the first purpose and trying to improve the effectiveness of fluoride by combining it with licorice, being the second. Research was planned in two stages - Invitro and invivo stage.

Health and medicinal plants Recently the practice of using medicinal plants for health benefits is increasing and dentistry is not an exception. Lei Cheng et al have summarized various natural products like Galla Chinensis, Propolis, Magnolia bark, Tea, Grapes, Coffee, Cacao and Hesperidin for their role either as antibacterial agents against common cariogenic organisms or for favorable shift from demineralization to remineralisation of enamel.[145] Commonly all natural products are made up of polyphenol compounds which means they contain at least 1 aromatic ring with 1 or more hydroxyl groups.[146] After thorough literature review, Licorice was chosen as the test agent in current study. It has been used for many of the general ailments.

Licorice is sweet herb which is moist by nature, has soothing effect and also protects as well as detoxifies liver, has anti inflammatory effects and is thus useful in arthritis and oral ulcers. In Greek it was used for gastric and peptic ulcers, in Asia and Europe for psoriasis. Licorice in Ayurveda is used to relive 'vata' and 'kapha' inflammation, opthmalogy problems, sore throat, gastric ulcers, inflammatory conditions of joints along with liver disorders.[147] Licorice has many phytochemicals, to name a few Triterpene Saponins, Flavanoids, amino acids, simple sugars, mineral salts and numerous other substances but most importantly it contains Glycyrrhizin, a triterpenoid component responsible for its sweet taste. Glycyrrhizin and glycyrrhetic acid together have antibacterial activity, flavonoid which contains liquiritin, isoliquiritin give its yellow colour, whereas glabridin and hispaglabridin give significant antioxidant property.[25] Licorice and its components are proven to be beneficial in common oral problems like Dental caries, Periodontitis, Apthous ulcers and Candidiasis.The glycyrrhizol A along with Glycyrrhizic acid have beneficial effect in dental caries [116] , Licorisoflavan A in periodontal disease [117] and Glycyrrhizin in candidiasis [148].

In vitro study- Licorice extract

In the present in vitro study phytochemical screening revealed presence of Carbhohydrates, Reducing Sugars, Terpenoids, Glycosides, Steroids, Tannins, Saponins, Anthroquiones, Flavanoids and Alkaloids. The antibacterial activity can be contributed to these secondary metabolites present in the licorice extract. Licorice extract in current study inhibited *S.mutans* effectively and similar finding is corroborated by other studies [149, 147]. Licorice consists of phytochemicals which are responsible for rendering antimicrobial and antiadherent property against *S.mutans* and thus can help in caries control[106]. Beneficial effects of a medicinal plant are based on quantities of different phytochemicals present in the extract and the procedure employed for extraction of active component from crude drug can be a decisive factor.In current study , cold maceration extract was significantly better than Soxhlet method. Phytochemicals are very sensitive to the external environment. Flavanoids and phenylpropanoids can break when present in solvents especially organic solvents. Other important component Glycosides degrade and are thermolabile and hence could be due to this reason that antibacterial activity of Soxhlet extract was lesser than cold maceration extract.[150] A comparison of various methods used for extraction of glycyrrhizin compounds and glycyrrhetic acid from root licorice stolons has been addressed by Sharad Visht [151] which reports the possibility to increase quantity of glycyrrhetic acid by altering pH of the extraction solvent.

In vitro study- Licorice varnish

Varnish is a resin mixture has a synthetic base, leaves a thin layer on surface after application. As long as the layer remains intact, the active agent will leach from it and benefit the tooth. We did a thorough review of literature but failed to get any previous reports of Licorice varnish. Thus the present study seems to be the first study which reports development of a novel Licorice varnish. Comparison was made between varnishes for antibacterial activity using an innovative method. MIC is 'gold standard' to evaluate how sensitive micro organisms are to the antimicrobials.[152] When varnishes were tested in the routine technique of agar well method, result was inconclusive and it may be attributed to evaporating nature of the varnish, since it was made up of alcoholic mixture. This constraint was overcome when varnish and BHI broth were added together and the susceptibility of *Streptococcus mutans* to the varnish was evident. De Luca et al [153] reported regarding estimation of MIC of a propolis varnish, where the varnish was diluted with water to reduce its viscosity. Since this method camouflages the inherent property of varnish, this particular method was not employed in current study. Any newer preventive tool will be successful in public programs only if it is affordable. Thus we compared the cost incurred for development of licorice varnish with the commercially available Fluoride varnish.LV is almost 6 times cheaper than FV. Most of the physical parameters of three varnishes were similar to each other except for shelf life which was lesser for LV. Improvising the Licorice varnish can be considered as scope for further research. Present study used 5 different combination varnishes which were tested and one among them combination varnish (80 % LV with 20 %FV) had failed to show antibacterial activity.

The exact cause as to why this particular combination, failed is difficult to speculate, as we need evidence from interaction studies between fluoride and licorice which is currently not available. This aspect was however beyond the scope of present research. When Scanning Electron Microscopy(SEM) images were studied, it became evident that all the varnishes formed layer on the tooth surface. The compactness of all the varnishes differed, as the composition of the varnishes was different. Bifluorid 12 consists of Ca and Na ions possess high affinity to fluoride ions. Licorice varnish is devoid of any calcium component and could be the reason behind the differed compactness.

Prevalence of ECC

Current study had representative sample of 407 children aged 3-4 years who were screened, and among them 73.21% prevalence of ECC was found. Prevalence of initial lesions was 48.64%. Higher prevalence of ECC was reported in current study compared to studies of Uganda 3.7 to 17.4% ,[154] Hong Kong 51% [155] and China 66%.[5]

It was lower compared to studies reported from south east Asia countries – Thailand 79% [156], Philipines 88% [8]. Prevalence of disease is more in socially disadvantaged, economically backward communities. Compared to national prevalence of India which is 49.6%, the present study witnessed higher ECC prevalence.

Prevalence rate inflated in comparison to the prevalence rate in same city in 2012.We can contemplate that easy exposure to cariogenic food and lack of oral hygiene may have contributed to escalated ECC prevalence in the current study. ECC

is affected by certain socio cultural beliefs as well as from psychosocial behavior. [157]

In the present study when oral hygiene practices were cross tabulated with ECC, a non significant association was seen with most of the parameters tested except for dentifrice used. Those children, in the habit of using tooth powder had significantly lesser caries compared to toothpaste using population. It may be attributed to higher abrasivity of the powder which might have aided in better cleansing capacity. However we should be warned, that overzealous application of a rough powder can cause abrasion of enamel which was not assessed in current study. Social class yet again emerged as significant factor, with those who belonged to upper class had lesser caries experience owing to regular brushing and parental assistance for brushing.

Maternal education - Higher the educational status of mother, lesser was the caries prevalence and these concurrent findings are reported by Duijster , Verrips 2014 study.[158] In the present study LOC score was dichotomized into LOC score less than 29.5 as Internal LOC and LOC score more than 29.5 as external LOC. Children whose parents had internal LOC were 1.8 times less likely to experience ECC compared to those whose parents had external or chance LOC. Similar to our results, Lencova et al [126] have reported that those parents who had internal LOC had 2.32 times lesser chance of experiencing ECC. Area under curve was 0.56 which is less than optimal, which reinforces the evidence that there are multiple causative factors responsible for caries and disease cannot be attributed to one particular causative agent. Logistic regression analysis disclosed that among all factors considered, only Locus of control had a significant relation. Thus educating parents and using psychosocial behavior therapy can also help in preventing ECC. [127]

Present interventional study was conducted on a representative sample. Obtaining permission and conducting a field trial in the preschool children is a challenging task and many such practical difficulties were encountered during the current study as well. Out of 20 schools which were approached, only 14 schools agreed to take part. Fear, ignorance and a sense of accountability to parents could be the probable reasons for denying the permission. Rice [159] has also expressed these views in the literature.

Prevalence of Initial lesions The screening phase required more than 4 months for the initial screening and recruitment of all the study subjects. Participants were almost equally distributed with respect to age, gender, socioeconomic status, Diagnodent scores, initial lesions,dmft score and *S.mutans* count at baseline. Prevalence of initial lesions was 48.64%. It was higher compared to a study reported by Guedes. [160] However in current study two different methods were used for diagnosing initial lesions -visual examination and Diagnodent pen where as Geudes reported use of only visual examination which was ICDAS. Conjugation of the two methods may have led to higher sensitivity and thus higher prevalence value in present study.

Unstimulated saliva or resting saliva is the saliva which is constantly present in oral cavity.[161] Stimulated saliva differs from unstimulated saliva in pH, concentration of inorganic ions and other biochemical components. Unstimulated saliva was used in current study to assess salivary *S.mutans*. Various other studies have reiterated similar method.[162-165] Thenisch et al [89] in their systematic review have stated that dental plaque findings were more consistent and showed higher values compared to saliva tests. However not all areas of the tooth are equally prone for harboring bacterial colonies and thus result obtained may not be generalizable.

Effect of varnishes on *S.mutans* count

Varnish is expected to have remineralising potential and antibacterial activity both.

Fluoride varnish and its effect on *S.mutans* count In current study full mouth application of varnish was employed as full mouth application had given better results than single tooth application for *S.mutans* reduction. [166] In current study when salivary *S.mutans* count was compared pre and post intervention, no significant difference found with Fluoride varnish group. Our results corroborate the result of other studies reported. [6] A meta-analysis compared topical fluoride and other chemical agents like antimicrobials and reported some degree of reduction in count of *S.mutans* but however current study failed to get any significant difference. Paek et al [167] reported significant reduction of common cariogenic micro organism *S.mutans* count after intensive fluoride regime, but however this reduction did not affect caries outcome in anterior teeth and caries continued to progress in posterior teeth. All these studies show that even when fluoride varnish reduced *S.mutans* count, however the remineralizning effect of varnish was not evident. This could have been because of dilution of varnish in saliva after 6 hours

However many contrary results have been published.[165,168] Baygin et al [168] reported significant effect of Fluoride varnish on *S.mutans* count in disabled children. It is well recognized fact that fluoride has various actions on cariogenic bacteria. Fluoride inhibits enolase enzyme and thus causes imbalance of intracellular pH and extracellular matrix of the organism leading to lysis of the bacteria ultimately leading to its inhibition. [169]

Licorice varnish and its effect on *S.mutans* count

When licorice varnish group was assessed for its salivary *S.mutans* count, significant difference was found between pre post intervention scores. Since current study is a prototype study where licorice varnish was reported, comparison with similar studies was not possible. Licorice lollipop and licorice mouthwash have been reported in the literature.[116,170,149] When used licorice as mouthwash and lollipop, significant reduction of *S.mutans* count was noticed. Antibacterial action of licorice has been attributed to its phytochemical components – glycyrrzhin, glycyrrhizinic acid and glycyrrhizol A.[106] There are few studies which have reported effect of non fluoridated agents for prevention of caries. These agents include Arginine,Tricolsan, Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP), Xylitol, and Chlorhexidine and with Placebo or Fluoride which have been reported by Wang et al.[171] The effectiveness of CPP ACP was borderline, when compared to fluoride. However none of the studies published so far have used any of the electronic objective methods like Diagnodent to diagnose, monitor progression or regression of dental caries which has been used in current study.

Combination varnish and its effect on *S.mutans* count

Combinations of mouthwashes and varnishes are tested as synergistic activity of two components is expected to improve the efficacy in contrast to use of single drug. Significant reduction of *Streptococcus mutans* cfu/ml $\log_{10}$ scores were found when pre post comparison was done. Literature has many studies which have used combination of two drugs. Paul et al [172] compared Fluoride varnish with Chlorhexidine thymol varnish in dental plaque. Significant reduction of *S.mutans* cfu was observed when compared at 1 month post baseline. However results failed to vary

significantly after 3 months in either groups. In another study Liptak et al[173] compared chlorhexidine fluoride varnish with chlorhexidine thymol varnish on pit and fissure *S.mutans* count and both of them reduced *S.mutans* count significantly from the baseline counts.

It is also to be noted that in current study, only one micro organism *S.mutans* was studied and cultured. Overall, the varnishes may have acted on other organisms like *S.sobrinus* and *Lactobacillus* which are important for further progression of the disease. However the present study did not assess if the varnishes exerted any antibacterial activity against *S.sobrinus* and *Lactobacillus* and helped in overall oral health promotion.

Remineralising potential of the varnishes

Incipient lesions

An optimal clinical method for monitoring early caries lesions would permit longitudinal quantification of early mineral loss or gain.[174] As the awareness regarding Minimal Intervention Dentistry (MID) is increasing, there seems to be paradigm shift in our approach towards management of dental caries. When MID is planned for dental caries, it becomes imperative that we should be able to monitor the loss or gain of mineral ions on the tooth surface. Thus to make preventive dentistry more practical and feasible, more and more emphasis is being placed on identification of the carious lesions in the earliest phase. Identification of initial lesion is the crucial step if preventive therapy is planned.

Incipient lesions The dental caries can be considered as a slow progressive chronic disease and if diagnosed at the earliest stage, the disease is amenable for non surgical

intervention. The earliest surface change in enamel is evident as white spot lesion. It results due to continuous exposure of tooth surface to the external cariogenic challenge basically as a drop in salivary pH and loss of mineral ions from tooth surface. Tooth is covered by pellicle and plaque which acts as a reservoir for the inorganic ions. [161]

Today we have many diagnostic aids and Diagnodent has been reported widely.

DIAGNODENT It works on the laser fluorescence and is very convenient to use as well as to monitor either progression or regression of caries lesion. In current study, Diagnodent pen was used as a tool for identification of initial lesions during screening as well as during baseline and final evaluation post intervention. All the precautions were taken during examination to minimize false positive results. Instrument was calibrated after every 10 students to minimize instrument error. Sterilization of the tip was done using 70% isopropyl alcohol. Since the Diagnodent reading is a continuous reading from 0-99, the actual value was recorded for study purpose. Later on during statistical analysis it was grouped as per Lussi's classification. In current study initial lesions were diagnosed with Diagnodent and baseline Diagnodent scores were represented in quintiles. The 25th, 50th and 75th quintile value of Diagnodent scores were 12, 15 and 18 respectively. A similar study is corroborated by Ferreira et al (175) who reported 7.4 as baseline Diagnodent score. In current study those subjects who were likely to develop ECC were selected and we assume that the Diagnodent score of the present study subjects was higher compared to Ferreria et al study. [175]

In the post intervention, Diagnodent scores reduced to 6, 9 and 22 in 25th, 50th and 75th percentile respectively and they were statistically significant compared

to baseline. It is to be noted that Diagnodent score in 75th percentile increased from 18 at baseline to 22 post intervention. The post intervention score was influenced by extreme values of the Diagnodent scores which were observed in the cases where in intervention had failed. We had a total of 30 subjects who accounted for failure of therapy. When we analysed the data for success rate of remineralization based on location of the tooth we observed that varnish had acted equally in all the 3 groups on all the tooth surfaces.

However logistic regression proved that if a maxillary anterior tooth had initial decay, there was more chance for remineralization of the tooth. Varnish acts better on smooth surface than on occlusal surfaces as reported by Honkala et al. [176] This has been studied in detail by Pearce. [177] The way fluoride acts remains unchanged whether the target area is smooth surface or pit and fissures. But however, invariably it is observed that fluoride fails in remineralising caries of pit and fissures. The reason is the, inaccessibility of fluoride to reach bottom of the fissures. Fissures are more prone for plaque buildup, presence of food substrate and though fluoride may also be available, but the conditions in the fissures favour demineralization. Thus the small initial lesions in which remineralisation would have begun, usually crumble out due to masticatory forces and thus we often witness that fluoride is not a very good remineralising agent for occlusal caries.

Fluoride varnish

The frequency of fluoride varnish has been reported to vary from once in 3 months to once in 6 month. There is no fixed protocol to be followed. It depends on an individual's risk assessment apart from the choice of the fluoride agent used. Cochrane review suggests use of either two or four applications per year for reduction

in caries prevalence.[23] In current study four applications with interval of 3 months were carried out. However Marinho reported that bimonthly application of this product is more effective than tri monthly or semi annually.[178]

Fluoride varnsih in current research, was successful in remineralising initial lesions in almost 67% of subjects. When the Diagnodent values decreased by minimum 4 units than the baseline value, the lesion was considered as remineralized.[133]

In current study Diagnodent scores were categorized into 4 groups based on Lussi classification with smallest category being less than 4.[140] However at baseline none of the mean scores of initial lesions were less than 4 score. Mean Diagnodent score among 3 groups was not different statistically at baseline.

Novel Licorice varnish and Combination varnish

The present research was based on formulating and testing a novel varnish.Varnish is easy to use, not technique sensitive, delivers concentrated drug at the target area thus this form of drug delivery becomes the most accepted preventive modality for high risk groups especially children. When we reviewed literature about use of natural products which have been imbibed in the varnish formulation we encountered very sparse literature. However it is noteworthy that of late, varnish has been tried and tested using natural products, to name a few, these are Miswak varnish, Propolis varnish, Propolis based Chitosan varnish, and Chitosan Nano particles varnish. [179,180,153] In current study all three varnishes were compared for antibacterial activity, licorice and combination varnish were better than fluoride varnish. Another study reported by De Luca et al [153] compared chitosan based varnish containing

propolis extract in varying concentrations of 5%, 10% and 15% however similar result to chlorhexidine varnish was found in zone of inhibition against *S.mutans*. Franca et al compared the release pattern of propolis based chitosan varnish as well as antibacterial activity against *S.mutans* and concluded that chitosan based propolis varnish was significantly better than chlorhexidine varnish.[180] Remineralizing capacity of the licorice varnish was assessed using Diangodent scores in the present research.

Wassesl et al [179] compared remineralising capacity by estimating Calcium ion dissolution. Natural products performed inferior to sodium fluoride varnish when remineralizing ability was being compared and this may be attributed to the composition of natural products which are devoid of any calcium or inorganic constituent. Thus combination of fluoride with natural product can have relevance in clinical dentistry.

However when effectiveness was compared for remineralising abilities, the licorice varnish performed better as compared to combination. Fluoride varnish was least effective of all the three varnishes. Contrary to this Wassel & Khattab [179] concluded that 5% Sodium varnish was significantly better when compared to other natural product varnishes.

Viscosity of dental varnish could be an important physical parameter. We have compared the viscosity of the varnishes which was estimated using Viscometer. Surprisingly we did not encounter reports which have addressed comparison of viscosity parameters. Wassel et al [179] reported that Propolis varnish was more viscous than Chitosan and Miswak varnish and thus was less effective which may have resulted due to partial release of fluoride ions. But however it is not clear as to how viscosity was assessed by Wassel et al.

Incidence of new carious lesions

In present research caries incidence ranged from 35% to 27% among varnish groups although no significant difference in incidence was found across groups. A total of 61% had no new caries incidence. Our results are in accordance with Douglass et al study.[181] Our study reports the mean caries incidence as 0.8737 ± 1.48741 where as Douglass reported caries incidence to be 0.7 either for 2 or 4 fluoride applications per year .However it would not be pragmatic to ascertain the varnishes as sole entity responsible for remineralization of the lesions. There are many factors which need to be taken in to account like adherence to post varnish application instructions, taking care of oral hygiene to be few. Oral hygiene is undoubtedly an important precursor for caries.

Oral hygiene can be measured in a number of objective ways using indices like Oral Hygiene Index Simplified Miglani et al. [132], Van Strydonck 2004 index [182], Axelsson & Lindhe 1987 [183] debris index. OHIS was used in current research. Instructions about Oral hygiene were given at the baseline to all the parents. They were also taught about the initiation of oral hygiene at the earliest, using proper oral hygiene aids. They were also sensitized regarding amount of toothpaste to be used such as, of 'rice grain size' amount for less than 3yrs old and not more than pea size for 3-6yr old children.[184] All these measures will play a decisive role in preventing ECC. In corroboration to our study Millan et al[185] also used simplified OHI to measure oral hygiene. Mean Oral hygiene score at the baseline was 1.0354 ±1.12370 and post intervention it reduced to 0.829 ± 0.99164 which was statistically significant. Millan et al [185] study reported mean oral hygiene score to be 0.92 and difference found was not significant at the conclusion of two years.

Oral hygiene and ECC- There was significant improvement in oral hygiene in Licorice and Combination group which can be modified and it does have an impact on overall success or failure of the preventive therapy which is quite evident from the result of current research. Oral hygiene emerged as a very highly significant factor in logistic regression analysis with an odds ratio of 5.9.Oral hygiene index has been measured by Esha Jain et al[149] with an odds ratio of 0.69 and no statistical significance was found.

Adverse events- Neither we nor parents encountered any adverse events post application of the varnish. All the parents had contact details of the investigator and they were well informed to call the investigator in case of any unlikely event occurring after application of varnish. But throughout the study period none of the parents called or informed about any sort of discomfort arising out of varnish application. Hence we can believe that all the three dental varnishes were well tolerated by the children. Majority of the children liked the taste and smell of the varnishes.

The present study witnessed 19% of dropout rate. Dropout was due to change of schools by the children and migration of the family from the city. Though drop outs in the field study are common, it can threaten the validity of any study result. Drop outs as high as 31% have been reported by Millan et al,[185] 45% at end of 3 years by Plonka et al [6] and 44.6% by Aruda et al. [186] Though sample size was inflated at baseline anticipating the dropout, but the observed dropout was higher than what was expected. Every effort was made to recall the students and collect the information related to study parameters.

Telephonic calls were made to recall the students, parents were also reminded about the visit.But in spite of all these efforts however dropout was observed. On a brighter note, the study provided chance to preschool children to avail the varnish application at their door steps along with this awareness was created about ECC among parents and teachers alike. Children also had an opportunity to learn and inculcate basic oral hygiene skills taught to them by the investigator.

Intention to treat analysis was applied to compensate for dropout rate. Interestingly the study results of both Per Protocol and Intention to treat analysis remained same. Licorice varnish was significantly better than fluoride varnish as far as remineralising potential is concerned.

Cost factor

Licorice is not only cheap and easily available but also has superior action on ECC. Whenever plant based modality is researched, few considerations should be kept in mind like sustainability, affordability and toxic potential to humans. Licorice varnish met all these requirements. We did compare the cost incurred in preparing licorice varnish against Bifluorid and combination varnish. Licorice varnish was more effective than other two varnishes and almost six times cheaper than fluoride varnish.

Limitations

In spite of the best efforts put to implement this research study, the study does suffer from some limitations which have been mentioned below.

- Use of a single organism that is *S.mutans*. Since salivary estimation had to be carried at both baseline and post intervention phases, a total of 400 samples were analyzed. As microbiological investigations are expensive, other micro organism were not cultured or studied.

- Attrition rate – which was beyond our control.

- Compliance to the instructions by the children and parents – We are unsure as to how many parents and children actually adhered to the instructions given to them post application of the varnish. Non adherence to the instructions may be the reason for reduced effectiveness of the products, the extent of which remains undetermined.

- Some intra oral physiological changes were evident like spacing seen in the teeth due to growth of the mandible, which helped in easy clearance of the food particles and debris along with completion of the mineralization of the newly erupted primary teeth. These events may have helped in giving better resistance to the tooth against dental caries.

- Licorice and combination varnish had shorter shelf life which needs to explored and modified in future research.

- Hawthorne effect could probably be responsible for improved oral hygiene scores.

- Children in all groups were exposed to topical fluorides especially from toothpastes. There may have been some additive effect of toothpaste with varnish.

- Double blinding was not possible and feasible in the present field trial.

Strengths of the study – Following are the strengths

- Comparison of two different extraction procedures for licorice extract -Cold Maceration with Soxhlet extraction.
- Licorice varnish was prepared using root of a medicinal plant and this varnish was tested for physical parameters and evaluated for toxicity.
- Current research is the first one to use combination varnish made up of Licoirce Varnish and Fluoride Varnish.
- Five different combinations were prepared for testing Combination varnish.
- Minimal Inhibitory Concentration of varnish was estimated using a novel technique to overcome the inherent property of varnish – its viscosity.
- Current study assessed effectiveness of the standard varnish as well as novel varnish in real life situations.
- It is a parallel arm design; hence possibility of contamination effect is reduced.
- Sample size was adequate and is representative of the preschool children of Belagavi city.
- Varnish application covered entire dentition of all the selected children.
- Awareness was created among parents and teachers regarding Early Childhood Caries.
- There was an active interaction between child, parent, school teachers and the investigator.
- Use of a positive control which is Fluoride Varnish group.
- In a span of 1 year, 4 applications of varnish were applied at 3 monthly intervals.
- Salivary samples were used for estimation of *Streptococcus mutans.*

- Diagnodent scoring was used to assess remineralisation potential of the varnishes.
- Nyvad's index was used in combination with Diagnodent score to assess remineralisation of the initial lesion post intervention.
- Varnish was applied at the door steps of the children; printed instructions were given along with reminder calls being made to the parents to reinforce the instructions.
- Children also received free dental health card for the comprehensive dental care requirements post intervention.

Future scope

There are many avenues for further research; specific areas which can be explored, understand the mechanism of how Licorice varnish helps to improve remineralisation potential of the tooth surface. Interaction studies at molecular level are required to understand the relation between Licorice and fluoride varnish. Antibacterial spectrum of licorice varnish. Bias can be reduced by employing double blind studies. Stability studies of Licorice Varnish can be undertaken to assess if Licorice Varnish is equally efficacious at different temperatures and at varying salivary pH values.

Public Health Significance

The present research is valuable in terms of its utility to public in general and to preschool children in specific. It was designed to deliver an affordable, accessible oral health care product to the community at large. It attempted to find an alternative to fluoride varnish. This alternative agent had time tested root extract of a medicinal

plant, Licorice. Drug delivery is an important parameter and when the drug has to be delivered to the youngest members of the society, the efficacy and toxicity should be well thought. Delivering the licorice extract in the dosage form of the varnish was able to strike the right balance between the benefit availed and risk incurred. Fluoride varnish which is considered as Benchmark control for caries prevention was combined with Licorice varnish and was compared for its effectiveness as Combination varnish.

6. CONCLUSION

In the current study, Licorice extract and Licorice varnish both exhibited antibacterial action against standard strain of *Streptococcus mutans* in an invitro study. FV, LV and CV were similar to one another in all the physical parameters tested. Combination varnish with the composition of 50% FV and 50% LV was chosen after assessing all other concentrations of CV.

Parental locus of control was significantly associated with caries experience of their children. Children of parents who had Internal LOC had 1.8 times more chance to be caries free compared to their counterparts whose parents had External Locus of control.

When baseline and post intervention Diagnodent scores were compared for remineralisation potential, scores differed significantly in post intervention phase. When inter group comparison was done for remineralising potential, significant difference between Licorice varnish and Fluoride varnish was found. When mean salivary *Streptococcus mutans* scores were compared between baseline scores and post intervention scores, significant difference was observed with Licorice varnish and Combination varnish and non significant difference with Fluoride varnish. Logistic regression revealed good oral hygiene and location of the initial lesion in the anterior region of the oral cavity as significant factors which were associated with success of the intervention. It is noteworthy that, though the study witnessed dropouts, the conclusions of both the analysis - intention to treat as well as per protocol remained same.

7. SUMMARY

Current research was an interventional study which was aimed to compare effectiveness of three varnishes namely Licorice, Fluoride and Combination varnish on initial lesions of ECC. Study had two phases – 1st phase- Invitro phase and 2nd phase – Invivo phase. Invitro study was scheduled from from 12th June 2014 to 28th Dec 2014. Trial was registered at Central Trial Registry India with trial no. CTRI/2015/10/006305. Invivo part was scheduled from 24th April 2015 to 10th October 2016. Antibacterial activity of root extract of licorice was estimated during invitro study against standard strain of *Streptococcus mutans* ATCC 25175. Licorice root were purchased from KLE Ayurveda Pharmacy. Roots were authenticated from Botanist, ICMR RMRC, Belagavi. Roots were powdered and extract was prepared in two different methods using these roots. Extract was obtained from two commonly used methods – Cold maceration and Soxhlet method. These experiments were conducted in Pharmacy college of KLE Society at Pharmaceutics and Pharmacognosy department. Phytochemical screening of licorice extract was performed. MIC of both the extract were assessed against standard strain of *Streptococcus mutans* 25175 by broth dilution method. Experiment was repeated 3times and mean values were considered for assessment. This was followed by preparation of the varnish. Licorice varnish was prepared along with the various concentrations of combination varnish. Evaluation of the varnishes was made for physical properties like pH, rate of evaporation, viscosity, film forming ability and color matching.

Film forming ability was assessed by painting 20 microlitre of varnish on extracted anterior teeth and examined under scanning electron microscopy. Minimal inhibitory concentration of varnishes was assessed against standard strain of

Streptococcus mutans 25175.Routinely used methods like agar well method and broth dilution were attempted but the results were inconclusive. Hence a newer method to assess MIC of varnishes was tried which gave satisfactory results. Safety of three varnishes were assessed by carrying out Toxicity study on- Primary Gingival Fibroblasts.;Normal, Adult (HGF) ATCCPCS-201-018 . Once the varnishes were prepared and tested they were poured in an amber colored bottle which was maintained at normal temperature till further use.

All the required permissions were obtained. Sample size was based on eta square; the required sample size was 62 in each group which was rounded off to 65 in each group.

Study was pilot tested on 12 preschool children of Sidrameshwar anganwadi, Belagavi. During pilot study method for collection of saliva sample, identification of initial lesions using Nyvad's index and Diagnodent pen, application of dental varnish and study questionnaire were standardized. Training and calibration of the investigator was done for recording the relevant indices like Nyvad's index, OHI simplified and for the recording of initial lesions using Diagnodent pen under supervision of guide. A self-designed proforma collected socio demographic, brushing habits and LOC information. The study sample of 195 subjects was selected from 14 randomly selected schools.

Field study had 2 phases- Screening phase and interventional phase. Screening phase –This phase consisted collection of data pertaining to parental perception regarding LOC and dental caries status and brushing habits among their young ones.

On predetermined date; parents were called to attend oral health talk for about 20-25 minutes, delivered by principal investigator on "How best to prevent tooth decay in your young ones?"Before the lecture parents were asked to fill study questionnaire. During the lecture, parents were sensitized regarding various topics related to oral hygiene. After the screening, parents of those children who satisfied inclusion criteria were informed about the initial lesions. After parental consent, child was recruited into the study.

Intervention phase- Out of the total 407 children screened, 198 satisfied inclusion criteria, selected children were randomized to three arms using computer generated random allocation. The intervention phase was performed in mobile dental van which included following steps - recording of OHI Score, recording of Diagnodent score of initial lesion, Recording of Nyvad's index, collection of saliva sample and application of respective varnishes and post application instructions were given. Respective varnishes were reapplied at 3rd month, 6th month and 9th month. One year post intervention Diagnodent scores, Nyvad's scores were reassessed. Saliva sample was collected for estimation of *Streptococcus mutans* count. All children received instructions on oral health along with demonstration of brushing technique. Every study participant received a toothbrush and fluoridated toothpaste containing 450 ppm sodium monofluorophosphate along with free referral card of the institute. The data was subjected to analysis using SPSS version 20 ((SPSS Inc, Chicago IL). p value lesser than 0.05 was considered significant.

Descriptive statistics was used for summarizing data. Chi square test, Wilcoxson sign rank test, ANOVA test, Mann Whitney U test, Logistic regression test and Intention to treat were carried out. Following are the results of current study.

Cold maceration extract had mean MIC value of 1.8mg/ml±0.145 and Soxhlet method had mean MIC value of 3.80 mg/ml±0.097 .When unpaired T test was applied result was significant, with p value lesser than 0.0001 with 4 degree of freedom and t value of -19.180. Hence cold maceration extract was used for further study. Both the extracts contained similar phytochemical constituents. Licorice extract had antibacterial action on *Streptococcus mutans* with a MIC value of 2mg/ml. Various combinations tested were 50% Licorice Varnish + 50% Fluoride Varnish, 60% Licorice Varnish + 40% Fluoride Varnish, 75% Licorice Varnish + 25% Fluoride Varnish and 60% Fluoride Varnish + 40% Licorice Varnish and all these combinations showed antibacterial activity to *Streptococcus mutans*. Since 50% fluoride varnish and 50% licorice varnish was practical and feasible to prepare, further investigations were carried out with this combination. Varnishes were similar in all the parameters assessed. Licorice varnish was less viscous than fluoride varnish though no significant difference noted.

Screening for initial lesions was done on 407 preschool children. Prevalence rate of ECC was 73.21% whereas, initial lesions was 48.64% which was equally distributed among males and females. Parental responses for LOC questionnaire was analyzed and 29.5 was estimated as cut off point for LOC score. Children whose parents had internal LOC had 1.8 times more chances of being caries free then parents who had External LOC. Logistic regression was carried out, with dental caries as a dependent variable. Among all the factors analyzed LOC score had statistically significant association with ECC.

The interventional phase had 198 subjects who were randomized to varnish groups. Among 198 subjects, 103 were males and 95 were females. Majority of them were from upper middle class. No significant difference was seen with oral hygiene aid used, parental assistance for oral hygiene measures, frequency of brushing, material used for brushing and frequency of changing toothbrush with age gender and socio economic status.

Among the total sample of 198 subjects, 64 subjects belonged to Fluoride varnish group, 68 to Licorice varnish and 66 to Combination varnish group. They were similar at baseline for various parameters like caries experience, mean Diagnodent scores and *Streptococcus mutans* count. Baseline Diagnodent scores and post intervention scores differed significantly with respect to various tooth surfaces like pit and fissures, smooth surfaces, anterior region and posterior region. Diagnodent scores were presented as percentiles, and comparison was done between baseline score and post intervention score.

In Fluoride varnish group, at 25th percentile Diagnodent score changed from 3 at baseline to 2 post intervention, 4 to 2.5 and 6 to 4 at 50th and 75th percentile respectively. Similarly in Licorice varnish group scores changed from 3 to 1, 3 to 2 and 3 to 2 at 25th, 50th and 75th percentile. In combination varnish group, scores changed from 3 to 2, 3 to 3 and 3 to 4 at 25th, 50th and 75th percentile respectively. When pre post comparison was done scores differed significantly.

Similarly when Diagnodent scores were compared significant difference found in post intervention phase among 3 groups. Pair wise analysis revealed significant difference with Licorice varnish group. Post intervention Nyvad's score showed significant difference with 87.5% of initial active lesions being converted to inactive

lesions in Licorice varnish group, followed by 70.9% and 60.7% in Combination varnish and FV respectively. Caries incidence during 1 year study period was not significantly different between groups.

Mean baseline salivary *Streptococcus mutans scores* and post intervention scores differed significantly in Licorice and Combination varnish groups. Mean Salivary *Streptococcus mutans* scores changed from 3.2±0.5 to 3.1±0.4 ($p>0.05$) in Fluoride varnish group, 3.29±0.8 to 3.06±0.35 ($p<0.05$) in Licorice varnish group and 3.11±0.87 to 2.81±0.48 ($p<0.05$) in Combination varnish group when baseline scores were compared with post intervention scores.

On pre post comparison, oral hygiene score showed significant improvement in Licorice and Combination varnish group however we failed to observe it in Fluoride varnish group. When rate of remineralization was compared among the 3 groups, remineralization rate of 93.6% in Licorice varnish group, 83.6% in Combination varnish and 67.9% in Fluoride varnish was observed which was statistically significant.

Logistic regression analysis was performed with success of the intervention as a dependent factor with factors like oral hygiene, dmft, incidence, varnish applied, and position of the tooth in oral cavity as the independent factors. Good oral hygiene along with presence of initial lesion in the anterior region were significantly associated with success of the intervention.

The present study reported 8 dropouts in Fluoride varnish group, 20 in Licorice varnish and 11 in Combination varnish group. Dropouts were due to change of the school and migration from the city. Intention to treat was performed and its

conclusions were similar to per protocol analysis. No adverse events were encountered. Taste and smell of varnishes was liked by most of the participants. Thus the present study concluded that Licorice varnish was significantly better than Combination varnish and FV in remineralising the initial lesions. However when effectiveness of varnishes on *Streptococcus mutans* was compared significant difference was observed in Licorice and Combination varnish and non significant difference was seen in Fluoride varnish group. Children whose parents had internal LOC had higher chances of being caries free

8. BIBILOGRAPHY

1. Davies G, Neville J, Rooney E, Robinson M, Jones A, Perkins C. National Dental Epidemiology Programme for England: oral health survey of five-year-old children 2012; A report on the prevalence and severity of dental decay. 2013.
2. Dye BA, Thornton-Evans G, Li X, Iafolla TJ. Dental Caries and Sealant Prevalence in Children and Adolescents in the United States, 2011-2012 Key findings Data from the National Health and Nutrition Examination Survey, 2011-2012.
3. Do LG SA. Oral health of Australian children. The national child oral health study 2012-2014. Adelaide (Australia): University Adelaide Press. 2016;370.
4. Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH. Diagnosing and reporting early childhood caries for research purposes. A report of a workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration, and the Health Care Financing Administ. J Public Health Dent. 1999;59(3):192–7.
5. Zhou Y, Lin HC, Lo E, Wong M. Risk indicators for early childhood caries in 2-year-old children in southern China. Aust Dent J. 2011;56:33–9.
6. Plonka KA, Pukallus ML, Barnett AG, Holcombe TF, Walsh LJ, Seow WK. A longitudinal case-control study of caries development from birth to 36 months. Caries Res. 2013;47(2):117–27.
7. Fan C, Wang W, Xu T, Zheng S. Risk factors of early childhood caries among children in Beijing: A case-control study. BMC Oral Health. 2016;16(1):98.
8. Duangthip D, Gao SS, Lo ECM, Chu CH. Early childhood caries among 5- to 6-year-old children in Southeast Asia. Int Dent J. 2017;67(2):98–106.

9. Ganesh A, Muthu MS, Mohan A, Kirubakaran R. Prevalence of Early Childhood Caries in India – A Systematic Review. Indian Journal of Pediatrics. 2018:3;276–86.

10. Prakash P, Subramaniam P, Durgesh BH, Konde S. Prevalence of early childhood caries and associated risk factors in preschool children of urban Bangalore, India: A cross-sectional study. Eur J Dent. 2012 ;6(2):141–52.

11. Prakasha Shrutha S, Vinit GBG, Giri KY, Alam S. Feeding practices and early childhood caries: a cross-sectional study of preschool children in kanpur district, India. ISRN Dent. 2013;2013:275193.

12. Jose B, King NM. Early childhood caries lesions in preschool children in Kerala, India. Pediatr Dent. 2003;25(6):594–600.

13. Sankeshwari R, Ankola A V, Tangade S, Hebbal I. Feeding habits and oral hygiene practices as determinants of early childhood caries in 3- to 5-year-old children of Belgaum City, India. Oral Health Prev Dent. 2012 ;10(3):283–90.

14. Edelstein BL. Solving the problem of early childhood caries: A challenge for us all. Archives of Pediatrics and Adolescent Medicine. 2009;163(7):667–8.

15. Chu CH. Treatment of early childhood caries: a review and case report. General dentistry. 2000. p. 142–8.

16. Petersen PE, Estupinan-Day S, Ndiaye C. WHO's action for continuous improvement in oral health. Bulletin of the World Health Organization. 2005;83(9):642.

17. Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013;4(1):29–38.

18. Seow WK. Early Childhood Caries. Pediatric Clinics of North America. 2018. p. 941–54.

19. Seow WK. Biological mechanisms of early childhood caries. Community Dent Oral Epidemiol. 1998;26(1 Suppl):8–27.

20. Manton DJ. Diagnosis of the early carious lesion. Aust Dent J. 2013 ;58 Suppl 1:35–9.

21. Bader JD, Shugars DA. A systematic review of the performance of a laser fluorescence device for detecting caries. Journal of the American Dental Association. 2004;135(10) : 1413–26.

22. Gao SS, Zhang S, Mei ML, Lo EC-M, Chu C-H. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health. 2016 ;16(1):12.

23. Marinho VCC, Worthington H V, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane database Syst Rev. 2013 Jan;7:CD002279.

24. Weyant RJ, Tracy SL, Anselmo TT, Beltrán-Aguilar ED, Donly KJ, Frese WA, et al. Topical fluoride for caries prevention: executive summary of the updated clinical recommendations and supporting systematic review. J Am Dent Assoc. 2013 ;144(11):1279–91.

25. AA.VV. Glycyrrhiza glabra monograph. Altern Med Rev. 2005;10(3):230–7.

26. Segal R, Pisanty S, Wormser R, Azaz E, Sela MN. Anticariogenic activity of licorice and glycyrrhizine I: Inhibition of in vitro plaque formation by Streptococcus mutans. J Pharm Sci. 1985 ;74(1):79–81.

27. Marsh HW, Richards GE. The Multidimensionality of the Rotter I-E Scale and its Higher-order Structure: An Application of Confirmatory Factor Analysis. Multivariate Behav Res. 1987 ;22(1):39–69.

28. Tinanoff N. Introduction to the Early Childhood Caries Conference: initial description and current understanding. Community Dent Oral Epidemiol. 1998 ;26(1 Suppl):5–7.

29. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986 ;50(4):353–80.

30. Vadiakas G. Case definition, aetiology and risk assessment of early childhood caries (ECC): a revisited review. Eur Arch Paediatr Dent. 2008 ;9(3):114–25.

31. Frejeskov. Dental caries disease and its clinical management. Wiley-Blackwell; 2nd edition; 2003. 21-35pages.

32. Gomez J, Tellez M, Pretty IA, Ellwood RP, Ismail AI. Non-cavitated carious lesions detection methods: A systematic review. In: Community Dentistry and Oral Epidemiology. 2013;41(1): 55–66.

33. Dirks OB. Posteruptive Changes in Dental Enamel. J Dent Res. 1966 8;45(3):503–11.

34. Carvalho JC, Ekstrand KR, Thylstrup A. Dental Plaque and Caries on Occlusal Surfaces of First Permanent Molars in Relation to Stage of Eruption. J Dent Res. 1989;68(5):773–9.

35. Ekstrand KR, Ricketts DNJ, Kidd EAM, Qvist V, Schou S. Detection, Diagnosing, Monitoring and Logical Treatment of Occlusal Caries in Relation to Lesion Activity and Severity: An in vivo Examination with Histological Validation. Caries Res. 1998;32(4):247–54.

36. Kidd EAM, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. In: Journal of Dental Research. 2004. Spec No C:C35-8.

37. Zandoná AF, Zero DT. Diagnostic tools for early caries detection. Journal of the American Dental Association. 2006;137:1675–84.

38. Guerrieri A, Gaucher C, Bonte E, Lasfargues JJ. Minimal intervention dentistry: Part 4. Detection and diagnosis of initial caries lesions. Br Dent J. 2012;213(11):551–7.

39. Kühnisch J, Dietz W, Stösser L, Hickel R, Heinrich-Weltzien R. Effects of dental probing on occlusal surfaces - A scanning electron microscopy evaluation. Caries Res. 2006;41(1):43–8.

40. Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. Caries Res. 1999;33(4):252–60.

41. Pitts N. "ICDAS" - An international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dental Health. 2004;21(3):193–8.

42. Dikmen B. ICDAS II Criteria (International Caries Detection And Assessment System). J Istanbul Univ Fac Dent. 2015;49(3):63.

43. Pitts NB. Modern concepts of caries measurement. J Dent Res. 2004 ;83 Spec No:C43-7.

44. Shoaib L, Deery C, Ricketts DNJ, Nugent ZJ. Validity and reproducibility of ICDAS II in primary teeth. Caries Res. 2009 ;43(6):442–8.

45. Ricketts Dnj, Kidd Eam, Smith Bgn, Wilson Rf. Clinical and radiographic diagnosis of occlusal caries. a study in vitro. J Oral Rehabil. 1995;22(1):15–20.

46. Hintze H, Wenzel A, Danielsen B, Nyvad B. Reliability of Visual Examination, Fibre-Optic Transillumination, and Bite-Wing Radiography, and Reproducibility of Direct Visual Examination Following Tooth Separation for the Identification of Cavitated Carious Lesions in Contacting Approximal Surfaces. Caries Res. 1998;32(3):204–9.

47. Diniz MB, Rodrigues JA, Neuhaus KW, Cordeiro RCL, Lussi A. Influence of examiner's clinical experience on the reproducibility and accuracy of radiographic examination in detecting occlusal caries. Clin Oral Investig. 2010 8;14(5):515–23.

48. Pretty IA. Caries detection and diagnosis: Novel technologies. J Dent. 2006 ;34(10):727-39.

49. Côrtes DF, Ellwood RP, Ekstrand KR. An in vitro comparison of a combined FOTI/Visual examination of occlusal caries with other caries diagnostic methods and the effect of stain on their diagnostic performance. Caries Res. 2003;37(1):8–16.

50. Betrisey E, Rizcalla N, Krejci I, Ardu S. Caries diagnosis using light fluorescence devices: VistaProof and DIAGNODENT. Odontology. 2014 7;102(2):330–5.

51. Bader JD, Shugars DA, Bonito AJ. Systematic reviews of selected dental caries diagnostic and management methods. J Dent Educ. 2001 ;65(10):960–8.

52. Hibst R, Paulus R, Lussi A. Detection of occlusal caries by laser fluorescence: Basic and clinical investigations. Med Laser Appl. 2001;16(3):205–13.

53. Kühnisch J, Bücher K, Henschel V, Hickel R. Reproducibility of DIAGNOdent 2095 and DIAGNOdent Pen measurements: Results from an in vitro study on occlusal sites. Eur J Oral Sci. 2007 ;115(3):206–11.

54. Kavvadia K, Seremidi K, Reppa C, Makou M, Lagouvardos P. Validation of fluorescence devices for evaluation of white spot lesions in orthodontic patients. Eur Arch Paediatr Dent. 2018 13;19(2):83–9.

55. Theocharopoulou A, Lagerweij MD, van Strijp AJ. Use of the ICDAS system and two fluorescence-based intraoral devices for examination of occlusal surfaces. Eur J Paediatr Dent. 2015 ;16(1):51–5.

56. Rodrigues JA, Hug I, Diniz MB, Lussi A. Performance of fluorescence methods, radiographic examination and ICDAS II on occlusal surfaces in vitro. Caries Res. 2008;42(4):297–304.

57. Gripp VC, Schlagenhauf U. Prevention of early mutans streptococci transmission in infants by professional tooth cleaning and chlorhexidine varnish treatment of the mother. Caries Res. 2002;36(5):366–72.

58. Ismail AI. Prevention of early childhood caries. Community Dent Oral Epidemiol. 1998;26(1 Suppl):49–61.

59. American Academy on Pediatric Dentistry, American Academy of Pediatrics. Policy on early childhood caries (ECC): classifications, consequences, and preventive strategies. Pediatr Dent. 2008;30(7 Suppl):40–3.

60. Harris NO, Christen AG. Primary Preventive Dentistry, Fourth Edition. Applet Lange. 6th ed. Upper Saddle River, N.J Pearson Education; 1995;259–315.

61. Collins FM. The Development and Utilization of Fluoride Varnish Educational Objectives. 2014.

62. Strohmenger L, Brambilla E. The use of fluoride varnishes in the prevention of dental caries: A short review. Oral Dis. 2001 ;7(2):71-80.

63. Øgaard B, Seppä L, Rolla G. Professional Topical Fluoride Applications—Clinical Efficacy and Mechanism of Action. Adv Dent Res. 1994;8(2):190–201.

64. Horowitz HS. The effectiveness of community water fluoridation in the United States. J Public Health Dent. 1996;56(5):253–8.

65. Aoba T. The effect of fluoride on apatite structure and growth. Crit Rev Oral Biol Med. 1997;8(2):136–53.

66. Fejerskov O, Thylstrup A, Larsen MJ. Rational use of fluorides in caries prevention: A concept based on possible cariostatic mechanisms. Acta Odontol Scand. 1981;39(4):241–9.

67. Weintraub JA. Fluoride varnish for caries prevention: comparisons with other preventive agents and recommendations for a community-based protocol. Spec Care Dentist. 2003;23(5):180-6.

68. Bader JD, Shugars DA, Bonito AJ. Systematic reviews of selected dental caries diagnostic and management methods. J Dent Educ. 2001;65(10):960–8.

69. Helfenstein U, Steiner M. Fluoride varnishes (Duraphat): a meta-analysis. Community Dent Oral Epidemiol. 1994 ;22(1):1–5.

70. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2002;(3):CD002279.

71. Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JDB, et al. Fluoride varnish efficacy in preventing early childhood caries. J Dent Res. 2006;85(2):172–6.

72. Ahovuo-Saloranta A, Forss H, Hiiri A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev. 2016 18;(1):CD003067.

73. Hiiri A, Ahovuo-Saloranta A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in children and adolescents. Cochrane database Syst Rev. 2010 ;(3):CD003067.

74. Lenzi TL, Montagner AF, Soares FZM, De Oliveira Rocha R. Are topical fluorides effective for treating incipient carious lesions? A systematic review and meta-analysis. J Am Dent Assoc. 2016;147(2):84–91.

75. Du M, Cheng N, Tai B, Jiang H, Li J, Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment HHS Public Access. Clin Oral Investig. 2012;16(2):463–8.

76. Lippert F. Fluoride release from fluoride varnishes under acidic conditions. J Clin Pediatr Dent. 2014;39(1):35–9.

77. Carvalho DM, Salazar M, Oliveira BH de, Coutinho ESF. Fluoride varnishes and decrease in caries incidence in preschool children: a systematic review. Rev Bras Epidemiol. 2010 ;13(1):139–49.

78. Roberts JF, Longhurst P. A clinical estimation of the fluoride used during application of a fluoride varnish. Br Dent J. 1987;162(12):463–6.

79. Sharma PR. Allergic Contact Stomatitis from Colophony. Dent Update. 2006 ;33(7):440–2.

80. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005;43(11):5721–32.

81. Van Houte J, Gibbs G, Butera C. Oral Flora of Children with "Nursing Bottle Caries." Journal of Dental Research. J Dent Res. 1982 ;61(2):382-5

82. Marsh PD, Martin M V, Lewis MAO, Williams D. Oral Microbiology E-Book. 2009.

83. Nissegard Russell NM. Oral Microbiology and Immunology. Second edi. WB Saunder Company Ltd , London UK; 1994. 1-477 p.

84. Singla D, Sharma A, Sachdev V, Chopra R. Distribution of Streptococcus mutans and Streptococcus sobrinus in dental plaque of Indian pre-school children using PCR and SB-20M agar medium. J Clin Diagnostic Res. 2016;10(11):ZC60-ZC63.

85. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol. 2008;46(4):1407–17.

86. Agnello M, Marques J, Cen L, Mittermuller B, Huang A, Chaichanasakul Tran N, et al. Microbiome Associated with Severe Caries in Canadian First Nations Children. J Dent Res. 2017;96(12):1378–85.

87. Ma C, Chen F, Zhang Y, Sun X, Tong P, Si Y, et al. Comparison of oral microbial profiles between children with severe early childhood caries and caries-free children using the human oral microbe identification microarray. PLoS One. 2015;10(3):e0122075.

88. Burton L. Edelstein, Steven D. Ureles, Arlene Smaldone. Very High Salivary Streptococcus Mutans Predicts Caries Progression in Young Children. Pediatr Dent. 2016;38(4):325–30.

89. Thenisch NL, Bachmann LM, Imfeld T, Leisebach Minder T, Steurer J. Are mutans streptococci detected in preschool children a reliable predictive factor for dental caries risk? A systematic review. Caries Res.;2006 ;40(5):366–74.

90. Twetman S, Dhar V. Evidence of Effectiveness of Current Therapies to Prevent and Treat Early Childhood Caries. Pediatr Dent. 2015;37(3):246–53.

91. Lawrence HP, Binguis D, Douglas J, McKeown L, Switzer B, Figueiredo R, et al. A 2-year community-randomized controlled trial of fluoride varnish to prevent early childhood caries in Aboriginal children. Community Dent Oral Epidemiol. 2008 ;36(6):503–16.

92. Milgrom P, Tut OK. Evaluation of pacific islands early childhood caries prevention project: Republic of the marshall islands. J Public Health Dent. 2009;69(3):201–3.

93. Lin C, Coors S, Rambob I, Tinanoff N, Grossman LK. Evaluation of an early childhood caries prevention program at an urban pediatric clinic. Pediatr Dent. 2008;30(6):499–504.

94. Slade GD, Bailie RS, Roberts-Thomson K, Leach AJ, Raye I, Endean C, et al. Effect of health promotion and fluoride varnish on dental caries among Australian Aboriginal children: Results from a community-randomized controlled trial. Community Dent Oral Epidemiol ; 2011;39(1):29–43.

95. Ramos-Gomez FJ, Gansky SA, Featherstone JDB, Jue B, Gonzalez-Beristain R, Santo W, et al. Mother and youth access (MAYA) maternal chlorhexidine, counselling and paediatric fluoride varnish randomized clinical trial to prevent early childhood caries. Int J Paediatr Dent. 2012;22(3):169–79.

96. Li Y, Tanner A. Effect of Antimicrobial Interventions on the Oral Microbiota Associated with Early Childhood Caries. Pediatr Dent. 2015;37(3):226–44.

97. Garcia R, Borrelli B, Dhar V, Douglass J, Gomez FR, Hieftje K, et al. Progress in Early Childhood Caries and Opportunities in Research, Policy, and Clinical Management. Pediatr Dent. 2015 ;37(3):294–9.

98. Liberio SA, Pereira ALA, Dutra RP, Reis AS, Araújo MJAM, Mattar NS, et al. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee Melipona fasciculata Smith. BMC Complement Altern Med. 2011;11(1):108.

99. Obolentseva G V., Litvinenko VI, Ammosov AS, Popova TP, Sampiev AM. Pharmacological and therapeutic properties of licorice preparations (A review). Pharmaceutical Chemistry Journal. 1999; (33): 427–34.

100. Dr Gyanendra Pandey Vol II Chavkhamba Publication ND. Dravyaguna Vigyan. 456-468 p.

101. Van Rossum TGJ, Vulto AG, Hop WCJ, Schalm SW. Glycyrrhizin-induced reduction of ALT in European patients with chronic hepatitis C. Am J Gastroenterol. 2001 ;96(8):2432–7.

102. Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, et al. The long term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer. 1997 ;79(8):1494–500.

103. Das SK, Das V, Gulati AK, Singh VP. Deglycyrrhizinated liquorice in aphthous ulcers. J Assoc Physicians India. 1989 ;37(10):647.

104. Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T. Anti-Helicobacter pylori flavonoids from licorice extract. Life Sci. 2002 9;71(12):1449–63.

105. Armanini D, De Palo CB, Mattarello MJ, Spinella P, Zaccaria M, Ermolao A, et al. Effect of licorice on the reduction of body fat mass in healthy subjects. J Endocrinol Invest. 2003;26(7):646–50.

106. Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis. 2012 ;18(1):32–9.

107. Takahashi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Research. 2008;42(6): 409–18.

108. Koo H, Xiao J, Klein MI. Extracellular Polysaccharides Matrix — An Often Forgotten Virulence Factor in Oral Biofilm Research. Int J Oral Sci. 2009;1(4):229–34.

109. Azaz E, Segal R. Glycyrrizin as gelling agent. Pharm Acta Helv. 1980;55(7):183–6.

110. Sela MN, Steinberg D, Segal R. Inhibition of the activity of glucosyltransferase from Streptococcus mutans by glycyrrhizin. Oral Microbiol Immunol. 1987 ;2(3):125–8.

111. Gedalia I, Stabholtz A, Lavie A, Shapira L, Pisanti S, Segal R. The effect of glycyrrhizin on in vitro fluoride uptake by tooth enamel and subsequent demineralization. Clin Prev Dent. 1986;8(2):5–9.

112. Deutchman M, Petrou ID, Mellberg JR. Effect of Fluoride and Glycyrrhizin Mouthrinses on Artificial Caries Lesions in vivo (Short Communication). Caries Res. 1989;23(3):206–8.

113. Steinberg D, Sgan-Cohen HD, Stabholz A, Pizanty S, Segal R, Sela MN. The anticariogenic activity of glycyrrhizin: preliminary clinical trials. Isr J Dent Sci. 1989;2(3):153–7.

114. Söderling E, Karjalainen S, Lille M, Maukonen J, Saarela M, Autio K. The effect of liquorice extract-containing starch gel on the amount and microbial composition of plaque. Clin Oral Investig. 2006 ;10(2):108–13.

115. Peters MC, Tallman JA, Braun TM, Jacobson JJ. Clinical reduction of S. mutans in pre-school children using a novel liquorice root extract lollipop: a pilot study. Eur Arch Paediatr Dent. 2010 ;11(6):274–8.

116. Hu C-H, He J, Eckert R, Wu X-Y, Li L-N, Tian Y, et al. Development and evaluation of a safe and effective sugar-free herbal lollipop that kills cavity-causing bacteria. Int J Oral Sci. 2011 ;3(1):13–20.

117. La VD, Tanabe S, Bergeron C, Gafner S, Grenier D. Modulation of matrix metalloproteinase and cytokine production by licorice isolates licoricidin and licorisoflavan A: potential therapeutic approach for periodontitis. J Periodontol. 2011;82(1):122–8.

118. Choi E-M. The licorice root derived isoflavan glabridin increases the function of osteoblastic MC3T3-E1 cells. Biochem Pharmacol. 2005;70(3):363–8.

119. Millsop JW, Fazel N. Oral candidiasis. Clin Dermatol. 2016 Jul;34(4):487–94.

120. Fatima A, Gupta VK, Luqman S, Negi AS, Kumar JK, Shanker K, et al. Antifungal activity of Glycyrrhiza glabra extracts and its active constituent glabridin. Phyther Res. 2009;23(8):1190–3.

121. Messier C, Grenier D. Effect of licorice compounds licochalcone A, glabridin and glycyrrhizic acid on growth and virulence properties of Candida albicans. Mycoses. 2011;54(6):e801–6.

122. Muñoz-Corcuera M, Esparza-Gómez G, González-Moles MA, Bascones-Martínez A. Oral ulcers: clinical aspects. A tool for dermatologists. Part II. Chronic ulcers. Clinical and Experimental Dermatology. 2009;34(4): 456–61.

123. Martin MD, Sherman J, van der Ven P BJ (2008). A controlled trial of a dissolving oral patch concerning glycyrrhiza (licorice) herbal extract for the treatment of aphthous ulcers. Gen Dent. 2008;56:206–210.

124. Pine CM, Adair PM, Petersen PE, Douglass C, Burnside G, Nicoll AD, et al. Developing explanatory models of health inequalities in childhood dental caries. Community Dent Health. 2004;21(1 Suppl):86–95.

125. Marsh HW, Richards GE. The Rotter locus of control scale: The comparison of alternative response formats and implications for reliability, validity, and dimensionality. J Res Pers. Academic Press; 1986;20(4):509–28.

126. Lencová E, Pikhart H, Broukal Z, Tsakos G. Relationship between parental locus of control and caries experience in preschool children - cross-sectional survey. BMC Public Health. 2008;8:208-14.

127. Albino J, Tiwari T, Henderson WG, Thomas JF, Braun PA, Batliner TS. Parental psychosocial factors and childhood caries prevention: Data from an American Indian population. Community Dent Oral Epidemiol. 2018;46(4):360–8.

128. Chase I, Berkowitz RJ, Proskin HM, Weinstein P, Billings R. Clinical outcomes for Early Childhood Caries (ECC): the influence of health locus of control. Eur J Paediatr Dent. 2004;2:76–80.

129. Twetman S. Prevention of early childhood caries (ECC)--review of literature published 1998-2007. Eur Arch Paediatr Dent.2008; 9(1):12–8.

130. Freires IA, Rosalen PL. How Natural Product Research has Contributed to Oral Care Product Development? A Critical View. Pharm Res. 2016 ;33(6):1311–7.

131. Gyanendra Pandey Vol II Chavkhamba Publication ND. Drvyaguna Vigyan. chavkhamba publication, New Delhi; 456-468 p.

132. Miglani DC, Beal JF, James PM, Behari SA. The assessment of dental cleanliness status of the primary dentition using a modification of the simplified oral hygiene index(OHIS-M). J Indian Dent Assoc. 1973 ;45(12):385–8.

133. Instructions for use Diagnodent pen 2190. Kavo Dental Excellence.

134. Kumar N, Gupta N, Kishore J. Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012. Indian J Public Health.2012 ;56(1):103–4.

135. WHO Oral Health Surveys - Basic Methofd. World Heal Organ. 2013;1.137.

136. Trease, G.E, Evans W. pharmacognosy. 14th ed. WB Saunder Company Ltd , London UK; 1996. 612 p.

137. Cockerill FR, Wikler M a., Alder J, Dudley MN, Eliopoulos GM, Ferraro MJ, et al. Performance standards for antimicrobial disk susceptibility tests ; approved atandard. Clinical and Laboratory Standars Institute - NCCLS. 2012. 76 p.

138. Shah T, Joshi K, Mishra S, Otiv S, Kumbar V. Molecular and cellular effects of vitamin B12 forms on human trophoblast cells in presence of excessive folate. Biomed Pharmacother. 2016;84:526–34.

139. Denise Polit and Cheryl Tatano Beek.Nursing Resesarch – Principles and methods.7th edition.Lippincott publication. Pg 496-500.

140. Lussi A, Imwinkelried S, Pitts N, Longbottom C, Reich E. Performance and reproducibility of a laser fluorescence system for detection of occlusal caries in vitro. Caries Res. 1999;33(4):261–6.

141. O. T Jenkins. Human saliva: Clinical Chemistry and Microbiology. Library of congress cataloging in publication.

142. Miller EK, Vann WF. The use of fluoride varnish in children: a critical review with treatment recommendations. J Clin Pediatr Dent. 2008;32(4):259–64.

143. Al-Jumaily1* EFA, AL-Seubehawy1 HMZ, Al-Toraihy2 and FA. Isolation and Identification of Streptococcus mutans (H5) produced glucosyltransferase and cell-associated glucosyltransferase isolated from dental caries. Int J Curr Microbiol Appl Sci. 2014;3(6):850–64.

144. Davies GN. Early childhood caries - a synopsis. Community Dent Oral Epidemiol. 1998;26(S1):106–16.

145. Cheng L, Li J, He L, Zhou X. Natural products and caries prevention. In: Caries Research. 2015;49:38–45.

146. Yoo S, Murata RM, Duarte S. Antimicrobial traits of tea- and cranberry-derived polyphenols against streptococcus mutans. Caries Research. 2011;45 (4):327–35.

147. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, et al. Antimicrobial potential of Glycyrrhiza glabra roots. J Ethnopharmacol. 2008;116(2):377–80.

148. Utsunomiya T, Kobayashi M, Ito M, Pollard RB, Suzuki F. Glycyrrhizin improves the resistance of MAIDS mice to opportunistic infection of Candida albicans through the modulation of MAIDS-associated type 2 T cell responses. Clin Immunol. 2000 ;95(2):145–55.

149. Jain E, Pandey RK, Khanna R. Liquorice root extracts as potent cariostatic agents in pediatric practice. J Indian Soc Pedod Prev Dent.2013;31(3):146–52.

150. Sankeshwari R, Ankola A, Bhat K, Hullatti K. Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against Streptococcus mutans? J Sci Soc. 2018;45(2):67-71.

151. Sharad Visht GK. A comparison between different methods for extraction of glycyrrhetic acid from licorice stolons. Int J pharma Prof Res.2012;3(2):622–6.

152. Andrews JM, Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48 Suppl 1:5–16.

153. De Luca MP, Franca JR, Macedo FAFF, Grenho L, Cortes ME, Faraco AAG, et al. Propolis varnish: antimicrobial properties against cariogenic bacteria, cytotoxicity, and sustained-release profile. Biomed Res Int. 2014;2014:348647.

154. Masumo R, Bardsen A, Mashoto K, Åstrøm AN. Prevalence and socio-behavioral influence of early childhood caries, ECC, and feeding habits among 6-36 months old children in Uganda and Tanzania. BMC Oral Health. 2012 ;12:24-29.

155. Department of Health HongKong. Oral_Health_Survey_2011_WCAG_20141112_(EN_Full). 2011;

156. Jienmaneechotechai S, Ungchusak C DS et al. Thailand. Conference proceedings of International Dental Conference on “Caries Control throughout Life in Asia”. Krabi, Thailand; 2013;268–286.

157. Quiñonez RB, Keels MA, Vann WF, McIver FT, Heller K, Whitt JK. Early childhood caries: analysis of psychosocial and biological factors in a high-risk population. Caries Res. 2001;35(5):376–83.

158. Duijster D, Verrips GHW, Van Loveren C. The role of family functioning in childhood dental caries. Community Dent Oral Epidemiol. 2014;42(3):193–205.

159. Rice M, Bunker KD, Kang DH, Howell CC, Weaver M. Accessing and recruiting children for research in schools. West J Nurs Res.2007;29(4):501–14.

160. De Amorim RG, Leal SC, Bezerra ACB, De Amorim FPLG, De Toledo OA. Association of chlorhexidine and fluoride for plaque control and white spot lesion remineralization in primary dentition. Int J Paediatr Dent. 2008;18(6):446–51.

161. Mount GJ. Defining, classifying, and placing incipient caries lesions in perspective. Dental Clinics of North America. 2005;49(4): 701–23.

162. Gudipaneni RK, Vijay Kumarr R, Jesudass G, Peddengatagari S, Duddu Y. Term comparative evaluation of antimicrobial efficacy of short tooth paste containing lactoferrin, lysozyme, lactoperoxidase in children with severe early childhood caries: A clinical study. J Clin Diagnostic Res. 2014 ;8(4):ZC18-20.

163. Villavicencio J, Arango MC, Ordonez A, Contreras A, Villegas LM. Early childhood caries, salivary and microbiological aspects among 3- to 4-year-old children in Cali, Colombia. Eur Arch Paediatr Dent. 2018;19(5):347–52.

164. Patidar D, Sogi S, Singh V, Shinu P, Loomba A, Patidar DC. Salivary levels of Streptococcus mutans and Streptococcus sanguinis in early childhood caries: An in vivo study. J Indian Soc Pedod Prev Dent. 2018;36(4):386–90.

165. Patel PM, Hugar SM, Halikerimath S, Badakar CM, Gokhale NS, Thakkar PJ, et al. Comparison of the effect of fluoride varnish, chlorhexidine varnish and casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) varnish on salivary streptococcus mutans level: A six month clinical study. J Clin Diagnostic Res. 2017;11(8):ZC53-ZC59.

166. Ribeiro LGM, Hashizume LN, Maltz M. The effect of different formulations of chlorhexidine in reducing levels of mutans streptococci in the oral cavity: A systematic review of the literature. Journal of Dentistry. 2007;35(5): 359–70.

167. Paek AE, Li Y, Wang Z, So P, Janal MN, Herman NG, et al. Caries outcome following an intensive fluoride varnish treatment regimen for children at high risk for early childhood caries. Int J Paediatr Dent. 2018;28(3):291–9.

168. Baygin O, Tuzuner T, Kusgoz A, Senel AC, Tanriver M, Arslan I. Antibacterial effects of fluoride varnish compared with chlorhexidine plus fluoride in disabled children. Oral Health Prev Dent. 2014;12(4):373–82.

169. Rošin-Grget K. The cariostatic mechanisms of fluoride. Acta Med Acad. 2013 ;42(2):179–88.

170. Almaz ME, Sönmez IŞ, Ökte Z, Oba AA. Efficacy of a sugar-free herbal lollipop for reducing salivary Streptococcus mutans levels: a randomized controlled trial. Clin Oral Investig. 2017;21(3):839-845

171. Wang Y, Li J, Sun W, Li H, Cannon RD, Mei L. Effect of non-fluoride agents on the prevention of dental caries in primary dentition: A systematic review. PLoS One. 2017;12(8). e0182221

172. Paul S, Baranya Shrikrishna S, Suman E, Shenoy R, Rao A. Effect of fluoride varnish and chlorhexidine-thymol varnish on mutans streptococci levels in human dental plaque: A double-blinded randomized controlled trial. Int J Paediatr Dent. 2014;24(6):399–408.

173. Lipták L, Bársony N, Twetman S, Madléna M. The effect of a chlorhexidine-fluoride varnish on mutans streptococci counts and laser fluorescence readings in occlusal fissures of permanent teeth: A split-mouth study. Quintessence Int. 2016;47(9):767–73.

174. Tranaeus S, Shi XQ A-MB. Caries risk assessment: methods available to clinicians for caries detection. Community Dent Oral Epidemiol. 2005;33:265–73.

175. Ferreira JMS, Silva MFA, Oliveira AFB, Sampaio FC. Evaluation of different methods for monitoring incipient carious lesions in smooth surfaces under fluoride varnish therapy. Int J Paediatr Dent. 2008;18(4):300–5.

176. Honkala S, Elsalhy M, Shyama M, Al-Mutawa SA, Boodai H, Honkala E. Sealant versus fluoride in primary molars of kindergarten children regularly receiving fluoride varnish: One-year randomized clinical trial follow-up. Caries Res. 2015;49(4):458–66.

177. Pearce EIF, Larsen MJ, Coote GE. Fluoride in Enamel Lining Pits and Fissures of the Occlusal Groove-Fossa System in Human Molar Teeth. Caries Res. 1999;33(3):196–205.

178. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane database Syst Rev. 2002 ;(3):CD002279.

179. Wassel MO, Khattab MA. Antibacterial activity against Streptococcus mutans and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. J Adv Res. 2017 ;8(4):387–92.

180. Franca JR, De Luca MP, Ribeiro TG, Castilho RO, Moreira AN, Santos VR, et al. Propolis--based chitosan varnish: drug delivery, controlled release and antimicrobial activity against oral pathogen bacteria. BMC Complement Altern Med. 2014;14:478.

181. Douglass JM. Fluoride varnish when added to caregiver counseling reduces early childhood caries incidence. J Evid Based Dent Pract. 2011 Mar;11(1):46–8.

182. Van Strydonck DAC, Demoor P, Timmerman MF, Van Der Velden U, Van Der Weijden GA. The anti-plaque efficacy of a chlorhexidine mouthrinse used in combination with toothbrushing with dentifrice. J Clin Periodontol. 2004 ;31(8):691–5.

183. Axelsson P, Lindhe J. Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. J Clin Periodontol. 1987 Apr;14(4):205–12.

184. Sun H Bin, Zhang W, Zhou X Bin, Sun, HB; Zhang, W; Zhou X. Risk factors associated with early childhood caries. Chin J Dent Res. 2014;20(2):97–104.

185. Muñoz-Millán P, Zaror C, Espinoza-Espinoza G, Vergara-Gonzalez C, Muñoz S, Atala-Acevedo C, et al. Effectiveness of fluoride varnish in preventing early childhood caries in rural areas without access to fluoridated drinking water: A randomized control trial. Community Dent Oral Epidemiol. 2018 ;46(1):63–9.

186. Arruda AO, Senthamarai Kannan R, Inglehart MR, Rezende CT, Sohn W. Effect of 5% fluoride varnish application on caries among school children in rural Brazil: a randomized controlled trial. Community Dent Oral Epidemiol. 2012 ;40(3):267–76.

ANNEXURES

ANNEXURE I - CTRI REGISTRATION

Clinical Trial Details (PDF Generation Date :- Mon, 15 Oct 2018 04:02:54 GMT)

CTRI Number	CTRI/2015/10/006305 [Registered on: 26/10/2015] - **Trial Registered Prospectively**
Last Modified On	03/12/2014
Post Graduate Thesis	Yes
Type of Trial	Interventional
Type of Study	Ayurveda Preventive Dentistry
Study Design	Randomized, Parallel Group, Active Controlled Trial
Public Title of Study	A clinical trial to compare effect of licorice, fluoride and combination of both on early stage of tooth decay in 3-4 year old preschool children.
Scientific Title of Study	Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial

Secondary IDs if Any

Secondary ID	Identifier
NIL	NIL

Details of Principal Investigator or overall Trial Coordinator (multi-center study)

Details of Principal Investigator	
Name	Dr Roopali Sankeshwari
Designation	Reader
Affiliation	KLEVK Institute of dental sciences
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	Email	docrups@gmail.com

Source of Monetary or Material Support

Source of Monetary or Material Support
> Nil

Primary Sponsor

Primary Sponsor Details	
Name	Dr Roopali Sankeshwari
Address	Department of Public Health Dentistry KLEVK Institute of dental sciences Belgaum Nehru nagar 590010
Type of Sponsor	Other []

Details of Secondary Sponsor

Name	Address
NIL	NIL

Countries of Recruitment

List of Countries
India

Sites of Study

Name of Principal Investigator	Name of Site	Site Address	Phone/Fax/Email
Dr Roopali Sankeshwari	Randomly selected private and public preschools of Belgaum	Belgaum city Belgaum KARNATAKA	9844837197 docrups@gmail.com

Details of Ethics Committee

Name of Committee	Approval Status	Date of Approval	Is Independent Ethics Committee?
Ph.D Ethical committee (Human) KLE University Belgaum	Approved	29/04/2014	No

Regulatory Clearance Status from DCGI

Status	Date
Not Applicable	No Date Specified

Health Condition / Problems Studied

Health Type	Condition
Healthy Human Volunteers	initial dental caries

Intervention / Comparator Agent

Type	Name	Details
Comparator Agent	Fluoride varnish	0.5 ml varnish will be applied every 3 months at 0,3,6 and 9th month
Intervention	Licorice varnish, combination of fluoride and licorice varnish	0.5 ml varnish will be applied every 3 months at 0,3,6 and 9th month

Inclusion Criteria

Inclusion Criteria	
Age From	3.00 Year(s)
Age To	4.00 Year(s)
Gender	Both
Details	• Child with at least one initial caries lesion as per Nyvad classification. • Children with dental caries experience (dmft score) in the range of 3-6.

Exclusion Criteria

Exclusion Criteria

	Details:	• Children with any GIC restorations, pit and fissure sealants. • Developmental alterations like hypoplasia or fluorosis. • Use of orthodontic device or appliance. • Being under medical treatment or taking any kind of medication and • Parents not willing to give consent.
Method of Generating Random Sequence	Coin toss, Lottery, toss of dice, shuffling cards etc	
Method of Concealment	Sequentially numbered, sealed, opaque envelopes	
Blinding/Masking	Participant Blinded	
Primary Outcome	**Outcome**	**Timepoints**
	• Remineralization of initial caries – Minimum 4 points change in diagnodent score • Change in salivary Streptococcus mutans count before and after the intervention at the end of 1 year.	• at the end of 1 year
Secondary Outcome	**Outcome**	**Timepoints**
	NIL	NIL
Target Sample Size	**Total Sample Size=**196 **Sample Size from India=**196	
Phase of Trial	Phase 3	
Date of First Enrollment (India)	15/12/2014	
Date of First Enrollment (Global)	No Date Specified	
Estimated Duration of Trial	**Years=**2 **Months=**5 **Days=**30	
Recruitment Status of Trial (Global)	Not Applicable	
Recruitment Status of Trial (India)	Not Yet Recruiting	
Publication Details		
Brief Summary	This study is a randomized, single blind parallel group trial comparing the effectiveness of Licorice varnish, fluoride varnish and the combination of both on initial lesions of Early childhood caries in 3-4 year old preschool children of Belgaum city. The primary outcome measures will be Remineralization of initial dental carious lesions and change in Salivary Streptococcus mutans count after 12 months. Research hypothesis would be that the effect of licorice varnish on initial carious lesions would be non inferior to the effect of fluoride varnish. Varnishes would be applied on randomly selected preschool children at baseline,3rd , 6th,9th month respectively. Diagnodent instrument would be used to measure the extent of changes in the demineralized lesion after the intervention.	

ANNEXURE II -RECORDING OF OHIS INDEX

Oral Hygiene Index Simplified (OHIS) for primary dentition was given by Miglani et al.(1973).Examination method and scoring system were essentially the same as used by Greene and Vermillion in their Oral Hygiene Index.

1. Buccal surface of second upper primary molar
2. Lingual surface of second lower primary molar
3. Labial surface of upper right
4. Labial surface of lower left primary central incisor

- These teeth were assessed for debris and calculus and were scored in the range of 0-3.
- When any of these teeth were missing, a comparable adjacent molar or opposite central incisor tooth was substituted.
- No score was assigned unless two out of six possible surfaces or their substitutes had been examined.

Debris Index

Scores	Criteria
0	No debris or stain present
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface

Calculus index

Scores	Criteria
0	No calculus present
1	Supragingival calculus covering not more than third of the exposed tooth surface
2	Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
3	Supragingival calculus covering more than two third of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both

Calculation

- Debris index Simplified (DI- S) = $\frac{\text{Total scores}}{\text{Number of surfaces examined}}$
- Calculus index Simplified (CI-S) = $\frac{\text{Total scores}}{\text{Number of surfaces examined}}$
- OHIS = DI S + CI S

ANNEXURE III

1. Recording of Nyvad's index:-

- Cotton roll isolation would be used.
- Caries examination will be conducted in mobile dental unit.
- All the teeth would be recorded using plane mouth mirrors and standard explorers after drying the teeth with a blast of compressed air for 3-5 mins per surface
- Activity will be measured using 3 scores of Nyvad's scale

Score	Category	Criteria
0	Sound	Normal enamel translucency and texture
1	Active caries (intact surface)	Surface of enamel is whitish / yellowish opaque with loss of luster; generally covered with plaque. No clinically detectable loss of substance. smooth surface – caries lesion typically located close to gingival margin.
4	Inactive caries (intact surface)	Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the probe is moved gently across the surface. Smooth surface- caries lesion typically located at some distance from gingival margin.

ANNEXURE IV

Standard operating procedures for using Diagnodent Pen

- Laser fluroescence readings will be carried out using the point B specific for smooth surface.
- The maximum peak of the examined area will be recorded and a drawing of the lesion will be used for locating the site in future evaluations.
- The peak values of the readings will be noted as real values (L)and will be classified in agreement with Lussi scale which are

Surface examined	Normal	Minimal intervention	Restoration required
Occlusal	0-12	13-24	25 and above
interproximal	0-7	8-15	16 and above

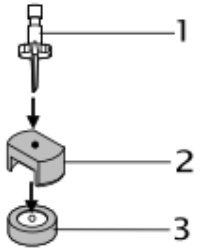
However some precautions are necessary to rule out false positive results like

1. The tips must be aligned correctly on the test surfaces
2. Thorough cleaning and drying without dehydration
3. Careful scanning of the entire surface with repeating beep pulses indicating good signal reception.

Calibration of the Diagnodent Pen

Calibration enables:

- The Diagnodent pen to be observed over a longer period.
- The comparison of diagnodent pen values from different diagnodent pens.
- The use of different probes with individual values.
- Calibration is required when the displayed value differs more than ±3 from the reference value when the reference is held down.



1. Only during calibration of approx probe 1, we should attach approx. attachment 2. to ceramic reference 3.
2. Press the menu button. The calibration icon appears.
3. Press save button. The calibration procedure starts.
4. For both probes, we can hear a tone then place the distal end of the tip vertically into the depression at the centre of the ceramic reference. Inconsistent readings will result if the probes are not at 90degree in all planes to the surface of the ceramic reference. As soon as the signal tone stops, calibration is over. Calibration is successful when the value in the display agrees with the reference value.(±3)

I. Standard Operating Procedures for Digital Weighing Scale

PURPOSE: To provide a procedure for operation of Digital balance in Dr. Prabhakar Kore Basic Science Research Centre.

SCOPE: Applicable to maintenance of all weighing balances of different capacities in Dr. Prabhakar Kore Basic Science Research Centre, Belgaum

RESPONSIBILITY: All the research technicians and Ph.D scholars working at Dr. Prabhakar Kore Basic Science Research Centre, Belgaum.

ACCOUNTABILITY: Manager QC.

1.0 HEALTH, SAFETY AND ENVIRONMENT: Electricity, Micro-organisms, Chemicals, Damaged containers.

i) Microbial contamination of body, clothing.

ii) Burns to skin and electrical shock.

iii) Chemical contamination of body, clothing.

2.0 Model: Digital Balance AUW 220D

3.0 PROCEDURE:

- Ensure that the instrument is clean and free from dust and placed in such a way that any vibrations do not affect it.
- Check the spirit level provided on the top of the balance
- Switch on the mains
- Press the power button provided on the instrument
- The instrument will do calibration **CAL2, CAL1, CAL0** and the digital screen will show 0.000 g reading.
- Press O/T button to change the display units from 0.0000 g to 0.00 mg

- Place the object to be weighed, on the platform of the balance.
- The accurate weight will be shown on the screen.
- The balance gives accuracy from 1mg to 220g with d=0.01g. (0-82gm) d=0.1mg (0-220 mg)
- Switch off the balance when not in use by pressing on/off button present on the instrument.

4.0 CALIBRATION

1. Switch ON the mains.

2. Switch ON the ON/OFF switch provided on the instrument.

3. Place 50g and 100g standard calibrated weights on the platform. Calibrate the machine.

4. If any variation of more than the standard limit is observed during calibration, weighing balance should be labeled as "out of order**".** Inform about the variance to the lab assistant. Do not use balance until all errors are rectified.

5. After rectification of error in the weighing balance, calibrate the weighing balance by repeating the same procedure and variation should fall in the standard limits.

6. All the balances should be serviced by authorized personnel on a quarterly basis. During each servicing, the balance should be cleaned and calibrated.

II. Standard Operating Procedures for Laminar Airflow Unit

PURPOSE: To lay down the procedure for Operation of Laminar Airflow Unit.

OBJECTIVE: To provide a procedure for operating Laminar Air Flow used to maintain the area for microbial analysis in Dr. Prabhakar Kore Basic Science Research Centre.

SCOPE: Applicable to maintenance of all LAF units of different capacities in Dr. Prabhakar Kore Basic Science Research Centre, Belgaum

RESPONSIBILITY: All the technicians, Research assistants, Research associates and Ph.D scholars working at Dr. Prabhakar Kore Basic Science Research Centre, Belgaum.

ACCOUNTABILITY: Head QA & QC

1.0 HEALTH, SAFETY AND ENVIRONMENT: Heat, Electricity, UV light, Micro-organisms, Chemicals, Damaged containers.

- Microbial contamination of body, clothing.
- Burns to skin and electric shocks.
- Chemical contamination of body, clothing.
- UV damage to eyes and skin.

2.0 Model: LAF 0913

3.0 Operation and cleaning of LAF

- Switch "OFF" the UV light.
- Clean the equipment with clean lint free cloth duster and spray 70% ethanol to LAF unit and entire area of LAF room.
- Switch "ON" the "AIR" and visible light switch situated on the control panel on right hand side of the instrument.
- Now check the LAF Manometer pressure, it should be within 10-20 mm of Water Gauge.
- Check the log record for burning hour of UV light, it should not exceed more than 2000 hours. UV tube should be replaced after specified burning period.
- Start the gas burner with the help of gas lighter and carry out the routine works.
- After completion of work, switch OFF the airflow and gas burner.
- Clean any remaining water or waste liquid material spilled on the laminar airflow platform properly with a dry cloth.

- Clean properly the working chamber from ceiling, followed by the side glass and then the platform of the chamber with sterile 70% ethanol.
- Spray sterile 70% ethanol and switch OFF the visible light and then switch ON the UV light till to start the next operation.
- If the LAF is used for aseptic filtration, clean the receiver pipe tank with hot WFI.
- Open the outlet of solution collection tank and collect the solution in plastic crate. Close the valve and transfer the crate for solution.
- 4.0 Cleaning of Pre filters
- Ensure that the LAF is switch off. Affix the tag on instrument "UNDER MAINTENANCE".
- Pre filter is situated on the backside of the LAF, unscrew and then remove the pre filter from LAF.
- Transfer the pre filter to washing area and blow the compressed air from reverse side to blow out all the dust particles.
- Wash the pre filter with DM water and then with liquid detergent solution. Finally wash again with DM water to remove the detergent solution.
- Remove the trapped water from the pre filter by jerking and finally rinse with 70% ethanol.
- Allow to dry the pre-filter at their original place and tighten the screw properly.
- 5.0 Precautions
- Take care to prevent any damage to the integrity of filter during cleaning. In case of observation of any damage to filters, immediately inform to your superior for further action.
- Instrument should be cleaned when the electrical connections of the equipment is in OFF position.

- Clean the laminar airflow chamber after every operation.
- Do not work when UV light is ON as it may cause eye damage.
- Maintain the level of the platform by adjusting the equipment from the base with the help of glass beads in such a manner that the platform level should remain horizontally flat. There should be ups and downs in any portion of the chamber.
- 6.0 Frequencies
- Daily for operation
- Fortnightly for pre filter cleaning
- Calibration: Yearly by external agency

III. Standard Operating Procedures for Labotech Bacteriological Incubator

PURPOSE: To provide a procedure of bacteriological incubator used to incubate bacterial cultures in Dr. Prabhakar Kore Basic Science Research Centre.

OBJECTIVE : To describe the procedure for calibration of bacteriological incubator.

SCOPE: Applicable to calibration of bacteriological incubators of different capacities in Dr. Prabhakar Kore Basic Science Research Centre, Belgaum

RESPONSIBILITY: All the technicians, Research assistants, Research associates and Ph.D scholars working at Dr. Prabhakar Kore Basic Science Research Centre, Belgaum.

ACCOUNTABILITY: Manager QC

1.0 HEALTH, SAFETY AND ENVIRONMENT: Heat, Electricity, Micro-organisms, Chemicals, Damaged containers.

- Microbial contamination of body, clothing.
- Burns to skin and electrical shock.
- Chemical contamination of body, clothing.
- UV damage to eyes and skin.

2.0 MODEL: M. No. BDI-54 Labotech bacteriological incubator

3.0 PROCEDURE:

A. General cleaning procedure

- Ensure that the power supply to the incubator is switched OFF.
- De-dust the incubator daily externally with a clean dry cloth.
- Once in a week remove adhered dust by wet mopping using soap solution. Afterwards wipe the surface with a clean dry cloth to remove the moisture.
- Mop the interior surfaces with a clean dry cloth, daily.

B. Operating procedure

- Ensure that the incubator is properly connected to the power supply.
- Switch „ON‟ the main switch and then the cabinet switch.
- Set the required temperature to 37°C by pressing the set knob and the soft keys.
- Monitor the temperature daily as per following procedure.
- Temperature shall be recorded which is displayed on LCD of controller of incubator.
- Observe the temperature shown on digital display. The temperature should not differ by +/- 2°C.
- Report any discrepancy observed during operation or temperature monitoring to Manager QC.
- Inform to Engineering Department for rectification and put the status label of „Under Maintenance‟.
- Maintain the record of incubator usage.
- Fill the temperature record regularly.

4.0 ABBREVIATIONS:

SOP – Standard Operating Procedure

IV. Standard Operating Procedures for Hot Air Oven:

PURPOSE : To provide a procedure for operation of Hot Air Oven in Dr. Prabhakar Kore Basic Science Research Centre..

SCOPE : Applicable to operation of weighing balances in Dr. Prabhakar Kore Basic Science Research Centre.

RESPONSIBILITY: All the technicians, research assistants,research associates and Ph.D scholars working at Dr. Prabhakar Kore Basic Science Research Centre.

1.0 HEALTH, SAFETY AND ENVIRONMENT : Heat, Electricity and Chemicals

i) Chemical contamination of body, clothing.

ii) Burns to skin and electrical shock.

2.0 MODEL: BDI-50 (Memmert Type)

3.0 PROCEDURE :

i) Ensure the cleanliness of the instrument.

ii) Open the ventilation knob provided on top of the oven.

iii) Switch “ON” the power supply.

iv) Electronic temperature controller displays the chamber temperature.

v) Set the required temperature by pushing the “PUSH” switch and first potentiometer knob clockwise or anticlockwise until the temperature comes to set one.

vi) Set the temperature with the help of second potentiometer knob.

vii) Release the “PUSH” switch.

viii) Indicator Bulb glows indicates that the power to the heater is “ON”.

ix) Switch “ON” the fan switch for air circulation.

x) Use rotary switch for precise control of temperature.

xi) Four positions of Rotary switch are available as follows:-

0 - Off position 1 - 5oC above ambient to 90oC,

2- 90oC to 150oC 3 - 150oC to 250oC

xii) Keep the switch on suitable markings as per requirements of temperature.

4.0 ABBREVIATIONS :

SOP : Standard Operating Procedure

No. : Number

V. Standard Operating Procedures for Vortex Mixer:

PURPOSE : To define the operation of the instrument for better and error free use in Dr. Prabhakar Kore Basic Science Research Centre

SCOPE : Applicable to mix the solution or to dissolve the various substance in to solvent in Dr. Prabhakar Kore Basic Science Research Centre.

RESPONSIBILITY: All the technicians, research assistants, research associates and Ph.D scholars working at Dr. Prabhakar Kore Basic Science Research Centre.

1.0 HEALTH, SAFETY AND ENVIRONMENT : Heat, Electricity, Micro-organisms, and Chemicals.

i) Microbial contamination of body, clothing.

ii) Burns to skin and electrical shock.

iii) Chemical contamination of body, clothing.

2.0 MODEL: RIVOTEK 50141022.

3.0 PROCEDURE :

i) Switch “ON” the power supply.

ii) Switch “ON” the instrument.

iii) Red light will glow.

iv) Put the test tube on the rubber pad.

v) Set the required speed with the help of the knob.

vi) On completion turn the speed knob anticlockwise.

vii) Switch “OFF” the instrument.

viii) Switch “OFF” the power supply.

4.0 ABBREVIATIONS: NIL

Standard operating procedures (SOP) for saliva collection

1. Subjects will be instructed not to eat or drink anything except water for at least 30 minutes before saliva collection.

2. Saliva collection will be done in the morning. Subject will be made to sit comfortably on a chair and saliva will be allowed to collect in the oral cavity.

3. He/she will be instructed not to swallow the saliva.

4. After two minutes, saliva will be collected into sterile disposable syringe and 0.5 ml will be injected into thioglycollate broth with hemin and vitamin K transport media.

5. Sample will be transported to laboratory immediately after collection and processed on the same day.

6. The sample will be vortexed (15 seconds, cyclomixer,) 1 loop will be inoculated on dry Mitis Salivarius agar medium and plates will be incubated at 37 ^{0} C in 5-10% CO2 jar for 48 hours.

7. After 48 hours colony characteristics will be studied and number of colony forming units of *Streptococcus mutans* in saliva will be determined.

Standard operating procedures (SOP) for varnish application

1. At baseline oral porphylaxsis will be done for all the subjects.
2. Varnish application will be performed quadrant wise sequentially starting from the lower arches and then continued to the upper arch.
3. Each subject will be instructed to rinse their mouth with plain water
4. Teeth will be cleaned with the help of cotton and tweezers.
5. Varnish will be applied to the teeth with the help of custom made pellets.
6. It will be allowed to dry for 1 minute.
7. Subjects will be instructed-
    - Not to drink for 2 hrs
    - Not to chew for 4 hrs
    - Avoid rough food for 24hrs.

ANNEXURE V STUDY PROFORMA

Association of locus of control with prevalence of early childhood caries among preschool children of Belgaum city.

Dr Roopali M Sankeshwari , Reader ,Dept of Public Health Dentistry.

Dr Anil V Ankola , Prof and Head ,Dept of Public Health Dentistry.

Dr Kishore Bhat, Consultant Microbiologist, Basic science research lab,

KLEVK Institute of Dental Sciences, Belgaum

Dear parents/caretakers,

This is a research project where we are trying to prevent dental decay in your young ones. The personal details obtained of you and your child through this proforma will be kept confidential. Children will not be harmed in any way through this project. Cost of the project is borne entirely by the researchers. Study results will be utilized only for the development of medical research. We request you to kindly give your consent .

We as parents are convinced that the procedure is not going to do any kind of injury or infection. I hereby give my consent to the investigator to carry out oral examination and being assured that the study is only for research purpose.

Signature of the parents/Guardian

Name of the child-	Class Sec
Date of birth- Age -	Gender – M/F
Father's name	Mother's name
Father's education	Mother's education
Father's occupation	Mother's occupation
Total Family income /month	Total number of family members
Contact no.	Address

Please read the following questions carefully and **tick ANY ONE** which according to you is appropriate.

Sl.no	Questions	1.strongly agree	2. Moderately Agree	3. Agree	4.Disagree	5.Moderately Disagree
1	As a family, we are confident that we can reduce the chances of our child getting tooth decay.					
2	As parents, it is our responsibility to prevent our child getting tooth decay.					
3R	It is the responsibility of the dentist to prevent our child getting tooth decay.					
4R	No matter what we do, our child is likely to get tooth decay.					
5	We can prevent tooth decay in our child by reducing sugary foods and drinks between meals.					
6R	It just happens that children get tooth decay.					
7	If we brush our child's teeth twice a day, we can prevent our child getting tooth decay in the future.					
8	If our child gets tooth decay, it is by chance.					
9R	It would not make any difference to our child getting tooth decay, if we helped him/her brush every day.					
10R.	Some people just naturally have soft teeth.					
11	As a family, we intend controlling how often our child has sugary foods or drinks between meals.					
12R.	It is just bad luck if our child gets tooth decay.					
13R	The dentist is the best person to prevent tooth decay in our child					

Oral hygiene practices of the child

Sl.no	Question	Response
1	How does your child clean his/her teeth?	i. Finger ii. Brush iii. Datum iv. Others
2	Child's teeth are cleaned by	i. Himself/herself ii. Parents
3	How often child's teeth are cleaned in a day?	i. Once ii. Twice iii. After every meal iv. Don't clean everyday
4	When are teeth cleaned?	i. Morning ii. Night iii. Both morning and night iv. Doesn't clean
5	What material is used to clean the teeth?	i. Toothpaste and brush ii. Toothpaste and finger iii. Tooth powder and brush iv. Tooth powder and finger
6	Name of the tooth paste or toothpowder	
7	How often is toothbrush changed?	i. 1-3 months ii. 3-6 months iii. 6 months

ANNEXURE VI

KUPPUSWAMY SOCIO ECONOMIC CLASSIFICATION 2012

(A)	**Education**	**Score**
1.	Profession or honors	7
2	Graduate or post graduate	6
3	Intermediate or post high school diploma	5
4	High school certificate	4
5	Middle school certificate	3
6	Primary school certificate	2
7	Illiterate	1
(B)	**Occupation**	**Score**
1.	Profession	10
2	Semi profession	6
3	Clerical,shop owner, farmer	5
4	Skilled worker	4
5	Semi skilled worker	3
6	Unskilled worker	2
7	Unemployed	1
(C)	**Family income per month in Rupees (2012)**	**Score**
1.	=30375	12
2	15188-30374	10
3	11362-15187	6
4	7594-11361	4
5	4556-7593	3
6	1521-4555	2
7	=1520	1
	Total score	**Socio economic class**
	26-29	Upper (I)
	16-25	Upper middle (II)
	11-15	Lower middle (III)
	5-10	Upper lower (IV)
	< 5	Lower (V)

ANNEXURE VII- DATA COLLECTION SHEET

Child's name	
Class **Section**	**Gender**

Modification of the Simplified Oral Hygiene Index

Debris score (DI-S)

55 51 65

85 71 75

DI-S=

Calculus score (CI –S)

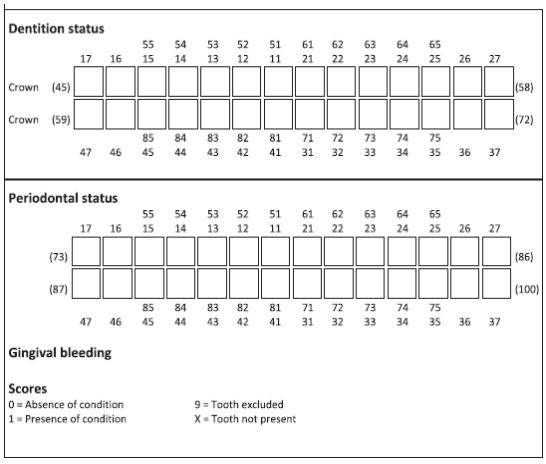
55 51 65

85 71 75

CI-S=

OHI-S = DI-S +CI-S =

WHO Dentition Status 2013



Primary teeth	Permanent teeth
	Status
A	0 = Sound
B	1 = Caries
C	2 = Filled w/caries
D	3 = Filled, no caries
E	4 = Missing due to caries
—	5 = Missing for any another reason
F	6 = Fissure sealant
G	7 = Fixed dental prosthesis/crown, abutment, veneer
—	8 = Unerupted
—	9 = Not recorded

Caries experience of the patient at Baseline Caries incidence At 1 year

IV. Recording of Initial lesions - Teeth selected for Diagnodent scoring

Teeth	**55**	**54**	**53**	**52**	**51**	**61**	**62**	**63**	**64**	**65**
Diagnodent scores at baseline(D1)										
Diagnodent scores after 1 year(D2)										
Teeth	**85**	**84**	**83**	**82**	**81**	**71**	**72**	**73**	**74**	**75**
Diagnodent scores at baseline (D1)										
Diagnodent scores after 1 year(D2)										

V.Proforma for recording Nyvad's scale

Teeth	**55**	**54**	**53**	**52**	**51**	**61**	**62**	**63**	**64**	**65**
Nyvad's score at baseline(N1)										
Nyvad's score after 1 year(N2)										
Teeth	**85**	**84**	**83**	**82**	**81**	**71**	**72**	**73**	**74**	**75**
Nyvad's score at baseline(N1)										
Nyvad's score after 1 year(N2)										

III.Salivary *Streptococcus Mutans* count. Sample no.

	Date	**Salivary streptococcus mutans count**
Baseline examination		
Examination at the end of the study		

Varnish applied

1.Fluoride Varnish
2.Licorice varnish
3. Combination varnish

ANNEXURE VIII

AUTHENTICATION CERTIFICATE

क्षेत्रीय आयुर्विज्ञान अनुसंधान केंद्र
REGIONAL MEDICAL RESEARCH CENTRE
भारतीय आयुर्विज्ञान अनुसंधान परिषद
Indian Council of Medical Research
नेहरु नगर, बेळगाम - ५९० ०१०.
Nehru Nagar, BELGAUM - 590 010.
Tel. : 0831-2475477-78
Fax : 0831-2475479
E-mail : oicrmrcblm@yahoo.co.in

Date: 22-01-2014

AUTHENTICATION

This is to authenticate that the plant material brought by Dr. Roopali Sankeshwari, Ph. D. Scholar, KLEU's VK Institute of Dental Sciences, Belgaum, is identified as ***Glycyrrhiza glabra*** **Linn. (Fabaceae).**

Harsha Hegde
Scientist 'C'

ANNEXURE IX

PHYTOCHEMICAL SCREENING

The ethanolic extracts obtained from both the techniques were subjected to preliminary phytochemical screening for qualitative detection of phyto constituents using standard procedures as described by Trease and Evans. [(Trease, G.E, Evans)]

Tests for steroids:

i) Salkowski Tests: Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour.

Tests for Flavonoids-

Alkaline reagent -Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of **flavonoids**.

Tests of Alkaloids: The extracts were mixed with ammonia and then extracted with chloroform solution. To this dilute hydrochloride acid was added. The acid layer was used for chemical tests for alkaloids.

i) Mayer's test (Potassium Mercuric Iodide): The acid layer with few drops of Mayer's reagent gives a creamy white precipitate.

iii) Hager's Test (Saturated solution of picric acid): The acid layer with Hager's reagent gives yellow precipitate.

ii) iv) Dragendroff's test (Solution of Potassium Bismuth Iodide): Acid layer with few drops of Dragendroff's reagent gives reddish brown precipitate.

Test for tannins:

Lead acetate test: To the **test** solution, a few drops of 10% **lead acetate** solution were added. Precipitate formation indicated the presence of **tannin**.

Ferric chloride test: To the **test** solution, a few drops of ferric chloride solution were added.

Tests for Saponins: Foam test: A small amount of extract is shaken with little quantity of water. The foam produced persists for 10 min. It confirms the presence of saponins.

Reducing sugars- Benedict's test- To test for the presence of reducing sugars, a sample was dissolved in boiling water followed by addition of a small amount of Benedict's reagent as the solution begins to cool. During the next four to 10 minutes, the solution should began to change colors from green, yellow, orange red and finally dark brown which confirms presence of reducing sugar.

Test for Anthroquinones – Borntrager's Test- Borntrager's test is employed for presences of anthraquinones. The drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides.

ANNEXURE X- ETHICAL CLEARANCE CERTIFICATE

KLE UNIVERSITY
(Formerly known as KLE Academy of Higher Education & Research, Belgaum)
[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956 vide Government of India Notification No.F.9-19/2000-U.3(A)]
'Accredited 'A' Grade by NAAC
Office of the Registrar, KLE University,
JNMC Campus, Nehru Nagar, Belgaum-590 010, Karnataka State, India
☎: 0831-2444444/2493779 FAX: 0831-2493777 Web: http://www.kleuniversity.edu.in E-mail: info@kleuniversity.edu.in

Ref.No.KLEU/Ethic/14-15/D-78 26th May 2014

To,
Dr.Roopali M Sankeshwari
Ph.D.Scholar,2013-14
K.L.E. University,
Belgaum.

Dear Research Scholar

The KLE University Ethics Committee on Human Subjects for Ph. D Research Project met on 29th April 2014 to consider your application for approval of the research project **"EFFECTIVENESS OF LICORICE VARNISH, FLUORIDE VARNISH AND COMBINATION OF BOTH, ON INCIPIENT LESIONS OF EARLY CHILDHOOD CARIES IN 3-4 YEAR OLD PRESCHOOL CHILDREN OF BELGAUM CITY – A RANDOMIZED CONTROLLED TRIAL "**

After review of the documents submitted by you and satisfactory explanations provided to the members, the committee has provided approval for this research project.

You are requested to report to Ethical Committee of the following:

1. Any deviation from or change of the protocol.
2. All serious adverse events.
3. Any changes in study documents.

DIRECTOR KLE ACADEMIC AFFAIRS

(Dr. Hema Dhumale)
Member Secretary,
Ph.D. Ethical Committee(Human),
K.L.E. University, Belgaum

(Dr. Sudha A. Raddi)
Chairman
Ph.D. Ethical Committee(Human),
K.L.E. University, Belgaum

CC to: - The Director Academic Affairs, KLE University
- The Director Research Foundation, KLE University
- The Registrar, KLE University
- Special Officer to Hon. Vice Chancellor, KLE University, Belgaum

ANNEXURE XI PERMISSION FROM ANGANWADI OFFICER

ಕರ್ನಾಟಕ ಸರ್ಕಾರ
(ಜಿಲ್ಲಾ ಪಂಚಾಯತ ಬೆಳಗಾವಿ)
ಶಿಶು ಅಭಿವೃದ್ಧಿ ಯೋಜನಾಧಿಕಾರಿಗಳ ಕಛೇರಿ,ಬೆಳಗಾವಿ ನಗರ
CDPO BELGAUM (URBAN) 590017
ದೂರವಾಣಿ ಸಂಖ್ಯೆ : 0831-2471579 ಫ್ಯಾಕ್ಸ/Fax:- 0831-2471579
Telephone:: e- mail:- cdpo_bgmu@ymail.com

ಸಂ:ಶಿಅಯೋ:ಬೆನ: ದಂತ:ಪರೀಕ್ಷೆ:2014-15: ದಿನಾಂಕ: 26/09/2014

ಪರವಾನಗಿ ಪತ್ರ

ವಿಷಯ:- 03 ರಿಂದ 04 ವರ್ಷದ ವಯೋಮಿತಿಯ ಮಕ್ಕಳ ದಂತ ಪರೀಕ್ಷೆ ಹಾಗೂ ಚಿಕಿತ್ಸೆ ಸಲುವಾಗಿ ಅನುಮತಿ ನಿಡುವ ಕುರಿತು.

ಉಲ್ಲೇಖ:- ಡಾ: ರೂಪಾಲಿ ಸಂಕೇಶ್ವರಿ, ಪಿ.ಎಚ್.ಡಿ ವ್ಯಾಸಾಂಗ ವಿದ್ಯಾರ್ಥಿ, ಕೆ.ಎಲ್.ಇ ಸಂಸ್ಥೆಯ ದಂತ ವಿಜ್ಞಾನ ಬೆಳಗಾವಿ ಇವರ ಪತ್ರ ದಿನಾಂಕ : 22-09-2014

ಬೆಳಗಾವಿ (ನಗರ) ಶಿಶು ಅಭಿವೃದ್ಧಿ ಯೋಜನಾ ವ್ಯಾಪ್ತಿಯಲ್ಲಿರುವ 3 ರಿಂದ 4 ವರ್ಷದ ಅಂಗನವಾಡಿ ಮಕ್ಕಳ ದಂತ ಪರೀಕ್ಷ ಮತ್ತು ಚಿಕಿತ್ಸೆ ಮಾಡುವ ಸಲುವಾಗಿ ಕೆ.ಎಲ್.ಇ ಸಂಸ್ಥೆ, ಬೆಳಗಾವಿಯ ದಂತ ವಿಜ್ಞಾನದ ವಿಭಾಗದ ಪಿಎಚ್ ಡಿ ವ್ಯಾಸಾಂಗದಲ್ಲಿರುವ ಡಾ: ರೂಪಾಲಿ ಸಂಕೇಶ್ವರಿ ಇವರಿಗೆ ಅನುಮತಿಸಲಾಗಿದೆ.

ಶಿಶು ಅಭಿವೃದ್ಧಿ ಯೋಜನಾಧಿಕಾರಿ
ಬೆಳಗಾವಿ (ನಗರ)

ಗೆ,

ಡಾ: ರೂಪಾಲಿ ಸಂಕೇಶ್ವರಿ,
ಪಿ.ಎಚ್.ಡಿ ವ್ಯಾಸಾಂಗ ವಿದ್ಯಾರ್ಥಿ, ಕೆ.ಎಲ್.ಇ
ಸಂಸ್ಥೆಯ ದಂತ ವಿಜ್ಞಾನ ಬೆಳಗಾವಿ.

ANNEXURE XII SAMPLE SIZE ESTIMATION

TABLE 20.7 Approximate Sample Sizes* Necessary To Achieve Selected Levels of Power for $\alpha = .05$ as a Function of Estimated Population Values of Eta-Squared

	POPULATION ETA-SQUARED									
POWER	.01	.03	.05	.07	.10	.15	.20	.25	.30	.35
GROUPS = 3										
.70	255	84	50	35	24	16	11	9	7	6
.80	319	105	62	44	30	19	14	11	9	7
.90	417	137	81	57	39	25	18	14	11	9
.95	511	168	99	69	47	30	22	16	13	11
GROUPS = 4										
.70	219	72	43	30	21	13	10	8	6	5
.80	272	90	53	37	26	17	12	9	7	6
.90	351	115	68	48	33	21	15	12	9	8
.95	426	140	83	58	40	25	18	14	11	9
GROUPS = 5										
.70	193	64	38	27	18	12	9	7	6	4
.80	238	78	46	33	23	15	10	8	7	5
.90	306	101	59	42	29	18	13	10	8	7
.95	369	121	72	50	34	22	16	12	10	8

*The values are the number of subjects *per group*.

role and their social support. The hypothesis is that women with greater social support are more accepting of the role transition to motherhood. Both variables are measured by scales that yield interval measures. How many women should be included in the study, given an α of .05 and power of .80?

In this example, the relationship between the two variables will be tested using Pearson's *r*. The estimated value of γ in this situation is ρ, that is, the expected population correlation coefficient.

Suppose we found an earlier study that correlated a measure of social support (the number of people subjects felt they could count on) with an observational measure of maternal warmth. The resulting correlation coefficient was .18, which we use as our estimate of ρ and hence of γ. Table 20-8 shows sample size requirements for various powers and effect sizes in situations in which Pearson's *r* is used. With an α of .05 and power of .80, the sample size needed in the study lies between 197 (effect size = .20) and 349 (effect size = .15). Extrapolating for an effect size of .18, we would need a sample of about 250 subjects. With a sample this size, we would wrongly reject the null hypothesis only 5 times out of 100 and wrongly retain the null hypothesis 20 times out of 100. To increase power to .95 (wrongly retaining the null hypothesis only 5 times out of 100), we would need a sample of about 400 women.

Reference

McFarland GK, Polit DF, Hungler BP. Nursing Research: Principles and Methods. The American Journal of Nursing. Lippincott publishers; 2006. 1515 p.

ANNEXURE XIII

PERMISSION LETTER FROM SCHOOLS

K.L.E. V. K. INSTITUTE OF D[illegible] SCIENCES, BELGAUM
(Constituent College of K.L.E. University, Belgaum)
DEPARTMENT OF PUBLIC [illegible] DENTISTRY

KLE

Dr Anil V. Ankola [illegible]
Professor and Head,
Department of Public Health Dentistry,
K.L.E.V.K. Institute of Dental Sciences,
Belgaum-590010.
Phone(+91-831-2473777
Mobile(+91-9343434455
Email: dr.ankola@yahoo.com

To,
~~Headmaster~~ Headmistress
G.G. Chitnis School
Belagavi

Sub- Regarding Dental Check Up and treatment of 3-4 year old preschool children.

Respected Sir/Madam,

This is to introduce Dr Roopali Sankeshwari working as Reader in the Department of Public Health Dentistry of KLEVK Institute of Dental Sciences Belgaum. She is conducting a study titled "Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial". She needs to examine the preschool children for any signs of initial dental decay followed by saliva sample collection and application of dental varnish. She needs parental consent for the same. I request you to grant her permission to examine preschool children of your school.

Thanking you once again.

Headmistress
G. G. Chitnis Eng. Med. High School
Tilakwadi - Belgaum.

Yours sincerely,
Dr Anil V Ankola

K.L.E.V.K. INSTITUTE OF DENTAL SCIENCES, BELGAUM
(Constituent College of K.L.E.University, Belgaum)
DEPARTMENT OF PUBLIC HEALTH DENTISTRY

KLE

Dr Anil V. Ankola MDS
Professor and Head
Department of Public Health Dentistry,
KLE VK Institute of Dental Sciences,
Belgaum 590010
Phone: +91-831-24*3***
Mobile: +91-9343434455
Email: dr.ankola@yahoo.com

To,
~~Headmaster~~ Headmistress
Love Dale Nursery School
Chanamma Nagar, Belagavi

Sub- Regarding Dental Check Up and treatment of 3-4 year old preschool children.

Respected Sir/Madam,

This is to introduce Dr Roopali Sankeshwari working as Reader in the Department of Public Health Dentistry of KLEVK Institute of Dental Sciences Belgaum. She is conducting a study titled "Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial". She needs to examine the preschool children for any signs of initial dental decay followed by saliva sample collection and application of dental varnish. She needs parental consent for the same. I request you to grant her permission to examine preschool children of your school.

Thanking you once again.

Yours sincerely,
Dr Anil V Ankola

LOVE DALE NURSERY
Opp. Hindustan Petroleum
Sambaji Road, Angol Extn
Tilakwadi, Belgaum-6

K.L.E. V. K. INSTITUTE OF DENTAL SCIENCES, BELGAUM
(Constituent College of K.L.E.University, Belgaum)
DEPARTMENT OF PUBLIC HEALTH DENTISTRY

KLE

Dr Anil V. Ankola MDS
Professor and Head,
Department of Public Health Dentistry,
K.L.E.V.K Institute of Dental Sciences,
Belgaum-590010,
Phone:+91-831-2473777
Mobile:+91-9343434455
Email: drankola@yahoo.com

To,
~~Headmaster~~ / Headmistress
Blooming Buds Nursery School
Belgaum.

Sub- Regarding Dental Check Up and treatment of 3-4 year old preschool children.

Respected Sir/Madam,

This is to introduce Dr Roopali Sankeshwari working as Reader in the Department of Public Health Dentistry of KLEVK Institute of Dental Sciences Belgaum. She is conducting a study titled "Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial". She needs to examine the preschool children for any signs of initial dental decay followed by saliva sample collection and application of dental varnish. She needs parental consent for the same. I request you to grant her permission to examine preschool children of your school.

Thanking you once again.

Yours sincerely,
Dr Anil V Ankola

Principal
Blooming Buds School
Belgaum.

List of the schools which participated in the study

1. Lovedale central school
2. Bright colors school
3. Shaikh central school
4. Lovedale preschool Channamma nagar
5. Tiny tots school
6. KLE Shishuvihar school
7. Lovedale school Sadashiv Nagar
8. Good shepherd central school
9. Chitnis school
10. Toonz Montessori
11. Samiti Public School
12. Sunflower Nursery, Angol
13. Bloomingbuds preschool
14. Balika Adarsha school

ANNEXURE XIV

SAMPLE COPY OF INVITATION LETTER SENT TO PARENTS

Dear Parents,

Oral health is an integral part of general health. Research has shown that children with Dental caries can have

- Significantly **lower height and weight**
- Iron deficiency **anemia**
- **Diminished ability** to learn
- Risk for **delayed physical growth** and development
- Higher **risk of new carious lesions** in both primary and permanent dentition

Thus it is important to PREVENT DENTAL CARIES IN TODDLERS. We are trying to reduce the chances of your young ones getting tooth decay. With this motto in our mind, we have organized an Interactive oral health talk on **"How best to prevent tooth decay in toddlers?"** We need your co operation for our endeavour.

Dr Roopali Sankeshwari

Reader , Dept of Public Health Dentistry

KLEVK Institute of dental sciences, Belgaum.

Time – 9th September 2015 at 10.30am

Venue- Blooming buds school

ANNEXURE XV – WHO Dentition Status 2013

The criteria for diagnosing a tooth status and the coding are as follows (codes applied to primary teeth are given in parentheses):

- **0 (A) Sound crown.** A crown was coded as sound if it showed no evidence of treated or untreated clinical caries. Thus, a crown with the following defects, in the absence of other positive criteria, were coded as sound

  • white or chalky spots; discolored or rough spots that were not soft to touch with a metal CPI probe;

  • stained enamel pits or fissures that did not have visible cavitation or softening of the floor or walls detectable with a CPI probe;

  • dark, shiny, hard, pitted areas of enamel in a tooth which showed signs of moderate to severe enamel fluorosis;

  • Lesions that, on the basis of their distribution or history, or on examination, appeared to be due to abrasion.

- **1 (B) Carious crown**. Caries was recorded as present when a lesion in a pit or fissure, or on a smooth tooth surface, had an unmistakable cavity, undermined enamel, or a detectably softened floor or wall. A tooth with a temporary filling, or one which was sealed but also had decay was included in this category. In cases where the crown was destroyed by caries and only the root was left, the caries was judged to have originated in the crown and was therefore scored as crown caries only. The CPI probe was used to confirm visual evidence of caries on the tooth surface(s). Where any doubt exists, caries was not recorded as present.

- **2 (C) Filled crown, with caries.** A crown was considered filled, with decay, when it had one or more permanent restorations and one or more areas that were decayed. No distinction was made between primary and secondary caries and the same code applied regardless of whether the carious lesions were in contact with the restoration.
- **3 (D) Filled crown, with no caries**. A crown was considered filled, without caries, when one or more permanent restorations were present and there was no caries anywhere on the crown. A tooth that had been crowned because of previous decay was recorded in this category. A tooth that has been crowned for reasons other than caries by means of a fixed dental prosthesis abutment was coded 7 (G).

- **4 (E) Missing tooth, due to caries**. This code was used for permanent or primary teeth that had been extracted because of caries and were recorded under coronal status. For missing primary teeth, this score was used only if the subject was at an age when normal exfoliation would not be a sufficient explanation for absence.
- **6 (F) Fissure sealant**. This code was used for teeth in which a fissure sealant had been placed on the occlusal surface, in pits or for teeth in which the occlusal fissure had been enlarged with a rounded or "flame-shaped" bur, and a composite material placed. If a tooth with a sealant had caries, it was coded as 1 or B.
- **7 (G) Fixed dental prosthesis abutment, special crown or veneer**. This code was used under coronal status to indicate that a tooth formsed part of a fixed bridge abutment. This code was also be used for crowns placed for reasons other than caries and for veneers or laminates covering the labial surface of a tooth, on which there was no evidence of caries or a restora- tion .

ANNEXURE XVI

INFORMED CONSENT FORM

Title of the study: Scientific Title

Effectiveness of Licorice varnish, Fluoride varnish and Combination of Both, on Incipient Lesions of Early Childhood Caries in 3-4 year old preschool children of Belgaum city- A randomized controlled trial.

Public title -A clinical trial to compare effect of licorice, fluoride and combination of both on early stage of tooth decay in 3-4 year old preschool children.

Objective / Purpose of the study:

You are being invited to participate in this study which is conducted to know if Licorice (Jesthmadhu) can prevent tooth decay in 3-4 year old preschool children. Principal investigators for this study are– Dr Roopali Sankeshwari, Dr Anil V Ankola.This project is not funded by any organization.

Procedures:

This project is a research project. We would like to collect some information about you and your child. This information will be kept confidential and will be coded without your name or any other identifying information about you. Oral examination of the preschool children will be done and those who suffer from initial dental decay will be invited to participate in this study. Initial tooth decay is a condition which is likely to progress if we do not prevent it. Preventive therapy of this study consists of following steps

- Initial stage of tooth decay is a stage which cannot be appreciated with normal examination. Hence a Thermometer like Digital instrument (Diagnodent) will be placed on child's tooth and it will show how much decay has occurred.
- After this child will be asked to spit saliva in a container.

- Following these steps teeth will be cleaned with cotton and a gum like medication (fluoride varnish/licorice varnish) will be painted on the teeth. This procedure will take 10-15 mins.
- Child will be instructed not to eat anything hard for 8 hours and not to brush for 24 hours after which he / she can resume routine brushing.
- Painting the teeth will be repeated 3 times at 3^{rd},6^{th} and 9^{th} month.
- At the end of one year, again saliva will be collected and digital instrument will be used to check if decay has stopped or progressed.

Risks and benefits:

Your child may get or may not get any benefit from the study. We will be examining the child at 3^{rd}, 6^{th}, 9^{th} month and at the end of 1year.If we find that decay is progressing, then child will be referred to KLEVK institute of dental sciences Belgaum for further treatment. Parents will be requested to take the child to the dental college and get the treatment done at concessional rates.

Alternatives:

There are other options available to treat initial caries which include drilling the teeth and filling it with restorative material.

Withdrawal:

Participation in this study is voluntary. If you don't wish to participate in this study, you will not lose any benefits to which you are entitled. You are free to withdraw your consent and discontinue participation in this study at any time.

Privacy and confidentiality:

Your child's photograph may be taken during the study. However your child's identity will not be revealed. All information collected will be coded so that your child's identity will not be known to others.

Financial incentives for publication:

The cost of the study will be borne by the researcher. There will be no payment to participants or their parents for participating in this study.

Authorization to publish the results:

The results of the study will be used for teaching and medical publications. However, the participant's identification will be kept confidential.

Questions:

If you have any questions about this study, please call Dr.Roopali Sankeshwari , Principal investigator at 9844837197 or Dr Anil Ankola, Head of the department of public health dentistry 0831 2444114 or Dr. Sudha Raddi, Chairman, Human Ethical Committee, KLE University, Belgaum , Phone.no. 0831-2444444.

Consent Statement: I am making a voluntary decision whether or not my child will participate in this study. My signature below indicates that I have given consent for my child to participate, and I have read (or been read) the information provided above and I was given the opportunity to ask questions and that they have been answered to my satisfaction and that I have received a copy of this signed consent form.

----------Participant's parents Name----------Participant's signature/Thumb print

----------Experimenter's name ----------Experimenter's Signature

----------Witness Name----------Witness signature

---------- Participant Name

ಸಮ್ಮತಿ ಪತ್ರ

೧. **ಶಿರೊನಾಮ**- ಇಫ಼ೆಕ್ಟೀವನೆಸ್ ಆಫ಼್ ಲಿಕೊರೈಸ ವಾರ್ನಿಶ್, ಫ಼್ಲೊರೈಡ ವಾರ್ನಿಶ್ ಆನ್ಡ್ ಕೊಂಬಿನೆಶನ್ ಆಫ಼್ ಬೊಥ್ ಓನ್ ಇನಸಿಪಿಯಂಟ ಲಿಜನ್ಸ್ ಆಫ಼್ ಅರ್ಲಿ ಚೈಲ್ಡುಡ ಕೆರಿಸ್ ಇನ್ ೩-೪ ಇಯ್ರ್ ಒಲ್ಡ್ ಪ್ರಿಸ್ಕೂಲ್ ಚಿಲ್ಡ್ರ್ನ ಆಫ಼್ ಬೆಲಗಮ ಸಿಟಿ- ಎ gÁåAಡಮೈಝ್ಡ್ ಕಂಟ್ರೊಲ್ಡ್ ಟ್ರಯಲ್.

೨. **ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ :** ನಿಮ್ಮನ್ನು ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಮಂತ್ರಿಸುತ್ತೇವೆ. ಈ ಸಂಶೋಧನೆಯ ಉದ್ದೇಶವೆನೆಂದರೆ, ಲಿಕೊರೈಸ (ಜೆಶ್ಟ್ ಮಧು)ವನ್ನು ೩-೪ ವಯಸ್ಸಿನ ಮಕ್ಕಳಲ್ಲಿ ಹಲ್ಲು ಹುಳಿಯುವುದನ್ನು ತಡೇಗಟ್ಟ ಬಹುದೆ? ಎಂಬುದನ್ನು ಕಂಡುಹಿಡಿಯುವುದಾಗಿದೆ.

.ಈ ಸಂಶೋಧನೆಯ ಸ್ಂಶೊಧಕರು ಡಾ|| ರೂಪಾಲಿ ಸಂಕೇಶ್ವರಿ ಮುಖ್ಯ ಸಂಶೋಧಕರು ಹಾಗು ಡಾ|| ಅನಿಲ್ ಅಂಕೋಲ ಪಿ.ಹಚ.ಡಿ ಗೈಡ.ಈ ಸಂಶೋಧನೆಗೆ ಯಾವುದೆ ಸಂಘಟಣೆಯ ಹಣಕಾಸಿನ ಸಹಾಯ ಮಾಡುತ್ತಿಲ್ಲ.

೩.**ಪ್ರಕ್ರಿಯೆಯ ವಿವರಣೆಗಳು**: ಪ್ರಿಸ್ಕುಲ ಮಕ್ಕಳ ಬಾಯಿ ತಪಾಸನೆ ಮಾಡಲಾಗುವುದು.ಮಕ್ಕಳಿಗೆ ಪ್ರಾರಂಬಿಕ ಹಂತದಲ್ಲಿ ಹಲ್ಲು ಹುಳಿಯುವುದು ಕಂಡು ಬಂದರೆ ಅಂಥಹ ಮಕ್ಕಳನ್ನು ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಮಂತ್ರಿಸಲಾಗುವುದು. ಪ್ರಾರಂಬಿಕ ಹಂತದಲ್ಲಿರುವ ಹಲ್ಲು ಹುಳಿಯುವುದುನ್ನು ತಡೆಗಟ್ಟದ್ದಿರೆ, ಅದು ಮುಂದುವರಿಯುವುದು.ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಹಲ್ಲುಹುಳುಕನ್ನು ತಡೆಗಟ್ಟುವ ವಿಧಾನವು ಈ ಕೆಳಕಂಡತೆ ವಿವರಿಸಲಾಗಿದೆ.

- ಪ್ರಾರಂಬಿಕ ಹಂತದಲ್ಲಿರುವ ಹಲ್ಲು ಹುಳುಕುನ್ನು ಸಾಧಾರಣ ಪರೀಕ್ಷೆಯಿಂದ ಗೊತ್ತಾಗುವುದಿಲ್ಲ.ಅದನ್ನು ಗೊತ್ತುಪಡಿಸುವುದಕ್ಕೆ ಡೈಗ್ನೊಡೇಂಟ ಎಂಬ ಉಪಕರಣವನ್ನು ಬಳಸಲಾಗುವುದು.ಇದನ್ನು ಹಲ್ಲಿನ ಮೇಲೆ ಇಟ್ಟಾಗ, ಹಲ್ಲು ಎಶ್ಟರಮಟ್ಟಿಗೆ ಹುಳುಕಾಗಿದೆ ಎಂದು ತಿಳಿಯಬಹುದು.
- ಇದಾದ ನಂತರ ಮಗುವಿನ ಜೊಲ್ಲನ್ನುತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು.
- ಇದರ ಬಳಿಕ, ಮಗುವಿನ ಹಲ್ಲುಗಳನ್ನು ಹತ್ತಿಯಿಂದ ಶುಚ್ಚಿಗೊಳಿಸಲಾಗುವುದು. ಲಿಕೊರೈಸ ವಾರ್ನಿಶ್, ಫ಼್ಲೊರೈಡ ವಾರ್ನಿಶ್ ಅಥವಾ ಕೊಂಬಿನೆಶನ್ ಯಾವುದಾದರೊಂದನ್ನು ಹಲ್ಲಿನ ಮೇಲೆ ಲೇಪಿಸಲಾಗುವುದು.ಈ ಪ್ರಕ್ರಿಯೆ ೧೦-೧೫ ನಿಮೀಷದವರೆಗೆ ಆಗಬಹುದು.
- ವಾರ್ನಿಶ ಹಚ್ಚಿದ ಬಳಿಕ ೮ ಘಂಟೆಗಳ ಕಾಲ ಮಕ್ಕಳು ಗಟ್ಟಿ ಆಹಾರ ತೆಗೆದುಕೊಳ್ಳಬಾರದು ಹಾಗೂ ೨೪ ಘಂಟೆಗಳ ಕಾಲ ಹಲ್ಲನ್ನು ಉಜ್ಜಬಾರದು.
- ವಾರ್ನಿಶನ್ನು ೩,೬ ಮತ್ತು ೯ನೇ ತಿಂಗಳಿಗೆ ಪುನಃ ಹಚ್ಚಲಾಗುವುದು.
- ಒಂದು ವರ್ಷದ ನಂತರ ಡೈಗ್ನೊಡೇಂಟ ಸ್ಕೊರನ್ನು ಮತ್ತೆ ನಮುದಿಸಲಾಗುವುದು.ಜೊಲ್ಲನ್ನು ಪುನಃ ತಪಾಸನೆಗೆ ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು.

೪. ಹಿಂತೆಗೆದುಕೊಳ್ಳುವುದು: ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸುವುದು ಸ್ವಇಚ್ಛೆ. ಈ ಸಂಶೋಧನೆಯಿಂದ ಯಾವಾಗ ಬೇಕಾದರು ಯಾವುದೇ ಕಾರಣ ನೀಡದೆ ಹಿಂತೆಗೆದುಕೊಳ್ಳಬಹುದು.

೫. ಲಾಭ ಮತ್ತು ಅಪಾಯಗಳು: ನಿಮ್ಮ ಮಗುವಿಗೆ ಈ ಸಂಶೋಧನೆಯದಿಂದ ಲಾಭ ದೊರೆಯಬಹುದು ಅಥವಾ ದೊರೆಯದೆಯಿರಬಹುದು.ಹಲ್ಲು ಹುಳುಕು ತಡೆಗಟ್ಟದೆ ಮುಂದುವರಿದಲ್ಲಿ ಪೋಷಕರಿಗೆ ಮುಂದಿನ ಚಿಕಿತ್ಸೆಗಾಗಿ ಕೆ. ಎಲ್. ಇ. ವಿ. ಕೆ. ದಂತ ಮಹಾವಿದ್ಯಾಲಯ ಬೆಳಗಾವಿಗೆ ಕಳುಹಿಸಲಾಗುವುದು.

೬ . ಗೌಪ್ಯ ಮತ್ತು ರಹಸ್ಯತೆ: ನಿಮ್ಮ ಹಾಗೂ ನಿಮ್ಮ ಮಗುವಿನ ಗುರುತನ್ನು ಗುಪ್ತವಾಗಿಡಲಾಗುತ್ತದೆ.ಸಂಗ್ರಹಿಸಿರುವ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು (ಕೋಡ್) ಕೊಡಲಾಗುತ್ತದೆ.ಇದರಿಂದ ನಿಮ್ಮ ಹಾಗೂ ನಿಮ್ಮ ಮಗುವಿನ ಗುರುತು ಬೇರೆಯವರಿಗೆ ಗೊತ್ತಾಗುವುದಿಲ್ಲ.

೭. ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಹಣಕಾಸಿನ ಸಹಯ: ಈ ಸಂಶೋಧನೆಯಗೆ ಬೇಕಾಗುವ ವ್ಯಚ್ಚವನ್ನು ಸಂಶೋಧಕರೇ ಕೊಡುತ್ತಾರೆ.ಭಾಗವಹಿಸುವವರಿಗೆ ಚಿಕಿತ್ಸೆ ಬಿಟ್ಟು ಬೇರೆ ಯಾವುದೇ ತರಹದ ಹಣಕಾಸಿನ ಸಹಾಯ ಮಾಡುವುದಿಲ್ಲ.

೮ ಸಂಶೋಧನೆಯ ಫಲಿತಾಂಶವನ್ನು ಪ್ರಕಟಿಸಲು ಅನುಮತಿ: ಈ ಸಂಶೋಧನೆಯ ಫಲಿತಾಂಶವನ್ನು ಕೇವಲ ವೈದ್ಯಕಿಯ ಸಂಶೋಧನೆಯದ ಅಧ್ಯಯನ ಸಲುವಾಗಿ ಉಪಯೊಗಿಲಾಗುವುದು. ಆದರೆ ಭಾಗವಹಿಸುವವರ ಗುರುತನ್ನು ಗುಪ್ತವಾಗಿಡಲಾಗುತ್ತದೆ.

೯ ಪ್ರಶ್ನೆಗಳು: ನಿಮ್ಮಗೆ ಯಾವುದೇ ತರಹದ ಸಂದೇಶವಿದ್ದಿಲ್ಲಿ ಈ ಕೆಳಗೆ ಕೊಟ್ಟಿರುವ ಜನರನ್ನು ಸಂಪರ್ಕಿಸ ಬಹುದು.

ಡಾ|| ರೂಪಾಲಿ ಸಂಕೇಶ್ವರಿ

ಮುಖ್ಯ ಸಂಶೋಧಕರು

ದೂರವಾಣಿ ಸಂಖ್ಯೆ –9844837197

ಡಾ|| ಅನಿಲ ವಿ ಅಂಕೊಲ

ಪಿ.ಹಚ.ಡಿ ಗೈಡ

ಕೆ. ಎಲ್. ಇ. ವಿ. ಕೆ. ದಂತ ಮಹಾವಿದ್ಯಾಲಯ

ಬೆಳಗಾವಿ

ದೂರವಾಣಿ ಸಂಖ್ಯೆ – 0831 2444114

ಡಾ|| ಸುಧಾ ರೆಡ್ಡಿ

ಪ್ರಾಂಶುಪಾಲರು ಹಾಗೂ ಚೇರಮನ್

ಮಾನವ ನೀತಿ ಶಾಸ್ತ್ರ ಸಮಿತಿ

ಕೆ. ಎಲ್. ಇ. ವಿಶ್ವವಿದ್ಯಾಲಯ

ಬೆಳಗಾವಿ

ದೂರವಾಣಿ ಸಂಖ್ಯೆ – 0831-2444444

೧೦. ಸಮ್ಮತಿ ವಾಖ್ಯೆ: ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸಂಪುರ್ಣವಾಗಿ ಹಾಗೂ ತೃಪ್ತಿಕರವಾಗಿ ಉತ್ತರಿಸಲಾಗಿದೆ.ನನಗೆ ಸಂಪುರ್ಣ ಮಾಹಿತಿ ಲಭ್ಯವಾಗಿದೆ.ನಾನು ಮೇಲೆ ಹೇಳಿರುವ ಎಲ್ಲಾ ನಿಭಂದನೆಗಳನ್ನು ಹಾಗೂ ಶರತ್ತುಗಳನ್ನು ಸಂಪುರ್ನವಾಗಿ ನನಗೆ ತೆಳಿದಿರುವ ಭಾಷೆಯಲ್ಲಿ ಓದಿ ಅರ್ಥಮಾಡಿಕೊಂಡು ನನ್ನ ಸಮ್ಮತಿಯನ್ನು ಕೊಡುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು-- ಭಾಗವಹಿಸುವವರ ಸಹಿ

ಸಂಶೋಧಕರ ಹೆಸರು ಸಂಶೋಧಕರ ಸಹಿ

ಸಾಕ್ಷಿದಾರರ ಹೆಸರು ಸಾಕ್ಷಿದಾರರ ಸಹಿ

ANNEXURE XVII CERTIFICATES FOR BEST PAPER PRESENTATIONS

Title - Assessing antibacterial activity and physical properties of Licorice varnish versus Fluoride and the Combination Varnish

Title - Comparison Of Remineralisation Potential Of Three Varnishes On Initial Lesions In Preschool Children- A Randomized Controlled Trial

Indian Association of Public Health Dentistry

Certificate of Excellence

CONFERENCE THEME WINNER

"Affordable Accessible Oral Health Care"

Awarded to

DR. ROOPALI MANOHAR SANKESHWARI

LM-129

Date : **30th November, 2018**

Venue : **Divya Jyoti College of Dental Sciences & Research, Modinagar**

Dr. R.K. Bali
President Emeritus

Dr. Aruna Devi M
Hon. President

Dr. Sabyasachi Saha
Hon. Gen. Secretary

ANNEXURE XVIII PUBLICATION

Original Article
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Evaluation of Physical Parameters of Novel Licorice Varnish Versus Fluoride and Combination Varnish: An In-Vitro Study

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Key words: Dental Varnish ▪ Early Childhood Caries ▪ Licorice ▪ Prevention of Tooth Decay ▪ *Streptococcus Mutans*.

Objectives. The aim of this study was to evaluate the physical properties of locally prepared Licorice varnish (LV), commercially available Fluoride varnish (FV) and a Combination of both Varnishes (CV). **Material and Methods.** LV was prepared using authenticated licorice roots. Commercially available FV (Bifluorid 12) was used as a positive control and CV was prepared in six different concentrations of both varnishes. Conventional antibacterial activity assessment, employing disc diffusion and broth dilution methods, was inconclusive. Therefore a novel assessment method was used, whereby the varnish was directly added to a mixture of Brain Heart Infusion broth with *Streptococcus mutans* and incubated. Physical parameters such as pH, rate of evaporation, viscosity, film forming ability, and cost incurred for preparation were assessed and compared. **Results.** FV, LV and CV (except the combination of LV 80% + FV 20%) showed antibacterial activity against *Streptococcus mutans*. All three varnishes formed films on the tooth surface as confirmed by Scanning Electron Microscopy. Mean pH was in the range of 4-4.5, viscosity 48-52 centipoise (cP), rate of evaporation was 150-160 seconds. They were comparable to each other in the physical parameters tested, except for the shelf life of LV. **Conclusion.** All three varnishes showed antibacterial activity against *Streptococcus mutans* which was established using an innovative method of antibacterial activity assessment. LV was most economical of all but had a shorter shelf life. The results of this study need to be evaluated through an in vivo study.

Introduction

Dental caries is the most common chronic infectious disease in childhood (1). Early Childhood Caries (ECC) is a devastating form of dental disease affecting the youngest members of society. Children's quality of life can be seriously affected by severe caries because of pain and discomfort which could lead to disfigurement, acute and chronic infections, and altered eating and sleeping habits, as well as the risk of hospitalisation, high treatment costs, and the loss of school days, with the consequent diminished ability to learn (2). The knowledge about dental caries has increased, but dentists worldwide are struggling to prevent ECC. Community-based preventive programs have to be developed and implemented urgently to achieve the World Health Organization (WHO) goals, and to improve oral health, health in general and the quality of life in particular (3).

The foremost of all caries preventive tools is Fluoride which can favourably al-

ter demineralisation and remineralisation processes, thus preventing caries (4). Long-lasting pharmaceutical formulations in the form of varnishes have been developed for the prevention of dental caries (5).Varnishes are easy to apply, have a high concentration of fluoride, and can be applied in a moist environment, and thus can be considered as the best preventive tool against ECC (6). The remineralising capacity and antibacterial activity of Fluoride against *Streptococcus mutans* have been reported and time tested, however, the cost and manpower required for fluoride varnish application necessitate the search for other alternative preventive tools. The gap in the knowledge base in this regard remains a topic for exploration, as in the present study.

Recently, there has been an increasing interest in herbal dentistry to overcome drawbacks of modern medicine such as the development of resistance to antibiotics, and side effects such as vomiting, diarrhoea, alteration of taste sensations, etc. (7). There has been growing interest in biologically active compounds, derived from natural products, which may have potential therapeutic uses in dentistry (8). Ayurveda has a wide range of medicinal plants which have been used to alleviate human suffering and promote general health and well being. Among the various medicinal plants used, Licorice – *Glycyrrhizaglabra* is one such plant. Licorice, known as the "Grandfather of Herbs," has been used by various cultures and is time tested. It is 50 times sweeter than sucrose, has been successfully used to relieve sore throat and gastric problems, and enhance memory in children. It is easily available, inexpensive, approved by the US FDA as GRAS (generally regarded as safe as per 21 CFR section, 21CFR 184-1408) and LD_{50} of *Glycyrrhizin* is 1.94 g /kg subcutaneously (9) indicative of a good safety profile.

Isolated use of fluoride has proved to be insufficient to prevent progressive mineral loss and consequent lesion formation in children at high risk for caries development (10). Hence the combination of fluoride with licorice varnish was undertaken with the idea that the combination may control plaque formation with reduced acidogenicity, and may also help in remineralization of initial lesions.

Thus the objective of the study was to compare the physical properties of all three varnishes. We also compared the cost incurred for preparation of the varnish.

Materials and Methods

The present study was carried out from Jan 2016 to April 2016 at the Dr. Prabhakar Kore Basic Science Research Centre, Belagavi. Ethical clearance was obtained from the Institutional Ethics Committee. Authenticated Licorice roots were used to prepare Licorice extract using the cold maceration method. The extract was filtered using a muslin cloth and Whatman No.1 filter paper. The filtrate was concentrated using an IKA Rotary evaporator at 40°C, and the resultant residue was kept in a refrigerator until further use. The extract obtained was assessed for its antimicrobial activity against *Streptococcus mutans* ATCC 25175 (procured from PGI Chandigarh) using the broth dilution method.

Licorice Varnish (LV)

The Faculty of Pharmacy guided the preparation of Licorice Varnish. All ingredients of *IP* (*Indian Pharmacopeia)* grade, were used for preparing the varnish (Table 1). A manual of operations was prepared, and the Good Laboratory Practices (GLP) guidelines were followed for the varnish preparation. LV was prepared by the addition of ethyl acetate to licorice extract in a sterile glass container. The extract was dissolved by keeping a glass container in a bath sonicator for 30 minutes. When the extract was completely dissolved,

Table 1. Ingredients Used for Preparing Licorice Varnish Along with Their Functions

Ingredient	Function	Manufacturer
Iso amyl propionate purchased from Sigma	Plasticizer	Sigma Aldrich, SAPC
Ethyl acetate	Solvent	Sigma Aldrich, SAPC
Collodion solution, gift sample from Sigma	Lacquer	Omatek Laboratories,Indore.
Fumed silica-Gift sample, Aerosil 200	Thickening agent	Pharma from Evonik Industries,Germany
Licorice extract	Antimicrobial activity	Indigenously prepared

Collodion solution, along with Iso Amyl Propionate was added. It was centrifuged again and fumed silica was added to this mixture. All the contents were centrifuged for 30 seconds and the mixture was transferred to an amber colored sterile bottle and labeled.

Fluoride Varnish (FV)

Commercially available Bifluorid12 varnish (VOCO Company, Germany. Lot no.1523310) was used as a positive control.

Combination Varnish (CV)

This was initially prepared by mixing 80% LV with 20% FV but when it failed, other combinations were tested. Combination varnish was prepared by mixing various concentrations of licorice varnish and fluoride varnish as described below: 50% LV + 50% FV; 60% LV + 40% FV; 75% LV + 25% FV; 60% FV + 40% LV; 75% FV + 25% LV. All the three varnishes were assessed for their antimicrobial activity and physical parameters.

Antimicrobial activity – The antimicrobial susceptibility test was performed according to the Agar diffusion method. *Streptococcus mutans ATCC 25175* was cultivated in Brain Heart Infusion (BHI) broth, and it was transferred after 18 hours to BHI agar containing 5% sucrose.

Disc diffusion method – The direct colony suspension method for the preparation of inoculums was followed. The inocula of *Streptococcus mutans* was prepared by collecting 3-4 colonies grown on agar after 24 hours and the number of microorganisms was calculated based on the standard turbidity of 0.5 McFarland, corresponding to $1.0x10^8$ colony forming units/ml. All the three varnishes, FV, LV and CV, were diluted to concentrations ranging from 100%, 50%, 25%, 12.5% and 6.25% with the help of distilled water. Twenty micro liters of the respective varnishes were transferred onto sterile filter papers (6.4 mm diameter) and placed on the agar plate. The plates were then incubated at 37°C for 48 hours anaerobically. The varnishes, being alcoholic mixtures, evaporated and did not diffuse through the agar plates (Figure 1).

Broth dilution method – The broth dilution method was attempted next. As a

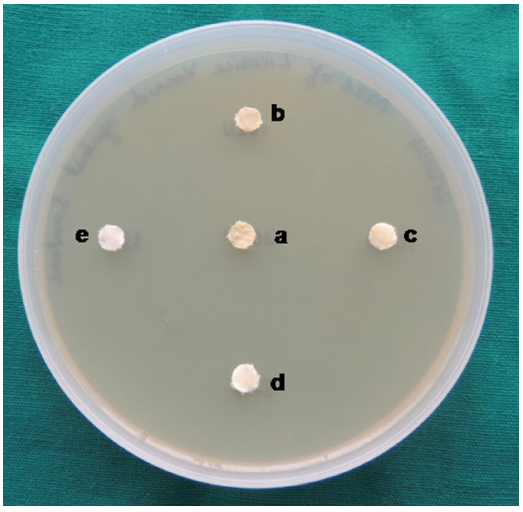

Figure 1. Disk diffusion method showing failure of varnishes (various dilutions: a=100%, b=50%, c=25%, d=12.5% and e=6.25%) to diffuse through agar medium.

procedural step, varnish was added to BHI broth, but as soon as the varnish was mixed with BHI broth a precipitate formed immediately annulling the chance of any further investigation. Since varnish is made up of resins, as soon as it was mixed with broth, a precipitate occurred (Figure 2). Since the result was inconclusive, this method was also discarded. The routine tests employed for testing the MIC of varnish failed and, hence there was a need to develop a novel method to assess the MIC of varnish.

Novel method – In this method, 0.5 ml of varnish was added directly to a mixture of 0.5 ml of BHI broth and *Streptococcus mutans*. One loop of this mixture was then spread over agar gel and incubated for 48 hours. A similar procedure was repeated with LV and CV and the results obtained are shown in Figure 3. As can be seen clearly, CV in a ratio of 80% LV and 20% FV, failed to show antimicrobial activity. All three varnishes were tested for physical properties by the principal investigator who was trained at KLE Dr. Prabhakar Kore Basic Science Research Centre.

Color matching – The freshly prepared LV was compared with the shade guide and the shade number was noted, along with the date of preparation. This helped us to assess the shelf life of the varnish. When performing the color matching, the investigator was wearing a vision aid, clear spectacles which are normally worn every day. The test area had the artificial natural daylight fluorescent illumination. The specimen was held at a distance of 25cms and was observed at perpendicularly. Color matching was done between the shade guide and the specimen. The intra observer agreement was calculated as the mean value of the highest percentage of identical scores for 6 specimen of the same shade, performed twice at an interval of one week: 1) Rate of evaporation – A sterile glass slide was taken and its weight was noted down. One hundred micro liters of varnish was then dispensed on it and evenly distributed, and kept on a digital weighing scale. A stop-watch was used to assess the time taken for the slide to return to its original weight. Viscosity was assessed using a CAP 2000 + Viscometer, Brookfield. Two ml of the varnish was placed on the viscometer plate and the test was run according to the manufacturer's instructions and values noted. 2) Film forming ability – Human Tooth samples of 3 mm thickness were obtained using hard tissue microtome. Fifty micro liters of the respective varnishes were applied us-

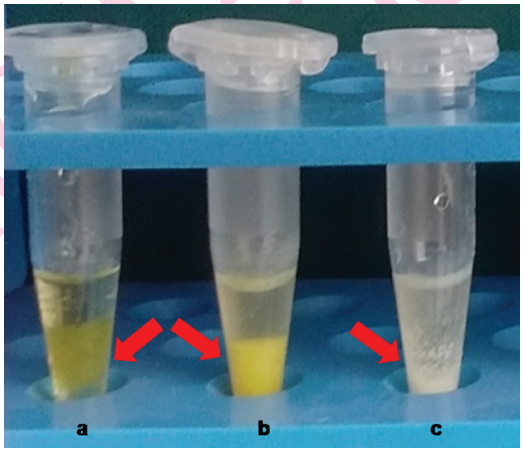

Figure 2. The broth dilution method showing the formation of precipitate upon addition of varnishes to Brain Heart Infusion broth: a=Licorice varnish; b=Fluoride varnish; c=Combination varnish.

Figure 3. New method for assessing the MIC of all three varnishes. Combination varnish (80% Licorice varnish and 20% Fluoride varnish) failed to show antibacterial activity.

ing the applicator tip. After the samples were completely dried, they were observed under a Scanning Electron Microscope (SEM). The morphology of the formed films on the tooth surface was studied using a JOEL Scanning Electron Microscope, Model JSM- 6360LV, operating in 15 kV acceleration voltage. 3) The safety of the LV was assessed by comparing the lethal doses of all the ingredients used with the actual concentration used for preparing the varnish (11-13).

Statistical Analysis

The data was analyzed using SPSS software version 20 (SPSS Inc Chicago, USA). The level of significance was set at 0.05. Intra examiner reproducibility was assessed using Cohen's Kappa for color matching. Triplicate values were used to determine the mean value for rate of evaporation and viscosity of the varnishes.

Results

Intra examiner Cohen's Kappa (k) for color matching was 0.86, confirming good reproducibility. The licorice extract, along with LV and FV, showed antimicrobial activity (Table 2). CV showed antimicrobial activity in all the tested permutations except 80% LV and 20% FV (Figure 4). All the combination of varnishes, that is (60% FV+40% LV), (40%FV+60%LV), (25%FV+75%LV), (75%FV+25%LV) and (50%FV+50%LV), were equally effective with regard to antibacterial activity. The combination varnish in the concentration of (50%FV+50%LV) was easier to prepare in terms of time and cost. Hence the physical parameters were assessed using this proportion and the results obtained are shown in Table 3. All the varnishes were acidic in nature.

Table 2. Results of Minimal Inhibitory Concentrations of Licorice Extract and the Three Varnishes Against *Streptococcus Mutans*

Test group	Antibacterial activity against *Streptococcus mutans*
Licorice extract	Positive - 2.0 mg/ml
Licorice varnish	Positive
Fluoride varnish	Positive
Combination varnish	
80% Licorice Varnish + 20% Fluoride Varnish	Negative
50% Licorice Varnish + 50% Fluoride Varnish	Positive
60% Licorice Varnish + 40% Fluoride Varnish	Positive
75% Licorice Varnish + 25% Fluoride Varnish	Positive
60% Fluoride Varnish + 40% Licorice Varnish	Positive

Table 3. Comparison of the Physical Parameters of All Three Varnishes

Parameters	Fluoride varnish	Licorice varnish	Combination varnish
Rate of evaporation (second)	150	156	160
pH	4	4.5	4.5
Viscosity (Pa*s)	48	52	49
Shelf life	Stable for 2 years	Shelf life 35 days	Shelf life 2 months
Cost	Rs 4500 per bottle*	Rs 700 per bottle; 6-7 times cheaper†	Approximately Rs 2500 per bottle

Pa*s=Pascal seconds; *Biflurid 12 Voco product; †Indigenously prepared; Rs=Indian Rupees.

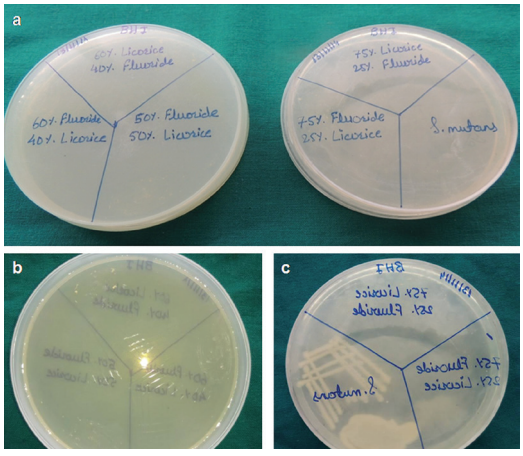

Figure 4: a) The combination varnish showed antimicrobial activity in all the tested permutations; b) No growth of *Streptococcus mutans* was seen in any of the combination varnishes; c) *Streptococcus mutans* growth was seen in the control group.

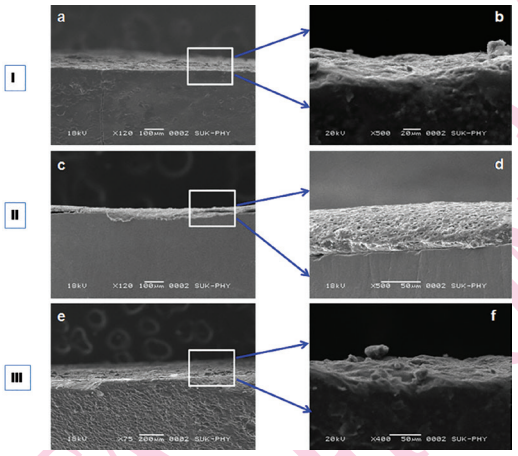

Figure 5. Morphological characterization of films formed on the tooth surface after application of varnishes. SEM images of I=Licorice varnish; II=Fluoride varnish; III=Combination varnish (a, c, e side view; b, d, f expansion view).

The safety profile of various components used in preparing LV is presented in Table 4.

Scanning Electron Microscopy (SEM) results - SEM images are presented in Figure 5. The images reveal the uniform film formation of all the three varnishes. The films were in intimate contact with the tooth. However, the compactness of the varnishes differed from one another.

Discussion

This study describes the development of a novel LV, as well as a new method to assess the MIC of varnish. The dental profession is currently faced with an enormous task of how to manage the huge burden of the consequences of the caries process amongst the world population (14). Providing care for preschool children can be stressful and troublesome (5). Hence, the focus is currently on minimally invasive approaches which can arrest caries progression. Fluoride is the most essential chemical agent used for dental caries prevention, and various topical fluoride interventions have been supporting this, with over six decades of experimental research (15). However, an increased resistance to caries from fluoride has been reported (16).

The present study compared locally prepared LV with FV and their combination. The antimicrobial activity was assessed using a novel method for LV. An extensive literature review showed that there were no

Table 4. Comparison Between the Toxic Values of Varnish Ingredients and the Actual Concentrations Used per Milliliter of the Varnish

Ingredient	Toxicity profile	Concentration*
Licorice extract	Animal studies – it does not cause genotoxicity, cytotoxicity or cellular toxicity. 1.94 gms/kg (29)	8 mg
Iso amyl propionate	Approximately 5 ml/kg (12)	0.1 ml
Aerosil	>3160 mg/kg body weight (Evonik MSDS datasheet)	10 mg
Ethyl acetate	11.3 g/kg body weight (12)	0.2 ml
Collodion solution	10 mg/kg (12)	0.2 ml

*Concentration used per milliliter of licorice varnish.

reports on licorice varnish and our study seems to be the first one. In the present study, licorice extract was found to have an effective antibacterial activity against *Streptococcus mutans,* and this result is in concordance with other studies (17, 18). Licorice contains alkaloids, flavonoids, saponins like *glycyrrhizic acid, glycyrrhizin* and stilbenes – *gancaonin G* which have antimicrobial and anti-adherent properties against *Streptococcus mutans* and thus can help in caries control (19). The minimum inhibitory concentrations (MIC) of licorice varnish provide evidence that, even when the licorice extract was mixed with the other constituents of varnish, the extract was able to sustain its antimicrobial activity. The licorice varnish can be considered as a pragmatic option to prevent dental caries.

Though *in vitro* studies have made it clear that licorice has good antimicrobial activity against *Streptococcus mutans* and other organisms, it has not been used in a therapeutic form which is practical and feasible in a field trial involving preschool children. MIC is considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and is therefore used to judge the performance of all other methods of susceptibility testing (20). Varnishes, being alcoholic mixtures, evaporated and failed to diffuse through the agar plates. Hence, the conventional tests employed for testing MIC of varnish did not give any results, and a new technique which could overcome this problem was needed.

The inability of the varnish to diffuse through agar medium was overcome by the novel method, and the varnish acted directly on *Streptococcus mutans,* thus demonstrating the antimicrobial potential of the varnish. Antimicrobial testing of propolis varnish has been reported (21) where the authors diluted propolis varnish in an ethanol-water solution at 20% in a proportion of 1:1 (75 mg/ml) to reduce the viscosity of the varnish. This technique though it provided satisfactory results, actually camouflages the inherent antimicrobial activity of the varnish. Thus this particular technique was not followed in the present study.

When the cost factor was compared, LV proved to be more economical compared to FV. It is about 6-7 times cheaper than FV (Bifluorid 12). A comparison of the three varnishes revealed that licorice and CV were comparable to FV in most of the parameters assessed. However, when shelf life was assessed, LV had a shorter shelf life. When the rate of evaporation was assessed, it was found to be slightly longer for LV, although the difference was not statistically significant. This could be attributed to the lower viscosity of LV.

Combination varnishes, such as fluoride with chlorhexidine, fluoride with cervitec, and chlorhexidine with xylitol, have been used in dentistry, as the combinations have been shown to increase the suppression period of *Streptococcus mutans* (22-24). Both Cervitec F and fluoride varnish performed similarly when their antimicrobial activity against *Streptococcus mutans* was compared (24). Contrary to this, MI varnish, a newer combination varnish with CPP-ACP and fluoride, was compared against plain fluoride varnish and chlorhexidine varnish, but chlorhexidine showed significantly better results than a combination of CPP-ACP and fluoride varnish for antibacterial activity against *Streptococcus mutans* (25). On the other hand, Gedalia (26) reported that when *Glycyrrhizin (Licorice)* was added to the APF solution, fluoride uptake increased and enamel solubility was reduced. To test this hypothesis, a CV was tested in the present study. In the present study, CV prepared by mixing 80% LV with 20% FV failed to show antimicrobial activity. This may be due to the high concentration of licorice (80%) varnish in the CV which probably antagonized the effect of fluoride varnish present

in a lower concentration (20%). Further, the interaction between the active constituents of licorice varnish with the sodium and calcium fluoride present in fluoride varnish may have resulted in the annulling of each other's antimicrobial activity. However, the exact reason why the combination varnish with 80% LV with 20% FV failed to show any antimicrobial activity remains unclear. An interaction study between licorice and fluoride varnish is probably needed to find the answer, however it was beyond the scope of the present research. FV has multiple effects such as remineralization of initial enamel lesions and inhibition of *Streptococcus mutans*. We can predict that when licorice is mixed with FV it probably enhances the antibacterial activity of FV. The inhibition of plaque biofilm formation is the key to successful control and prevention of dental caries (27). This may indirectly enhance the remineralizing potential of CV and at a cost much lower than FV. Will CV improve the efficiency of the Gold standard "FV "in preventing ECC? This question needs to be answered in future studies.

As shown by SEM analysis, varnish formulations formed a uniform layer on the tooth structure. FV (Bifluorid 12) contains 5.6% F, and both sodium fluoride and calcium fluoride, which could penetrate the tooth surface more effectively (28). Hence, this was used as a positive control in the present study. Both sodium and calcium ions are positively charged ions, and have a high affinity to the fluoride ion. This affinity makes CaF and NaF crystals more stable, thus enabling the compact nature of the varnish. However, it has been reported that since Bifluorid 12 has higher viscosity, it formed a thicker layer on the acrylic surface to which *Streptococcus mutans* adhered easily (29). On the other hand, LV had lower viscosity, and we can speculate that it would penetrate the enamel tags to a greater depth. Whether these properties make any significant impact on caries progression or biofilm formation needs to be assessed through *in-vivo* studies.

Safety Issues

Licorice has been used by various cultures for thousands of years and many previous studies have shown that it is a safe medicinal herb (30). Ames test using *S Typhimurium* TA 1535 revealed no genotoxicity; a cytotoxicity study with Promega's CellTiter-Glo Assay, using cell lines of Jurkat HOK68 CHO and BHK, revealed no cellular toxicity. A single dose acute toxicity test with mice confirmed *Glycyrrhiza glabra* to be non-toxic (30). All the ingredients used in the present study were of *IP* (Indian Pharmacopeia) Grade, and GLP (Good Laboratory Practices) guidelines were followed during the preparation of LV, thus ensuring the safety of LV and CV.

Dental caries is a multifactorial disease and many organisms cause this disease. However, in the present study, a single organism was used, that is, *Streptococcus mutans*. It would be interesting to find out if LV has broad spectrum antibacterial activity in future studies, by assessing its antibacterial activity against other oral pathogens. Nevertheless, such an investigation was beyond the scope of the present study. The shelf life of LV was found to be shorter than FV, and further studies with respect to optimization of methods for preparation of the varnish are required to improve the product.

Conclusion

LV, FV and CV (except 80% LV + 20% FV) showed antimicrobial activity against the standard strain of *Streptococcus mutans.* The viscosity, rate of evaporation, pH, and film forming ability of all three varnishes were comparable to each other. LV was the most economical of the three but had a shorter

shelf life. Future studies with *in vivo* study design are required to confirm these findings.

What is already known on this topic

ECC is pandemic in prevalence and is amenable to prevention. The Cochrane database recommends the use of fluoride varnish for the prevention of dental caries in young children. However, varnish is an expensive preventive tool, especially for developing countries where the prevalence of the disease is high, and resources to tackle it are limited and hence there is a need to search for an alternative indigenous product. Licorice has been used for various ailments for centuries and has been used in dentistry as a mouthwash and lollipop for its anti caries activity against Streptococcus mutans.

What this study adds

Although the anti-cariogenic properties of licorice have been suggested for over 30 years, it has not been tested in a dosage form that can be used in public health programs. Hence this study presents the details of licorice varnish its preparation and its comparison with fluoride varnish. A combination varnish may provide more benefits by suppressing the acidogenic bacteria in addition to accelerating the remineralization process of white spot lesions. The present research work also describes a novel way of assessing the MIC of a viscous substance such is "dental varnish".

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Authors' contributions: Conception and design: RS and AA; Acquisition, analysis and interpretation of data: RS, KB and UB; Drafting the article: RS and MR; Revising it critically for important intellectual content: KB, UB and MR; Approved final version of the manuscript: RS, AA, KB, UB and MR.

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References

1. Blanaid Daly, Paul Batchelor ET and RW. Essential Dental Public Health. 1st ed. London: Oxford University Press; 2002. p. 8.
2. Petersen PE, Estupinan-Day S, Ndiaye C. WHO's action for continuous improvement in oral health. Bull World Health Organ. 2005;83(9):642.
3. Sacic L, Markovic N, Arslanagic Muratbegovic A, Zukanovic A, Kobaslija S. The prevalence and severity of early childhood caries in preschool children in the Federation of Bosnia and Herzegovina. Acta Med Acad. 2016;45(1):19-25.
4. Rošin-Grget K. The cariostatic mechanisms of fluoride. Acta Med Acad. 2013;42(2):179-88.
5. Duangthip D, Jiang M, Chu CH, Lo EC. Non-surgical treatment of dentin caries in preschool children - systematic review. BMC Oral Health. 2015;15(1):44.
6. Marinho VCC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane database Syst Rev. 2013;7:CD002279.
7. Freires IA, Rosalen PL. How Natural Product Research has Contributed to Oral Care Product Development? A Critical View. Pharm Res. 2016;33(6):1311-7.
8. Groppo FC, De Cássia Bergamaschi C, Cogo K, Franz-Montan M, Motta RHL, De Andrade ED. Use of phytotherapy in dentistry. Phytotherapy Research. 2008;22(8):993-8.
9. Pandey G. Dravyaguna Vijnana: Materia Medica-Vegetable Drugs. Vol 2. Varanasi: Chaukhambha Krishnadas Academy; 2004.
10. Ullsfoss BN, Øgaard B, Arends J, Ruben J, Rölla G, Afseth J. Effect of a combined chlorhexidine and NaF mouthrinse: an in vivo human caries model study. Eur J Oral Sci. 1994;102(2):109-12.
11. Evonik Resource efficiency Gmbh| Product information AEROSIL* 200 Pharma [updated Aug 2012] [cited 2016 Jan 4]. Available from https://products-re.evonik.com/www2/uploads/.../AEROSIL-200-Pharma-EN.pdf.
12. Leonard BJ. Toxicological Aspects of Food Safety: Proceedings of the European Society of Toxicology Meeting held in Copenhagen, June 19-22, 1977. Berlin: Springer-Verlag; 1978.
13. World Health Organization. Evaluation of certain food additives (Seventyninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990. Geneva: World Health Organization; 2014.
14. Frencken JE, Peters MC, Manton DJ, Leal SC, Gordan VV, Eden E. Minimal intervention dentistry for managing dental caries - A review: Report of a FDI task group. Int Dent J. 2012;62(5):223-43.
15. Lopes RM, Domingues GG, Junqueira SR, Araujo ME, Frias AC. Conditional factors for untreated caries in 12-year-old children in the city of São Paulo. Public Health. 2013;27(4):376-81.
16. Sheiham A, Sabbah W. Using universal patterns of caries for planning and evaluating dental care. Caries Res. 2010;44(2):141-50.

17. Jain E, Pandey RK, Khanna R. Liquorice root extracts as potent cariostatic agents in pediatric practice. J Indian Soc Pedod Prev Dent. 2013;31(3):146-52.
18. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, et al. Antimicrobial potential of Glycyrrhiza glabra roots. J Ethnopharmacol. 2008;116(2):377-80.
19. Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis. 2012;18(1):32-9.
20. Andrews JM, Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(Suppl 1):S5-16.
21. De Luca MP, Franca JR, Macedo FAFF, Grenho L, Cortes ME, Faraco AAG, et al. Propolis varnish: antimicrobial properties against cariogenic bacteria, cytotoxicity, and sustained-release profile. Biomed Res Int. 2014;2014:348647.
22. Baygin O, Tuzuner T, Kusgoz A, Senel AC, Tanriver M, Arslan I. Antibacterial effects of fluoride varnish compared with chlorhexidine plus fluoride in disabled children. Oral Health Prev Dent. 2014;12(4):373-82.
23. Paula VAC, Modesto A, Santos KRN, Gleiser R. Antimicrobial effects of the combination of chlorhexidine and xylitol. Br Dent J. 2010;209(12):E19.
24. Lipták L, Bársony N, Twetman S, Madléna M. The effect of a chlorhexidine-fluoride varnish on mutans streptococci counts and laser fluorescence readings in occlusal fissures of permanent teeth: A split-mouth study. Quintessence Int. 2016;47(9):767-73.
25. Patel PM, Hugar SM, Halikerimath S, Badakar CM, Gokhale NS, Thakkar PJ, et al. Comparison of the effect of fluoride varnish, chlorhexidine varnish and casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) varnish on salivary streptococcus mutans level: A six month clinical study. J Clin Diagnostic Res. 2017;11(8):ZC53-9.
26. Gedalia I, Stabholtz A, Lavie A, Shapira L, Pisanti S, Segal R. The effect of glycyrrhizin on in vitro fluoride uptake by tooth enamel and subsequent demineralization. Clin Prev Dent. 1986;8(2):5-9.
27. Franca JR, De Luca MP, Ribeiro TG, Castilho RO, Moreira AN, Santos VR, et al. Propolis--based chitosan varnish: drug delivery, controlled release and antimicrobial activity against oral pathogen bacteria. BMC Complement Altern Med. 2014;14:478.
28. Bergström EK, Birkhed D, Granlund C, Sköld UM. Approximal caries increment in adolescents in a low caries prevalence area in Sweden after a 3.5-year school-based fluoride varnish programme with Bifluorid 12 and Duraphat. Community Dent Oral Epidemiol. 2014;42(5):404-11.
29. Pinar Erdem A, Sepet E, Kulekci G, Trosola SC, Guven Y. Effects of two fluoride varnishes and one fluoride/chlorhexidine varnish on Streptococcus mutans and Streptococcus sobrinus biofilm formation in vitro. Int J Med Sci. 2012;9(2):129-36.
30. Hu CH, He J, Eckert R, Wu XY, Li LN, Tian Y, et al. Development and evaluation of a safe and effective sugar-free herbal lollipop that kills cavity-causing bacteria. Int J Oral Sci. 2011;3(1):13-20.

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A Cross Sectional Study to Assess Relationship between Parental Locus of Control and Caries Experience in Preschool Children of Belgaum City

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ABSTRACT

Background- Preschool children are prone for dental caries and since they rely on parents for oral hygiene, it becomes imperative that parental perspective towards susceptibility to caries be assessed.

Objective - The present study aimed to evaluate existence of any association between parental Locus of Control (LOC) with their children's Early Childhood Caries (ECC) experience.

Method- Study comprised of 407 children and their parents. Data was collected using a self designed and validated questionnaire which included details of Socio demographic data, oral hygiene practice of the children and LOC (LOC) questionnaire. LOC consisted of thirteen questions adapted from Wallston's Multidimensional Health LOC. Responses for LOC were scored on 5 point likert scale. After obtaining consent, parents completed the questionnaire which was followed by dental examination of the child. Dmft was calculated form WHO Dentition Status. Multiple logistic regression and sensitivity analysis of the data was performed.

Result – As LOC scores increased, an increase in caries prevalence was observed. Area under Receiver Operating Characteristic curve with LOC as a criterion was 0.55 (95%CI 0.48-0.61), sensitivity and specificity were 0.82 and 0.28 respectively. Around 20.39% of parents had internal LOC and their children were 1.8 times less likely to suffer from dental caries as compared to others. When multiple logistic regression analysis was performed by adjusting age, gender, SES and total LOC score, a statistically significant association was seen with LOC score and ECC .p= 0.003.

Conclusion- Internal LOC is associated with decreased caries prevalence. Childhood caries requires broader pediatric and public health perspective than solely dental standpoint to effectively solve it.

Keywords - *dental caries, caries prevention, locus of control, parental perspective, preschool children.*

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INTRODUCTION

General health plays a vital role in maintaining oral health. Amongst various factors which influence health, mental health determines our perception towards health. Many psychological models have been studied in relation to health.[1–3]

Health Locus of Control (HLOC) was developed from social learning theory and refers to the degree of control that people believe they possess over their personal health. Locus of control (LOC) is multidimensional in form and is attributed to internal factors, external factors and chance factors.[4] People with various LOC like internal, external and powerful others believe that their health is influenced by their choices, powerful others or by luck factors respectively.

Early childhood caries(ECC) is defined as "the presence of one or more decayed missing teeth or filled tooth in a child less than 72months of age.[5] Parental attitudes are likely to play a role in achieving and maintaining a desired level of oral health in children.[6]

While Reisine [7] have found that parents with good internal LOC are more likely to have children who are caries free, but others have failed to obtain this association.[8-9] A recent meta analysis of psychosocial correlates with oral hygiene behavior also found that LOC was less likely to be related with tooth brushing frequency.[10] LOC is a subjective entity and results may vary from region to region depending on educational, cultural and social background. If we use some objective tool to segregate parents into those who need only reinforcement of educational programs and those who need reinforcement along with behavior modification therapies, we can probably tackle ECC in a better way. Thus the present study has been planned to fill this lacunae in the current knowledge with an aim to find an association between parental LOC with their children's ECC experience.

MATERIAL AND METHO

This was a cross sectional study conducted in Belagavi city of Karnataka state, India, in which a validated questionnaire was used. Required permissions and consents were obtained. Sample size was calculated using the formula $Z=4pq/d^2$ where d -allowable error, p= 63), q=37[11], d was set at 5% and hence the required sample size was 373, with 10% excess to allow for missing data, the final sample size was 410.

Pilot study was conducted on 10 participants to assess comprehensiveness, reliability and validity of the questionnaire. Self designed English questionnaire was translated to Kannada, Marathi by a person fluent in both the regional languages. It was then back translated to English to conform that same meaning was conveyed. Few questions were modified and the questionnaire was finalized. This was given to 10 mothers and their responses were noted. Again after one month, the same questionnaire was given to same 10 mothers to assess reliability of the questionnaire. To assess reliability of the data, LOC items were subjected to test retest scores which was 0.84 confirming good reliability.

DATA COLLECTION PROCEDURE

Participants aged 3 to 4 year old were recruited from various preschools of Belagavi city. List of all the preschools was obtained. Belagavi city was divided into - South, East, North and West zones. To achieve the required sample, five schools were randomly selected from each zone and approached for permission. On predetermined date; parents were invited for an oral health education program delivered by RMS on "How best to prevent tooth decay in your young ones?" Parents were sensitized regarding importance of primary dentition, tooth brushing, self examination for dental caries, proper feeding habits etc. After the talk, parents were invited to participate for examination of their children. Consenting parents were asked to fill the questionnaire which was followed by examination of the child. Inclusion criteria were children aged 3-4 year old. The final sample consisted of 407 parents with their children.

Questionnaire- It was self designed, had three parts – socio-demographic details, LOC questionnaire and oral hygiene practices. Socioeconomic status was classified as per Kuppuswamy's socio economic classification. [12]

Oral hygiene practice included details of presence/ absence of parental guidance, frequency of brushing, time of brushing, materials used and how often was a tooth brush changed?

LOC of parents – LOC was explored using a questionnaire which was standardized, validated in an international study. [13] Thirteen questions relevant to LOC were selected. Questions 1,2,5,7, 11 expressed Internal LOC, 3R, 9R and 13R- external LOC and 4R,6R,8R,10R, 12R represented beliefs in bad luck / Chance. Each item was measured using 5 point likert scale (1- strongly agree to 5 strongly disagree. The coding for the negatively formulated items for External Chance LOC were reversed so that for all items, lower scores reflected more positive attitude.

Children were examined in classroom by the principal investigator using CPI probe and mouth mirror.[14] Examiner was calibrated for 30 preschool children and inter examiner agreement was assessed using Cohen's Kappa which was >0.85. ECC was recorded using WHO Dentition status and dmft (d- decayed teeth, m- missing teeth, f- teeth filled because of decay) was calculated. Caries was recorded as present, when lesion in fissure, or smooth tooth surface, had an unmistakable cavity

Data analysis – Data was tabulated using SPSS 20 (Chicago, USA). Descriptive analysis was performed for LOC items. Association between LOC score and oral hygiene practices, socio demographic factors was assessed using Chi square and Fischer exact test when needed. LOC aggregate score was calculated for every parent and were arranged in increasing order. The cut off value to determine caries positive cases was obtained from Receiver Operating Curve. Multiple logistic regression was used with ECC as outcome variable and age, gender, SES, total LOC score as independent variable.

FINDINGS

Of the total 407 children, 119 were 3yrs old and 288 were 4 yrs old; 204-males, 203- females. Seventy parents belonged to upper class, 310 to middle class and 27 belonged to lower class as per Kuppuswamy classification. Almost 95.6% used brush with toothpaste, 52.3 brushed twice, 62.7% had parental assistance for brushing. Association of oral hygiene factors with ECC are described in Table 1

A ROC curve was generated to determine cut off value for LOC which was 29.5 with 82.6 as sensitivity and 28.4 as specificity values. Area under curve (AUC) for LOC in the detection of ECC was 0.55 (95% CI 0.48-0.61). When LOC score was dichotomized into <29.5 and >29.5 and compared against ECC, significant association was observed .Children of parents who had External LOC were at 1.8 times more prone for dental caries.(p=0.002) Table 2. But when LOC score was sorted into five quintiles and association with ECC was assessed, a non significant association was obtained (Table 2).However Odds Ratio for ECC showed increasing trend with increasing LOC score. When multiple logistic regression was performed significant association was seen with LOC score and ECC .p= 0.003.(Table 3)

Table 1:- Association of caries experience with oral hygiene factors and Socio economic status

Oral hygiene Factors		Early childhood caries		Total	Chi square test	
		0	>1		Chi square value	p-value
Frequency of changing toothbrush	<3months	80(26.9%)	217(73.1%)	297	1.34	0.51(NS)
	3-6 months	17(23.0%)	57(77.0%)	74		
	>6 months	12(33.3%)	24(66.7%)	36		
Kuppuswamy scale	Upper Class	24(34.3%)	46(65.7%)	70	5.26	0.15(NS)
	Middle	46(24.0%)	146(76.0%)	192		
	Lower middle	35(29.7%)	83(70.3%)	118		
	Lower class	4(14.8%)	23(85.2%)	27		
Teeth are Cleaned by	Self	37(24.3%)	115(75.7%)	152	0.74	0.39(NS)
	Parents	72(28.2%)	183(71.8%)	255		
Brushing frequency	Once daily	67(29.3%)	162(70.7%)	229	1.64	0.20(NS)
	Twice daily	42(23.6%)	136(76.4%)	178		

*p<0.05 statistically significant, p>0.05 non significant, NS

#Fisher's exact test

Table2:-Distribution of LOC scores in quintiles and their association with early childhood caries

Dichotomized LOC score		Early childhood caries		Total	Chi square value	p-value	Odds ratio (95% CI)
		0	>1				
Total	<=29.5	31	52	83	5.94	0.02*	1.88(1.13 – 3.14)
	>29.5	78	246	324			
LOC score sorted into five quintiles							
Total (quintile)	**<28.5**	23	40	63	2.16	0.14(NS)	-
	29 – 32.5	24	69	93			1.65(0.83-3.30)
	33 – 35.5	19	50	69			1.51(0.73-3.16)
	36 – 38.5	18	65	83			2.07(0.99-4.32)
	>39	25	74	99			1.70(0.86- 3.38)

*p<0.05 statistically significant, p>0.05 non significant, NS

#Fisher's exact test

Table 3:- Multiple logistic regression analysis between multiple risk factors and Early childhood caries

	B	S.E.	Wald	df	p-value	Odds ratio	95% C.I.for odds ratio	
							Lower	Upper
Age	0.24	0.25	0.92	1	0.34(NS)	1.27	0.78	2.07
Gender	-0.27	0.23	1.36	1	0.24(NS)	0.77	0.49	1.20
Kuppuswamy scale			4.24	3	0.24(NS)			
Upper class	0.46	0.31	2.23	1	0.14(NS)	1.58	0.87	2.89
Middle class	0.12	0.33	0.14	1	0.71(NS)	1.13	0.59	2.16
Lower class	0.92	0.61	2.28	1	0.13(NS)	2.52	0.76	8.34
Total LOC score	0.58	0.27	4.66	1	0.03*	1.78	1.06	3.00
Constant	0.23	0.38	0.39	1	0.53(NS)	1.26		

Variable(s): Age, Gender, Kuppuswamy Scale, Total LOC score adjusted.

*p<0.05 statistically significant, p>0.05 non significant, NS

#Fisher's exact test

DISCUSSION

The present study investigated association between LOC of parents with ECC experience in their children in a representative sample. Caries free children were more likely to belong to upper class, had parental assistance for brushing, brushed once daily regularly in the morning. These results support the evidence that family parameters do influence the onset of the disease. Parents with higher educational and socio economic position are more likely to recognize the importance of oral hygiene. Highest number of upper class children (34.5%) were caries free compared to only 14.8% in the lower class. When mother's education and caries status was assessed, non significant association was obtained. Interestingly 50% of the children whose mothers had university level education were caries free compared to 0% in mothers with primary education. Our results are similar to Duijster study. [15]

The results were clearer when LOC score was dichotomized with 29.5 as the cut off value. Children whose parents had more LOC score (had External or Powerful others score) were at least 1.8 times more likely to have ECC, whereas Lencova [16] et al reported that children of parents with internal LOC were 2.32 times less likely to suffer from ECC. The present study focused on 'd', 'm', and 'f' components separately. Irrespective of the LOC score, all the children who were examined had only untreated decay and no filled teeth. This suggests that factors which are responsible for availing treatment, perhaps need to be included in the LOC questionnaire. There could be several reasons for untreated caries, primarily being absence of any dental insurance in the country. India, being a developing country has limited resources and the GDP spent on health sector for the year 2017 is meager 1.2%, [17] and thus inclusion of dental insurance at the national level is an inconceivable thought as of now. Untreated decay status was seen unambiguously even for children whose mothers had university education. This can be explained better by the Acharya et al study which reports that, children who had high caries risk had mothers with high Internal LOC, indicating a high independence, may play a negative role by instilling a false sense of control about one's ability to maintain oral health, especially when it is not backed by adequate oral health knowledge of competence and awareness about disease. [18]

AUC obtained in the present study was 0.56 which is less than optimal, and is considered as poor. The study result reinforces the fact that dental caries is multifactorial and no single factor can be considered as causative factor. However social desirability bias among the parents might have also played a role. The cross sectional nature of the study restricts the exploration of change in parental behavior after exposing them to health education programs.

This study is first of its kind to assess association between parental LOC with ECC experience in the representative sample of India. The results of the logistic regression analysis reveal that LOC has an association with ECC. Thus we can contemplate that creating awareness by educating parents would be a cost effective tool for prevention of ECC.

CONCLUSION

Study result supports the hypothesis that parental LOC is associated with lesser caries experience in preschool children. The cut off value of LOC score was tabulated at 29.5.Lesser the score higher was the chance of children to be caries free. Children of parents with external LOC score was 1.8 times more likely to suffer from ECC after adjusting for other socio demographic factors.

Conflicting Interest - we **do not** have any conflict of interest.

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REFERENCES

1. Rosenstock IM, Strecher VJ, Becker MH. Social learning theory and the Health Belief Model. Health Educ Q 1988;15:175–83.
2. Rogers RW. A Protection Motivation Theory of Fear Appeals and Attitude Change1. J Psychol 1975;91:93–114.
3. Wallston KA, Wallston BS, DeVellis R. Development of the Multidimensional Health Locus of Control (MHLC) Scales. Health Educ Monogr 1978;6:160–70.
4. Wallston KA. The Validity of the Multidimensional Health Locus of Control Scales. J Health Psychol 2005;10:623–31.

5 Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH. Diagnosing and reporting early childhood caries for research purposes. A report of a workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration, and the Health Care Financing Administ. J Public Health Dent 1999;59:192–7.

6 Vermaire JH, Hoogstraten J, Van Loveren C, Poorterman JHG, Van Exel NJA. Attitudes towards oral health among parents of 6-year-old children at risk of developing caries. Community Dent Oral Epidemiol 2010;38:507–20.

7 Reisine S, Litt M. Social and psychological theories and their use for dental practice. Int Dent J 1993;43(3 Suppl 1):279–87.

8. Paulo S, Araraquara F, M G Brandão CI, Maria Gomes Brandão I, Moreira Arcieri R, Lúcia Mazza Sundefeld M, et al. Early childhood caries: the influence of socio-behavioral variables and health locus of control in a group of children. Dent Caries Oral Heal Child Welf 2006;22:1247–56.

9. Chase I, Berkowitz RJ, Proskin HM, Weinstein P, Billings R. Clinical outcomes for Early Childhood Caries (ECC): the influence of health locus of control. Eur J Paediatr Dent 2004;2:76–80.

10. Scheerman JFM, van Loveren C, van Meijel B, Dusseldorp E, Wartewig E, Verrips GHW, et al. Psychosocial correlates of oral hygiene behaviour in people aged 9 to 19 - a systematic review with meta-analysis. Community Dent Oral Epidemiol 2016;44:331–41.

11. Sankeshwari R, Ankola A V, Tangade S, Hebbal I. Feeding habits and oral hygiene practices as determinants of early childhood caries in 3- to 5-year-old children of Belgaum City, India. Oral Health Prev Dent. 2012;10:283–90.

12. Kumar N, Gupta N, Kishore J. Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012. Indian J Public Health 2012;56:103–4.

13. Pine CM, Adair PM, Petersen PE, Douglass C, Burnside G, Nicoll AD, et al. Developing explanatory models of health inequalities in childhood dental caries. Community Dent Health 2004;21(1 Suppl):86–95.

14. WHO Oral Health Survey Basic Methods. World Health Organization. 1997. p. 1–69.

15. Duijster D, de Jong-Lenters M, de Ruiter C, Thijssen J, van Loveren C, Verrips E. Parental and family-related influences on dental caries in children of Dutch, Moroccan and Turkish origin. Community Dent Oral Epidemiol 2015;43:152–62.

16. Lencová E, Pikhart H, Broukal Z, Tsakos G. Relationship between parental locus of control and caries experience in preschool children - cross-sectional survey. BMC Public Health 2008 Jan;8:208.

17. World Bank Data. Available from https://data.worldbank.org/country/india?view=chart (Accessed on 15 December 2017)

18. Acharya S, Pentapati KC, Singh S. Influence of socioeconomic status on the relationship between locus of control and oral health. Oral Health Prev Dent. 2011;9(1):9–16.

Original Article

Soxhlet versus Cold Maceration: Which Method Gives Better Antimicrobial Activity to Licorice Extract against *Streptococcus mutans*?

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Abstract

Purpose: Licorice is called "grandfather of herbs" and is being used for wide various ailments since time immemorial. However, its use in dentistry has been recently. Soxhlet and Cold maceration are the two commonly employed methods for extraction of drug from raw products. But which of two is gives better antibacterial property to licorice root remains unanswered. Hence, the present study has been planned with an aim to compare antibacterial activity of licorice root extracts obtained from two methods (Soxhlet and cold maceration) against *Streptococcus mutans*. It is an *in vitro* study. **Methodology:** Licorice roots were authenticated from recognized taxonomist. They were washed, dried completely, and coarsely powdered. The weighed powder was mixed with ethanol (100 mg in 500 ml). Two such mixtures were made. One was used for cold maceration procedure and the other was used for Soxhlet method. Extracts so obtained were assessed for their minimum inhibitory concentration against *S. mutans* ATCC 25175 in triplicates using broth dilution and disc diffusion method. Extracts were also compared for their phytochemical components. Descriptive analysis and unpaired *t*-test were performed. **Results:** Cold maceration extract at concentration of 1.95 mg/ml and Soxhlet method at 3.906 mg/ml showed inhibition of *S. mutans*. Both of them possessed the same phytochemical components. **Conclusion:** Licorice root extract obtained through cold maceration had significantly better antimicrobial activity against *S. mutans* than licorice extract obtained through Soxhlet method. Cold maceration method is relatively simple and does not involve complex instruments and yet yields better extract.

Keywords: *Cold maceration, licorice root extract, Soxhlet method*

Introduction

Licorice (*Glycyrrhiza glabra*) called as Madhuyashti, Mulethi, and Yastimadhu is an herbaceous perennial plant. Root consists of stolons and pieces of roots. It occurs in Southern Europe, Spain, Syria, Russia, Egypt, Arab, and Iran.[1] In India, it is reported to be cultivated in Baramulla, Srinagar, Jammu, Dehradun, Delhi, and South India. Licorice is obtained from the unpeeled, dried roots and stolons of *G. glabra* and *Glycyrrhiza uralensis*. Licorice contains several classes of secondary metabolites such as coumarin, flavonoids, isoflavonoids, pterocarpenes, saponins, and stilbenes which have been described in detail by Wang and Kondo.[2] Licorice has been used in Ayurveda since time immemorial. It is 50 times sweeter than sucrose and is used as diuretic, demulcent, mild laxative, aphrodisiac, expectorant, hemostatic, and intellect promoting.[3] The active chemical ingredients imparting the unique licorice taste are glycyrrhizic acid and its glucoside, glycyrrhizin (C42H62O16). These molecules are regarded as nearly synonymous as powerful organoleptic flavorants and impart characteristic licorice taste and aroma.[4,5]

Literature has a report of licorice being used for oral diseases such as dental caries, periodontal diseases, candidiasis, and recurrent aphthous ulcers.[6] It has also been used as a mouthwash and lollipop for control of tooth decay.[7,8] The aciduric mutans streptococci (MS) group, including *Streptococcus mutans* and *Streptococcus sobrinus*, are highly cariogenic and represent microorganisms most closely associated with dental caries. The oral cavity contains at least 52 genetic strains of *S. mutans*.[9,10] Some MS strains may have enhanced abilities to adhere and propagate in specific oral environment[11] including selective colonization of hard-tissue sites.[12] In addition, several MS strains have been

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known to concurrently colonize a single tooth, and single genotypes have been identified to colonize multiple sites within the dentition of individual patients.[13] Hence, *S. mutans* is considered as the main culprit for dental caries and was thus selected for the present study.

Ayurvedic products are polyherbal by nature and are found in the crude form. There are many extraction procedures which are available to obtain the desired drug. Most commonly followed extraction methods are Soxhlet method and cold maceration. Soxhlet extraction has been used for many decades, is very time-consuming, and requires relatively large quantities of solvents. It also needs Soxhlet extractor special equipment for the process.

On the other hand, cold maceration is a simple procedure which does not require any special armamentarium. Cold maceration always results in an odor similar to that in the original plant material without causing degradation of the thermolabile compounds present in the fraction due to the low extraction temperature similar to cold pressing.[14]

Although various methods have been described in the literature for extraction of crude drug, a scientific comparative study to know which method is superior over the other is lacking for licorice extract. Hence, the present study has been planned with an aim to compare the antibacterial activity of licorice extract obtained using cold maceration and Soxhlet method against *S. mutans*.

Methodology

The present study is an *in vitro* study, and ethical approval was obtained from the Institutional Ethics Committee No. 820 dated January 28, 2014, KLE University.

For licorice extract preparation, dried licorice roots were procured from KLE Ayurveda Pharmacy, Belgaum, Karnataka. Materials used in this *in vitro* study are as follows:

- Licorice roots
- Pure ethanol
- Standard strain of *S. mutans* ATCC 25175.

Licorice extract was prepared using two different methods which are cold maceration and Soxhlet method.

Cold maceration

Purchased licorice root specimen was authenticated from recognized botanist at Indian Council of Medical Research's Regional Medical Research Centre, Belgaum. After washing all the roots, they were dried in the shade for 3–4 days. Roots were cut into small pieces in a grinder and were grounded to coarse powder. One hundred grams of licorice powder was mixed with 500 ml of 100% ethanol in a conical flask. The mixture was stirred thoroughly with a glass rod. The conical flask was kept with intermittent shaking for 72 h. The mixture was filtered using muslin cloth and through Whatman No. 1 filter paper. The filtrate was concentrated using an IKA rotary evaporator at 40°C, and the resultant residue was kept in a refrigerator till further use.

Soxhlet method

One hundred grams of coarse powder of licorice root was packed in a muslin cloth bag and placed in the body of Soxhlet extractor. Then, 500 ml of ethanol (solvent) was poured in the round-bottom flask. The apparatus was then fitted with the help of clamps and stand to support the Soxhlet extractor, round-bottom flask, and condenser. The rubber tube connected to the tap water was attached to the condenser for continuous flow of water. The solvent was heated using the isomantle, which began to evaporate, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the plant extract. Once the level of solvent reached the siphon, it poured back into the flask and the cycle began again. The process was made to run for a total of 6 h. Finally, the extract was collected in the round-bottom flask. Once the process was finished, the ethanol was evaporated using IKA rotary evaporator at 40°C, leaving a small yield of extracted plant material (about 2–3 ml) in the glass-bottom flask. Extract was kept in a porcelain bowl till the remaining ethanol was completely evaporated. The content of extractable matter was calculated in mg/g of air-dried material using digital weighing balance. The extract was stored in the refrigerator till further use.

Phytochemical screening

The ethanolic extracts obtained from both the techniques were subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents using standard procedures as described by Trease and Evans.[15]

Preparation of inoculums

The microbial strain used for this study was procured from the Institute of Microbial Technology, Chandigarh (ATCC 25175). Stock cultures were maintained at 4°C on the slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria which were incubated for 24 h at 37°C.

Determination of antimicrobial activity of the licorice extract was done as per the Clinical Laboratory and Standard Institute guidelines.[16]

- The lowest concentration of the extracts that inhibits the growth of test organisms is the minimum inhibitory concentration (MIC). The MIC of the licorice extracts was determined by broth dilution method against bacterial culture
- MIC of the extract against *S. mutans* (ATCC 25175) by broth dilution method was carried. Media used was brain–heart infusion (BHI) broth. Culture/inoculum: *S. mutans*. Stock solution of the extract: 50% (500 mg in 1 ml of dimethyl sulfoxide).

- Extracts: Licorice extract-Cold maceration method (LC)
  Licorice extract-Soxhlet extraction method (LS).

Procedure

Nine dilutions of extract were done with BHI for MIC. In the initial tube, only 200 µl of extract was added. For dilutions, 200 µl of BHI broth was added into the next nine tubes separately. In the 2nd tube, 200 µl of extract was added which already contains 200 µl of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube, 200 µl was transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-8} dilution for each extract. From the maintained stock cultures of required organisms, 5 µl was taken and added into 2 ml of BHI broth. In each serially diluted tube, 200 µl of above culture suspension was added. The last tube contained only the media and culture suspension. The tubes were kept for incubation for 24 h at 37°C in bacteriological incubator and observed for turbidity. The experiment was repeated in triplicate for both LS and LC, to ascertain the antimicrobial activity.

Disc diffusion method was carried out to confirm the results of broth dilution method.

Results

Approximately 8 g and 6.5 g of licorice extract were obtained from 100 g of powder through cold maceration and Soxhlet method, respectively.

For broth dilution method of MIC, turbidity was seen in the ninth tube of cold maceration method and eight tube of Soxhlet method.

Further result was confirmed with the help of disc diffusion method in which the zone of inhibition was noted. All the experiments were repeated in triplicates, and the average value was taken.

Table 1 shows the MIC values of the triplicate experiments. The mean MIC value for cold maceration was 1.8 ± 0.145, and for Soxhlet method, it was 3.8 ± 0.097 [Table 2]. When unpaired *t*-test was applied, $P = 0.000$ with 95% confidence interval which concluded that there was a statistically significant difference between MIC values obtained through two methods [Table 3]. When phytochemical screening was performed, both the extracts showed the presence of components as shown in Table 4.

Discussion

Licorice has been used in Ayurveda for many ailments, and the literature search shows that it has good scope to be used in medicine and dentistry as an adjunct to allopathic drugs. Its effectiveness on general health problems has been well documented, but its effect on *S. mutans,* the main culprit in the initiation of dental caries, is sparsely reported.[17]

Table 1: Minimum inhibitory concentration values of the cold maceration and Soxhlet method

Experiment	Cold maceration (1)	Soxhlet method (2)
First time	1.953 mg/ml	3.906 mg/ml
Second time	1.783 mg/ml	3.715 mg/ml
Third time	1.664 mg/ml	3.781 mg/ml

Table 2: Mean and standard deviation of the minimum inhibitory concentration value

MIC value	*n*	Mean±SD	SE
Cold maceration (1)	3	1.800±0.145	0.0838
Soxhlet method (2)	3	3.800±0.097	0.056

MIC=Minimum inhibitory concentration, SD=Standard deviation, SE=Standard error

Table 3: Comparison of antimicrobial activity of licorice extract obtained by cold maceration and Soxhlet method (independent *t*-test)

Test for equality of means	*t*	df	Significant (two-tailed)
MIC value			
Equal variances assumed	−19.840	4	0.000
Equal variances not assumed	−19.840	3.48	0.000

MIC=Minimum inhibitory concentration

Table 4: Phytochemical test results of both the extracts

Phytochemical test	Cold maceration	Soxhlet method
Test for sterols and triterpenoids		
Salkowski test	+	+
Test for flavonoids		
Alkaline reagent test	+	+
Test for alkaloids		
Hager's test	+	+
Mayer's test		
Dragendorff's test		
Test for tannin		
Lead acetate test	+	+
Ferric chloride test		
Test for saponins		
Froth test	+	+
Test for reducing sugars		
Benedict's test	+	+
Test for anthraquinones		
Borntrager's test	+	+

+=Phytochemicals present

Ayurvedic drug formulation is known as pancavidhaa kasayaa and has description of five basic forms: Swarasa, Kalka, Kwatha, Sheeta, and Faanta. Ayurveda believes that a plant as a whole may not have therapeutic effect, and hence, the active ingredients need to be extracted from the whole plant.[18] The type of extraction procedure selected should be based on the nature of the constituents. If the constituents are thermolabile, then extraction procedures

such as cold maceration and percolation are preferred.[18] The major constituents of licorice are triterpenoids and flavonoids, apart from small quantities of pyrrolopyrimidine alkaloid and tetrahydroquinoline alkaloids.[19-21] The chemical components responsible for antioxidant and antibacterial activity present in *G. glabra* roots have been reported such as glycyrrhizin, glycyrrhizinic acid, glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, phaseollinisoflavan, hispaglabridin A and B, 3-hydroxyglabrol, and 3 methoxyglabridin, glabranin isomer.[22-24] Flavonoids and phenylpropanoids can degrade when kept in organic solvents. Glycosides tend to break up when exposed to higher temperatures as in case of Soxhlet extraction. We can contemplate that differences in MIC values observed in the present study could be attributed to differences in flavonoid concentration which is responsible for antibacterial activity of licorice to a large extent.

Solvent used for extract of crude product also plays an important role. Water and alcohol are the two commonly used solvents for extraction procedure. Water is considered as a universal solvent as it is inert, but it may not be useful in all circumstances, especially when the ingredients are insoluble in water. Hence, alcohol is considered a good choice for such conditions, and moreover, it has many advantages over water such as:

- Alcohol is neutral and hence extract products obtained from it are compatible with other products, small amount of heat is required to concentrate the alcoholic preparations, and it dissolves selective active constituents of the drug.[23] This postulate was tested by Ahmad *et al*.[24] and Jain *et al*.[8] who concluded that ethanolic extract of licorice had better antimicrobial activity. This might be attributed to the polar nature of the solvent ethanol which resulted in leaching of more active ingredients during extraction.

The phytochemical screening revealed the presence of carbohydrates, reducing sugars, terpenoids, glycosides, steroids, tannins, saponins, anthraquinones, flavonoids, and alkaloids. The presence of these secondary metabolites could contribute to the antibacterial activity of the extracts. It is interesting to note that these components were present in both the extracts, but however, the MIC value of both differed significantly. This difference could be attributed to the difference in solubility of both the extracts. In the present study, the cold maceration extract dissolved completely than extract obtained by Soxhlet method.

A comparison between different methods for extraction of glycyrrhetic acid from licorice stolons was reported by Sharad Visht[25] which concluded that extraction ratio of glycyrrhetic acid can be increased by changing pH of extraction solvent. In the present study, cold maceration proved to be better than Soxhlet method for extraction of licorice roots. The study result is of practical significance which demonstrates that licorice extract obtained from simple method of cold maceration is superior to the extract obtained from Soxhlet method which needs special apparatus for carrying out the procedure along with continuous supply of tap water for at least 24 h apart from requirement of personnel to monitor the procedure. Hence, future studies which are planned on licorice roots can directly adopt cold maceration method for obtaining extract.

The study has some limitations such as the use of single organism. It is a known fact that dental caries is a multifactorial disease and etiology has been attributed to various causative microorganisms. However, *S. mutans* is predominant of all cariogenic microorganisms, and hence, it was selected in the present study. In future, studies can be conducted on various other cariogenic microorganisms to know if licorice extract has broad-spectrum antimicrobial activity. Confirmatory tests such as thin-layer chromatography (TLC)/high-performance TLC can be carried out to know if there is any difference in the concentration of active ingredients of both cold maceration and Soxhlet extract.

Conclusion

The present study concludes that the licorice root extract obtained from cold maceration method showed significantly better antimicrobial activity than extract obtained from Soxhlet method against *S. mutans*.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Gyanendra Pandey . Drvyaguna Vigyan. Vol II. New Delhi: Chavkhamba Publication; 2004. p. 456-68.
2. Tao R, Tong Z, Lin Y, Xue Y, Wang W, Kuang R, *et al.* Antimicrobial and antibiofilm activity of pleurocidin against cariogenic microorganisms. Peptides 2011;32:1748-54.
3. Sastry DJ. Dravyagunn Vijnana. Varanasi: Chavkhamba Publication; 2013. p. 152-6.
4. Touyz LZ. *Liquorice* health check, oro-dental implications, and a case report. Case Rep Med 2009;2009:170735.
5. de Marsillac Mde W, Vieira Rde S. Assesment of artificial caries lesions through scanning electron microscopy and cross-sectional microhardness test. Indian J Dent Res 2013;24:249-54.
6. Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis 2012;18:32-9.
7. Peters MC, Tallman JA, Braun TM, Jacobson JJ. Clinical reduction of S. Mutans in pre-school children using a novel liquorice root extract lollipop: A pilot study. Eur Arch Paediatr Dent 2010;11:274-8.
8. Jain E, Pandey RK, Khanna R. *Liquorice* root extracts as potent cariostatic agents in pediatric practice. J Indian Soc Pedod Prev Dent 2013;31:146-52.

9. Napimoga MH, Höfling JF, Klein MI, Kamiya RU, Gonçalves RB. Tansmission, diversity and virulence factors of *Sreptococcus mutans* genotypes. J Oral Sci 2005;47:59-64.
10. Tabchoury CP, Sousa MC, Arthur RA, Mattos-Graner RO, Del Bel Cury AA, Cury JA, *et al.* Evaluation of genotypic diversity of *Streptococcus mutans* using distinct arbitrary primers. J Appl Oral Sci 2008;16:403-7.
11. Svensäter G, Larsson UB, Greif EC, Cvitkovitch DG, Hamilton IR. Acid tolerance response and survival by oral bacteria. Oral Microbiol Immunol 1997;12:266-73.
12. Baca P, Castillo AM, Baca AP, Liébana MJ, Junco P, Liébana J, *et al.* Genotypes of *Streptococcus mutans* in saliva versus dental plaque. Arch Oral Biol 2008;53:751-4.
13. Hardie JM. Oral *Streptococci*. In: Sneath PH, Mair NS, Sharpe ME, editors. Bergey's Manual of Systematic Bacteriology. Baltimore, MD: Williams and Wilkins; 1986. p. 1054-63, 12.
14. Wu C, Wang F, Liu J, Zou Y, Chen X. A comparison of volatile fractions obtained from *Lonicera macranthoides* via different extraction processes: Ultrasound, microwave, soxhlet extraction, hydrodistillation, and cold maceration. Integr Med Res 2015;4:171-7.
15. Trease GE, Evans W. Pharmacognosy. 14th ed. London, UK: WB Saunder Company Ltd; 1996. p. 612.
16. Cockerill FR, Wikler MA, Alder J, Dudley MN, Eliopoulos GM, Ferraro MJ, *et al*. Performance standards for antimicrobial disk susceptibility tests; approved standard. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2012. p. 76.
17. Sedighinia F, Safipour Afshar A, Soleimanpour S, Zarif R, Asili J, Ghazvini K, *et al.* Antibacterial activity of *Glycyrrhiza glabra* against oral pathogens: An *in vitro* study. Avicenna J Phytomed 2012;2:118-24.
18. Savrikar SS, Ravishankar B. Bhaishajya kalpanaa - the ayurvedic pharmaceutics - an overview. Afr J Tradit Complement Altern Med 2010;7:174-84.
19. Rao KV. A review on licorice. Anc Sci Life 1993;13:57-88.
20. Fukai T, Tantai L, Nomura T. Isoprenoid substitute flavonoid from *Glycyrrhiza glabra*. Phytochemistry 1996;43:531-53.
21. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, *et al.* Antimicrobial potential of *Glycyrrhiza glabra* roots. J Ethnopharmacol 2008;116:377-80.
22. Gupta A, Maheshwari DK, Khandelwal G. Antibacterial activity of *Glycyrrhiza glabra* roots against certain gram-positive and gram-negative bacterial strains. J Appl Nat Sci 2013;5:459-64.
23. Aulton M. Aultons Pharmaceutics :The design and manufacture of medicine. 4th ed. London: Churchill Livingstone; 2013.
24. Ahmad I, Mehmood Z, Mohammad F. Screening of some indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998;62:183-93.
25. Sharad Visht GK. A comparison between different methods for extraction of glycyrrhetic acid from licorice stolons. Int J Pharma Prof Res 2012;3:622-6.

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