Original Article

To Evaluate and Compare the Effects of Chlorhexidine Gel and *Ocimum sanctum* Gel on Salivary Amylase in 6–12 Years Old Children: An *In Vivo* Study

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Abstract

Introduction: To evaluate the effect of 4% *Ocimum sanctum* (in the form of a test gel) on human salivary amylase activity and to compare it with 1% Chlorhexidine digluconate gel in 6–12-year-old children. **Materials and Methods:** The present randomized controlled trial was conducted among 75 Primary school children of age 6–12 years. Children were selected by a simple random sampling technique and they were divided equally into three groups. Group A was treated by 4% *O. sanctum* gel, Group B by 1% Chlorhexidine digluconate gel, and Group C by placebo gel. Test gel was applied to the subjects. The gel was applied by the Tray technique method for 4 min. The saliva samples were collected within 2–3 min of application. All samples were then stored in a chamber at a temperature of −20°C until further processing. **Results:** The Mean age of participants was 10.60 ± 1.19 years. Mean Transmittance rate in Group A (4% *O. sanctum* gel) was 0.038 ± 0.001 and Mean Transmittance rate in Groups B and C were 0.053 ± 0.0006 and 0.057 ± 0.001, respectively. There was a significant difference in the transmittance rate between Groups A, B, and C ($P < 0.05$). Intragroup comparison between Groups A and B showed a significant difference in the transmittance rate ($P < 0.05$). The same was found between Groups B and C and Groups A and C ($P < 0.05$). **Conclusion:** 4% *O. sanctum* gel tremendously reduces the quantity of salivary amylase. Salivary amylase is one of the caries-inducing enzymes. Regular application of 4% *O. sanctum* gel in 6–12 years of children will help in reducing dental caries. Chlorhexidine also helps in reducing the salivary amylase, but not effective as 4% *O. sanctum* gel.

Keywords: 4% *Ocimum sanctum* gel, Chlorhexidine, Salivary amylase, Tulsi.

Introduction

Despite hundreds of research investigations for more than a century, dental hard and soft tissue pathologies still remain the most common disease of modern civilization. The use of herbal medicines continues to expand rapidly across the world. Many people take herbal medicines or herbal products now for their health care[1] due to its medicinal properties and less side effects. Herbal extracts have been used in dentistry for reducing inflammation, as antimicrobial plaque agents, for preventing the release of histamine, and as antiseptics, antioxidants, antimicrobials, antifungals, antibacterials, antivirals, and analgesics. They also aid in healing and are effective in controlling microbial plaque in gingivitis and periodontitis, and thereby improving immunity.[2]

Ocimum sanctum, also known as Holy basil and Tulsi, is the Queen of Herbs. It is the most sacred herb of India. *O. sanctum* has been revered in India for over 5000 years as a healing balm for the body. It holds a plethora of medicinally important compounds. A large number of Phytochemicals have been isolated from Tulsi leaves which contain eugenol, euginal, urosolic acid carvacrol.[3] Variety of other compounds, including saponins, phenols, tannins, and flavonide has also been isolated.[4] Among all these active principles Eugenol is believed to be responsible for antimicrobial effects.[5] The antimicrobial effect is due to linoleic acid which was effective against *Bacillus pumilus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[6]

Access this article online

Website: www.jrad.co.in

DOI: https://doi.org/10.53064/jrad.2024.16.5.714

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Received: Jun. 06, 2025; **Accepted:** Jul. 23, 2025; **Published:** Aug. 08, 2025

How to cite this article: Biradar S, Malik S, Jauhari D, Gupta S, Mishra R, Ghogare S. To Evaluate and Compare the Effects of Chlorhexidine Gel and *Ocimum sanctum* Gel on Salivary Amylase in 6–12 Years Old Children: An *In Vivo* Study. J Res Adv Dent 2025;16(5):1-7.

Chlorhexidine is one of the most commonly prescribed antimicrobial agents in the dental field. It has a long lasting antibacterial activity with a broad-spectrum of action and it has been shown to reduce plaque accumulation, gingival inflammation and bleeding.[7] It is cationic bisguanide with broad antibacterial activity, low mammalian toxicity and a strong affinity for binding to skin and mucous membranes.[8] The compound is strongly base and dicationic at pH levels above 3.5 with positive charges on either side of hexamethylene bridge. The dicationic nature of Chlorhexidine attributes to its affinity with anions. Clinical studies of chlorhexidine gels have been mainly conducted on children, and show that Chlorhexidine gel treatment in high caries-risk children showed significant reductions in dental decay.[9]

Salivary amylase is a principal digestive enzyme produced by the salivary glands, which plays an important role in the colonization and metabolism of Streptococcus, leading to the formation of dental plaque and caries in human beings. It has been identified as a constituent of the acquired pellicle and also acts as a receptor for microorganism adhesion on the tooth surface. It has the ability to bind on bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids, leading to dental caries.[10] Suppression of salivary amylase activity leads to decrease in risk of dental caries and other plaque-associated oral diseases.

There are no experiments comparing assessment of effect of 4% *O. sanctum* gel and 1% Chlorhexidine gel on salivary amylase in 6–12 year children, The present study was carried out to evaluate the effect of 4% *O. sanctum* (in the form of a test gel) on human salivary amylase activity and to compare it with 1% Chlorhexidine digluconate gel in 6–12 years of children.

Materials and Methods

The present Randomized controlled trial was conducted among 75 Primary school children of age 6–12 years. Children were selected by a simple random sampling technique and they were divided equally into three groups. Group Table 1 was treated by 4% *O. sanctum* gel, Group Table 2 by 1% Chlorhexidine digluconate gel, and Group Table 3 by placebo gel. The study was carried forward after ethical clearance from the institute. The study was conducted abiding by all human ethical principles as per the Declaration of Helsinki and the Guidelines of Good Clinical Practice were observed. This study was conducted after obtaining parent's verbal and written consent. Both genders were given equal opportunity to participate in the study. Subjects with any obvious oral mucosal lesions, dental caries, xerostomia, and those wearing any prosthetic and orthodontic appliance, and subjects on any oral and systemic medication were excluded from the study.

Table 1: Group-A (4% *Ocimum sanctum* gel)

Sample	Age	Sex	Before induction			After induction			Standard 100% transmittance
			1a	2a	3a	1a	2a	3a	
A1	11	Male	0.035	0.036	0.036	0.042	0.040	0.045	0.039
A2	10	Male	0.031	0.033	0.035	0.040	0.041	0.043	0.037
A3	11	Female	0.035	0.034	0.033	0.044	0.040	0.046	0.039
A4	12	Male	0.033	0.034	0.036	0.043	0.040	0.042	0.038
A5	10	Male	0.031	0.033	0.033	0.042	0.040	0.045	0.037
A6	8	Male	0.032	0.031	0.034	0.040	0.041	0.043	0.037
A7	12	Male	0.035	0.036	0.036	0.044	0.040	0.046	0.040
A8	11	Female	0.031	0.033	0.035	0.042	0.040	0.045	0.038
A9	10	Female	0.033	0.034	0.036	0.040	0.041	0.043	0.038
A10	11	Female	0.031	0.033	0.033	0.042	0.040	0.045	0.037
A11	9	Male	0.033	0.034	0.036	0.040	0.041	0.043	0.038
A12	12	Male	0.035	0.036	0.036	0.044	0.040	0.046	0.040
A13	11	Male	0.031	0.033	0.035	0.040	0.041	0.043	0.037
A14	10	Male	0.033	0.034	0.036	0.044	0.040	0.046	0.039
A15	11	Male	0.031	0.033	0.033	0.042	0.040	0.045	0.037
A16	12	Male	0.033	0.034	0.036	0.040	0.041	0.043	0.038
A17	10	Male	0.031	0.033	0.035	0.044	0.040	0.046	0.038
A18	8	Female	0.035	0.036	0.036	0.042	0.040	0.045	0.039
A19	12	Male	0.031	0.033	0.035	0.040	0.041	0.043	0.037
A20	11	Female	0.033	0.034	0.036	0.042	0.040	0.045	0.039
A21	10	Female	0.031	0.033	0.033	0.040	0.041	0.043	0.037
A22	11	Female	0.033	0.034	0.036	0.044	0.040	0.046	0.039
A23	9	Female	0.031	0.033	0.033	0.042	0.040	0.045	0.037
A24	12	Female	0.035	0.036	0.036	0.040	0.041	0.043	0.039
A25	11	Female	0.031	0.033	0.035	0.042	0.040	0.045	0.038

Table 2: Group-B (1% Chlorhexidine digluconate gel)

Sample	Age	Sex	Before induction			After induction			Standard 100% transmittance
			1b	2b	3b	1b	2b	3b	
B1	11	Male	0.047	0.047	0.053	0.059	0.060	0.060	0.054
B2	10	Male	0.043	0.051	0.048	0.057	0.059	0.059	0.053
B3	11	Female	0.048	0.045	0.049	0.059	0.057	0.057	0.053
B4	12	Male	0.047	0.049	0.051	0.059	0.057	0.057	0.053
B5	10	Male	0.043	0.051	0.048	0.059	0.057	0.057	0.053
B6	8	Male	0.047	0.047	0.053	0.057	0.059	0.059	0.054
B7	12	Male	0.047	0.049	0.051	0.059	0.057	0.057	0.053
B8	11	Female	0.051	0.043	0.048	0.059	0.057	0.057	0.053
B9	10	Female	0.049	0.047	0.051	0.057	0.059	0.059	0.054
B10	11	Female	0.045	0.048	0.049	0.059	0.060	0.060	0.054
B11	9	Male	0.051	0.048	0.043	0.059	0.057	0.057	0.053
B12	12	Male	0.051	0.048	0.043	0.057	0.059	0.059	0.053
B13	11	Male	0.049	0.043	0.048	0.059	0.057	0.057	0.052
B14	10	Male	0.047	0.049	0.051	0.057	0.059	0.059	0.054
B15	11	Male	0.043	0.051	0.048	0.059	0.060	0.060	0.054
B16	12	Male	0.045	0.048	0.049	0.057	0.059	0.059	0.053
B17	10	Male	0.047	0.047	0.053	0.059	0.057	0.057	0.053
B18	8	Female	0.048	0.045	0.049	0.057	0.059	0.059	0.053
B19	12	Male	0.051	0.043	0.048	0.059	0.057	0.057	0.053
B20	11	Female	0.049	0.043	0.048	0.057	0.059	0.059	0.053
B21	10	Female	0.047	0.049	0.051	0.059	0.060	0.060	0.054
B22	11	Female	0.043	0.051	0.048	0.059	0.057	0.057	0.052
B23	9	Female	0.051	0.048	0.043	0.057	0.059	0.059	0.053
B24	12	Female	0.049	0.043	0.048	0.059	0.060	0.060	0.053
B25	11	Female	0.043	0.051	0.048	0.059	0.057	0.057	0.052

Collection and storage of sample

The subject was made to sit comfortably in a calm and isolated room. He/she was asked to rinse the mouth thoroughly using distilled water for 10 min before sample collection to remove any food debris to avoid compromise by influencing pH and bacterial growth. The subjects were advised not to consume any food for at least 2 h before the experiment. Salivary samples were collected between 9.00 am and 12.00 pm. The diurnal variation of saliva is very low during this time. The same procedure was repeated for saliva sample collection after the application of test materials. All the saliva samples thus collected were then stored in a chamber at a temperature of −20°C.

Preparation of *O. sanctum* gel

The 4% *O. sanctum* gel was prepared by soaking the active component (4 g leaves) of the plant in warm water overnight (i.e., 12 h). After 12 h, the components were washed 8–10 times by distilled water and placed in a Soxhlet Apparatus for preparation of the extract. The obtained extract was then be filtered and 0.8 g of Carbapol 984P (Acropol) was sprinkled over this filtered extract and allow remaining there until it swells. 4% glycerine was then added to the extract and mixed well. The obtained mix was checked for pH and the pH will be adjusted between 6 and 7 with 10% NaOH.

Application of test material

Test gel was applied to the subjects. The gel was applied by the Tray technique method for 4 min. The saliva samples were collected within 2–3 min of application. All samples were then stored in a chamber at a temperature of −20°C until further processing.

Processing of samples

Estimation of salivary amylase was done based on the production of maltose from starch by salivary amylase. Maltose assay is stoichiometric and was used to estimate the amount of maltose formed. Saliva sample was centrifuged at 3000 rpm for 15 min and the supernatant was diluted with distilled water in a 1:10 ratio (Saliva 0.025 ± 0.225 mL distilled water) and was used as an enzyme source. One percent starch solution was used as substrate and it was prepared by adding 1 g of soluble starch powder to 100 mL of phosphate buffer (0.1N, pH 6.7).

This solution was mildly heated for dissolving starch and then it was cooled to room temperature and filtered. Six test tubes were taken for each case and those tubes will be arranged in "a" and "b" rows of 3 tubes each and were labeled accordingly 1a to 3a and 1b to 3b.1ml of substrate (10 mg/mL) was poured in each tube and 0.250 mL of supernatant, that is, enzyme source was added to each tube. Immediately, after the addition of enzyme source, 0.5 mL stop reagent was added to 1st row in all the tubes, and these were labeled as 0 h samples. Tubes in

Table 3: Group-C (Distilled water)

Sample	Age	Sex	Before induction			After induction			Standard 100% transmittance
			1c	2c	3c	1c	2c	3c	
C1	11	Male	0.049	0.053	0.055	0.062	0.069	0.064	0.059
C2	10	Male	0.048	0.052	0.054	0.063	0.067	0.061	0.058
C3	11	Female	0.053	0.048	0.050	0.065	0.061	0.066	0.057
C4	12	Male	0.051	0.049	0.047	0.067	0.068	0.062	0.057
C5	10	Male	0.054	0.052	0.049	0.062	0.069	0.064	0.058
C6	8	Male	0.048	0.051	0.053	0.063	0.065	0.061	0.059
C7	12	Male	0.048	0.049	0.052	0.064	0.061	0.063	0.056
C8	11	Female	0.047	0.048	0.052	0.061	0.068	0.063	0.057
C9	10	Female	0.054	0.052	0.049	0.067	0.068	0.062	0.059
C10	11	Female	0.047	0.049	0.051	0.065	0.061	0.066	0.057
C11	9	Male	0.048	0.050	0.052	0.063	0.067	0.061	0.057
C12	12	Male	0.047	0.049	0.051	0.062	0.064	0.063	0.056
C13	11	Male	0.049	0.052	0.053	0.061	0.063	0.065	0.057
C14	10	Male	0.047	0.048	0.052	0.067	0.065	0.064	0.057
C15	11	Male	0.050	0.053	0.054	0.064	0.061	0.063	0.058
C16	12	Male	0.049	0.052	0.053	0.065	0.061	0.066	0.058
C17	10	Male	0.048	0.049	0.052	0.062	0.064	0.063	0.056
C18	8	Female	0.051	0.049	0.047	0.063	0.067	0.061	0.056
C19	12	Male	0.047	0.049	0.051	0.066	0.061	0.065	0.057
C20	11	Female	0.050	0.053	0.054	0.065	0.063	0.061	0.058
C21	10	Female	0.054	0.052	0.049	0.064	0.065	0.067	0.059
C22	11	Female	0.049	0.053	0.055	0.068	0.062	0.067	0.059
C23	9	Female	0.047	0.049	0.051	0.064	0.061	0.063	0.056
C24	12	Female	0.050	0.053	0.054	0.067	0.065	0.064	0.059
C25	11	Female	0.049	0.052	0.053	0.063	0.067	0.061	0.058

Table 4: Distribution of study subjects according to gender

Groups	Male	Female	Total
Group A	14	11	25
Group B	14	11	25
Group C	14	11	25
Total	42	33	75

Table 5: Comparative evaluation of mean transmittance rate among Groups A, B, and C

Groups	Mean transmittance rate	
	Mean	SD
Group A	0.0381	0.00100
Group B	0.0532	0.00060
Group C	0.0575	0.00108
Significance "*P*"-value	0.01	

the 2nd row were incubated for 15 min at 37°C, at the end of which, the reaction was stopped by adding 0.5 mL 2N NaOH. 0.5 mL 3,5-dinitrisalisalicylic acid (DNSA) reagent was then added to all the tubes and mixed well.

All the tubes were kept in boiling water for 5 min. After cooling, 10 mL of distilled water was added to all tubes. The intensity of the color developed was measured at 520 nm in a Spectrophotometer (Systronics, India). A mixture containing starch solution, distilled water, and DNSA reagent was taken as a blank and used to set 100% transmittance. A standard graph of maltose was prepared and used as a reference to measure the corresponding amount of maltose formed per mL of saliva (mg/mL).

Statistical analysis

Statistical analysis was done with the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp.). Data comparison was done by applying specific statistical tests to find out the statistical significance of the results. Shapiro–Wilk normality test showed data were not normally distributed and thus non-parametric tests were applied to check the significant difference between two groups.

Results

The present Randomized controlled trial was conducted among 75 Primary school children of age 6–12 years. A total of 75 subjects were randomly allocated to 3 groups (Groups A, B, and C) using a pre-determined computer-generated random allocation plan (Table 4). Permutted Block Randomization method was used. Concealment of treatment was possible by using simple block randomization. This was open labeled trial. The mean age of participants was 10.60 ± 1.19 years. Mean

Transmittance rate in Group A (4% *O. sanctum* gel) was 0.038 ± 0.001 and Mean Transmittance rate in Groups B and C were 0.053 ± 0.0006 and 0.057 ± 0.001, respectively. Kruskal–Wallis non-parametric test for Analysis of Variance was applied to test the difference in transmittance rate between three groups (Table 5). The test revealed that there was a significant difference in the transmittance rate between Groups A, B, and C ($P < 0.05$). Intragroup comparison between Group A and B showed a significant difference in the transmittance rate ($P < 0.05$). The same was found between Groups B and C and Groups A and C ($P < 0.05$).

Discussion

Salivary amylase has been detected in the saliva of many omnivorous animals and a few herbivores. In contrast, obligate carnivores, such as cats, never have salivary amylase. Animals feeding on unripe fruits, seeds, roots, and bulbs, all rich in starch, exhibit higher salivary amylase activity. This supports the notion that a key role of salivary amylase is starch digestion. Human salivary amylase activity is highest among primates.

The presence of amylase in blood results from a very active circulation process, a balance between the rate of entry and the rate of clearance. When the parotid glands, a major source of plasma amylase in rats, are removed, the resting level of salivary-type amylase does not change, and an increase is still found to occur on feeding. Other sources of the enzyme compensate their loss. One of the sources might be the liver, which also produces low amounts of "salivary" amylase and can secrete amylase into the plasma. Moreover, in rats, the circulating levels of amylase (and lipase) are related to their presence in the intestinal lumen. Internalization by enterocytes and progression of the absorbed enzymes along a transcytotic pathway allows them to reach the blood circulation. In this study, amylase was present in higher concentrations in the intestinal mucosa and in blood after feeding, as it has been observed by others. This type of internalization and transfer has not been studied for oral epithelium; it would be interesting to know if it occurs in the oral mucosa.

Salivary amylase is a principal digestive enzyme produced by the salivary glands, which plays an important role in the colonization and metabolism of Streptococcus, leading to the formation of dental plaque and caries in human beings. It has been identified as a constituent of the acquired pellicle and also acts as a receptor for microorganism adhesion on the tooth surface. It has the ability to bind on the bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids, leading to dental caries.[10]

Amylase could serve digestive or non-digestive purposes in the blood. The serum amylase concentration is very low compared (ng/mL) with its concentration in secretory glands (millimolar). Nevertheless, it is sufficient to detect enzymatic activity and because of the high volume of blood, this concentration still represents a substantial amount in the range of 1–10% of tissue levels. It is also possible that the circulation of amylase through the bloodstream provides a means of transporting these proteins from one organ to another one. When labeled exocrine pancreatic proteins were injected into the bloodstream of conscious rats, the majority (approximately 97%) were taken up by a variety of body tissues, particularly kidney, liver, spleen, and lung.[11]

Ramamurthy *et al*., stated that *O. sanctum* has got antimicrobial, anti- inflammatory properties which could be used in the treatment of oral diseases, such as gingivitis and periodontitis.[12] In present study 4% *O. sanctum* gel application effectively reduced the amount of salivary amylase produced than 1% Chlorhexidine digluconate gel and distilled water in 6–12 years of children. The mean age group in the present study is 10.6 years.

In the present study, 1% Chlorhexidine digluconate gel application effectively reduced the amount of salivary amylase produced, but it is less effective than that of 4% *O. sanctum* gel. The use of chlorhexidine for caries prevention has been a controversial topic among dental educators and clinicians. In several reviews, it has been concluded that the most persistent reduction of mutans streptococci has been achieved by chlorhexidine varnishes, followed by gels and, lastly, mouth rinses. Furthermore, the evidence for using different chlorhexidine modes or a combination of chlorhexidine-fluoride therapy for caries prevention has been "suggestive but incomplete." Variable study designs and lack of data in high-risk children and adults support the need to continue conducting randomized, well-controlled clinical trials and to search for a practical, effective mode of antimicrobial treatment that augments the known effect of fluoride treatments.

At present, the only chlorhexidine-containing products marketed in the United States (US) are mouthrinses containing 0.12% chlorhexidine. Based on the available reviews, chlorhexidine rinses have not been highly effective in preventing caries, or at least the clinical data are not convincing. Due to the present lack of long-term clinical evidence for caries prevention and reported side effects, chlorhexidine rinses should not be recommended for caries prevention. Due to the inconclusive literature and sparse clinical data on gels and varnishes, their use for caries prevention should also be studied further to develop evidence-based recommendations for their clinical role in caries prevention. Since dental caries is a disease with a multifactorial etiology, it is currently more appropriate to use other established, evidence-based prevention methods, such as fluoride applications, diet modifications, and good oral hygiene practices.

Autio-Gold in 2018, stated that recent findings indicate that the effect of an antimicrobial agent for reducing the levels of mutans streptococci or plaque reduction may not always correlate with eventual caries reduction. The clinically important outcome is proven reductions in caries. Many advances in the treatment and prevention of dental caries have been introduced over the past century. The use of chlorhexidine in caries prevention has been referred to as a non-surgical management of dental

caries and has represented the modern medical model of caries treatment. However, there is a lack of consensus on evidence-based treatment protocols and controversy regarding the role of chlorhexidine in caries prevention among dental educators and clinicians. There is a need to standardize guidelines to optimize evidence-based non-surgical disease management to provide appropriate care.[13]

Maheshwari *et al*.[14] in 2012 stated that Tulsi is an important symbol of the Hindu religious tradition. Although the word "Tulsi" gives the connotation of the incomparable one, its other name, Vishnupriya means the one that pleases Lord Vishnu. Found in most of the Indian homes and worshipped, its legend has permeated the Indian ethos down the ages. Known in English as Holy Basil and botanically called *O. sanctum*, Tulsi belongs to the plant family Lamiaceae. It has made an important contribution to the field of science from ancient times, as also to modern research due to its large number of medicinal properties. Tulsi has been described as of two types-vanya (wild) and Gramya (grown in homes). In view of these facts, an attempt has been made to review on the various pharmacological activities of OS based on the experimental and clinical studies reported in different literatures.

Different parts of the plant are used in Ayurveda and Siddha Systems of Medicine for prevention and cure of many illnesses and everyday ailments, such as common cold, headache, cough, flu, earache, fever, colic pain, sore throat, bronchitis, asthma, hepatic diseases, malaria fever, as an antidote for snake bite and scorpion sting, flatulence, migraine headaches, fatigue, skin diseases, wound, insomnia, arthritis, digestive disorders, night blindness, diarrhea, and influenza. The leaves are good for nerves and to sharpen memory. Chewing of Tulsi leaves also cures ulcers and infections of the mouth.[15]

The chemical composition of Tulsi is highly complex, containing many nutrients and other biologically active compounds, the proportion of which may vary considerably between strains and even between the plants of the same field. Furthermore, the quantity of many of these constituents is affected by differing growing, harvesting, processing, and storage conditions, which are not yet well understood. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum*, perhaps is largely responsible for the therapeutic potential of Tulsi.[10]

On the other hand, early childhood caries (ECC) is a multifactorial disease associated with several social, psychological, and behavioral mediating factors, this study revealed a significant but inverse correlation between salivary alpha-amylase level and ECC.[16]

In the present study, as the 4% *O. sanctum* gel helps in reducing the salivary amylase formed in 6–12 years of children, it is a promising herbal remedy for reducing the rate of dental caries.

There are at least 50 macromolecular components in saliva, such as immunoglobulin (Ig)A and IgG which play an important role in colonization and metabolism of the oralbacteria[6] perhaps, some of these components may have either synergistic or antagonistic effects on salivary alpha-amylase activity and thus, may strengthen or weaken the link between alpha-amylase and ECC.[17]

The age group of children included in the present study was 6–12 years. The mean age of participants in Groups A, B, and C was 10.60 ± 1.19. Mean Transmittance rate in Group A (4% *O. sanctum* gel) was 0.038 ± 0.001. It states that there is a reduction in the salivary amylase on application of 4% *O. sanctum* gel and the Chlorhexidine gel.

Boehlke *et al*. in 2015, stated that the Salivary amylase enzyme seems to play an important role in caries initiation and oral bioadhesion phenomena,[18] this is in accordance with the present study. Till date, there are no studies depicting the relation between 4% *O. sanctum* gel and salivary amylase, and also the Chlorhexidine gel. This gel effectively reduces the amount of salivary amylase. Alpha-amylase is one of the major components of saliva. It is a calcium-dependent metalloenzyme which can hydrolyze starch to glucose and maltose; consequently, having an important role in binding to bacteria.[17]

Limitations of study

This randomized control study was limited to a small number of pediatric subjects, the age group of participating trial was in the limit 6–12 years, and the 4% *O. sanctum* gel was used only in one setting.

Conclusion

As 4% *O. sanctum* gel is one of the herbal gels having a lot of medicinal properties and no side effects. 4% *O. sanctum* gel tremendously reduces the quantity of salivary amylase.

Salivary amylase is one of the caries-inducing enzymes. Regular application of 4% *O. sanctum* gel in 6–12 years of children will help in reducing the dental caries. Chlorhexidine also helps in reducing the salivary amylase, but not effective as 4% *O. sanctum* gel.

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