Effect of herbal water, ozonated water, water and chlorhexidine mouthrinses on oral health status of children- A randomized controlled trial

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PEDODONTICS AND PREVENTIVE DENTISTRY

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KSR INSTITUTE OF DENTAL SCIENCE AND RESEARCH

DEPARTMENT OF PEDODONTICS AND PREVENTIVE DENTISTRY

CERTIFICATE

This is to certify that the dissertation titled **"Effect of herbal water, ozonated water, water and chlorhexidine mouthrinses on oral health status of children- A randomized controlled trial"** is a bonafide workdone by Dr. JIJO MON, Postgraduate student, during the course of the study for the degree of "Master of Dental Surgery" in Department of Pedodontics and Preventive Dentistry, KSR Institute of Dental Science and Research, Tiruchengode during the period of 2016-2019.

Date: 24/1/19

Place: Tiruchengode

Dr. G.S. Kumar, M.D.S.,

Principal

PRINCIPAL,
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR,
THOKKAVADI POST,
TIRUCHENGODE - 637 215

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Date: 23/1/19

Place: Tiruchengode

Dr. Sharath Asokan, M.D.S., Ph.D

Professor and Head

PROFESSOR AND HOD
DEPARTMENT OF PAEDODONTIA
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH
K.S.R. KALVI NAGAR POST
TIRUCHENGODE - 637 215

DECLARATION BY THE CANDIDATE

TITLE OF DISSERTATION	Effect of herbal water, ozonated water, water and chlorhexidine mouthrinses on oral health status of children- A randomized controlled trial
PLACE OF STUDY	K.S.R Institute of Dental Science and Research
DURATION OF COURSE	3 Years (2016-2019)
NAME OF THE GUIDE	Dr. Sharath Asokan, M.D.S., Ph.D
HEAD OF THE DEPARTMENT	Dr. Sharath Asokan, M.D.S., Ph.D

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from the principal, K.S.R Institute of Dental Science and Research, Tiruchengode. In addition, I declare that no part of this work will be published either in print or electronic without the guide who has been actively involved in this dissertation. The author has the rights reserved for publishing the work solely with prior permission of the principal, K.S.R Institute of Dental Science and Research, Tiruchengode.

Head of the Department **Signature of candidate**

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INTRODUCTION

Dental caries is a major oral health problem in most industrialized countries, affecting 60% to 90% of school children and the vast majority of adults. In recent years, the prevalence of dental caries has steadily declined in most western countries. The most common reason for the decline of dental caries is attributed to the widespread use of fluorides. But, it has shown an increasing trend in developing countries including India.[10]

Research in the field of caries prevention has been focusing on ways for reducing or totally eradicating cariogenic flora from the oral cavity. Studies have shown that caries can be prevented by regular tooth brushing and flossing. However, various studies have shown that by mechanical means alone it is difficult to eliminate Streptococcus mutans (S. mutans) from pits, fissures, and approximal surfaces. For effective caries control, it is better to combine these strategies with the chemoprophylactic agents. These agents are generally delivered as mouth rinses, toothpastes, gels and varnishes.[10]

Mouthrinses have been popularly used as a supplementary oral hygiene aid. There are various mouth rinses that are available to reduce the S. mutans count. Among them, the most common is chlorhexidine (CHX).

The bisbiguanide chlorhexidine has been studied extensively for over 20 years and is currently the most potent chemotherapeutic agent against S. mutans and most effective antiplaque agent. It has bactericidal activity both Gram positive and Gram negative bacteria. CHX is bacteriostatic at low concentrations and bactericidal at high concentrations. It has a broad antimicrobial spectrum thus making it effective against many oral bacterial species. It is more effective against mutans streptococci than against Streptococcus sanguis and lactobacilli.[4]

But the long term use of CHX has few disadvantages such as teeth staining, altered taste sensation, supragingival calculus formation, and desquamation of oral mucosa have restricted its usage in pediatric age group.[22,50,61] Many commercially available CHX mouthrinses possess few adverse effects, and this necessitated the search for alternatives.[4]

One effective and safe alternative to CHX mouthwash are herbal products which have lesser side effects, cheaper and locally available.[54] Natural herbs when used in mouthwashes, have shown significant advantages over the chemical ones.[5,36] For thousands of years, humans have sought to fortify their health and cure various ailments with herbal remedies.[35] Many herbal extracts are commercially available. Few examples are HiOra (Terminalia bellirica, piper betle, salvadora persica, peppermint and cardamom), Herboral (Neem pan, tulsi, mayphal, khadir chhal, pudina, lawang oil), Freshol (Staphysagria, chamomilla, Echinacea, plantago, tulsi and cistus) and Allfresh (Aloe, tea tree, fennel, clove, cardamom, basil, peppermint and glycerine). With growing evidence of the connection between oral health and whole body health, herbal medicines with their naturally occurring active ingredients offer a gentle and durable way for the restoration of health in the least harmful way.[47] The use of natural products is a comprehensive remedy that includes promotive and preventive strategies in the maintenance of health.

Tulsi is an aromatic shrub from the basil family Lamiaceae (*Tribe ocimeae*). Tulsi is one of the best examples of Ayurveda’s holistic lifestyle approach to health. Tulsi tastes hot and bitter and is said to penetrate the deep tissue and dry tissue secretions.[3] Different parts of *Ocimum sanctum* Linn (known as Tulsi), have been used for various medicinal purposes. Tulsi has long been recognized as possessing antioxidant properties, as a COX_2 inhibitor and to provide protection from radiation poisoning and cataracts. A decoction prepared from Tulsi plant is immunomodulatory, hepatoprotective, antipyretic, analgesic and is also used as a diaphoretic in malarial fever. Tulsi has also been used as an important pot herb in traditional practices for a number of ailments and diseases. Its antimicrobial activity has also been tested against plaque and salivary S. mutan and it is found to be effective.[3]

Another alternative for CHX one can think of is the use of ozone. From various literatures, ozone has being discussed in dentistry as a possible alternative antiseptic agent. It is a powerful non-antibiotic biocide that is effective as a gas and can be dissolved in water. Its antimicrobial spectrum includes a wide variety of bacteriae, viruses and fungi. Its mechanism of action is by inducing oxidation of the cell wall and cytoplasmic membranes that leads to the lysis of the microorganism. Ozonated water is found to be very effective in inhibiting bacterial growth in biofilms. Recent investigations have reported that both gaseous and aqueous forms of ozone are equally effective as anti-microbial agents against oral pathogens. The effectiveness of ozone is currently a subject of intensive research in the treatment of oral diseases.[9,13,14,28] Ozonation has been used as one of the methods for treating water in Europe. In France, many municipal drinking water facilities have used ozone as the primary disinfectant since 1906.[40] Even though ozonated water is a powerful antimicrobial agent against bacteria, fungi, protozoa and viruses, less attention has been paid to its antibacterial activity in bacterial biofilms.[55] To consider ozone as a potential antimicrobial agent against oral pathogens, it is important to compare its effectiveness with established agents currently used in dentistry.

Hence, the aim of this study was to evaluate the antibacterial effect of herbal water, ozonated water and chlorhexidine on salivary streptococcus mutans level and to compare their effect on the oral health status of children.

AIMS

AIMS

- To evaluate the antibacterial effect of herbal water, ozonated water and chlorhexidine on salivary streptococcus mutans level
- To compare the effectiveness of herbal water, ozonated water, chlorhexidine and water on the oral health status of children.

REVIEW OF LITERATURE

Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T (2004)[55] examined the effect of ozonated water on oral microorganisms and dental plaque. Almost no microorganisms were detected after being treated with ozonated water (4 mg/l) for 10 seconds. To estimate the ozonated water-treated S. mutans, bacterial cells were stained with Live/Dead BacLight™ Bacterial Viability Kit. Fluorescence microscopic analysis revealed that S. mutans cells were killed instantaneously in ozonated water. The disruption of cells were found when S. mutans was treated with ozonated water under electron microscopy. The number of viable S. mutans remarkably decreased when the experimental dental plaque was exposed to ozonated water. It strongly inhibited the accumulation of experimental dental plaque *in vitro*. Almost no viable bacterial cells were detected, when dental plaque samples from human subjects were exposed to ozonated water *in vitro*. These results suggest that ozonated water could be useful in reducing the infections caused by oral microorganisms in dental plaque.

Haffajee AD, Yaskell T, Socransky SS (2008)[27] investigated mouthrinses antimicrobial effectiveness against predominant oral bacteria, as determined by the minimum inhibitory concentration (MIC). Specifically, they evaluated a herbal mouthrinse (manufactured by Natural Dentist with ingredients - spring water, vegetable glycerin, aloe vera gel, echinacea, goldenseal, calendula, citric acid, grapefruit seed extract, natural flavors, Poloxamer 4 and vitamin B12), an essential oil rinse (Listerine Cool Mint) and a 0.12 % CHX rinse. They assessed the inhibitory effects of the three test agents against 40 oral bacteria at concentrations of 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 micrograms per milliliter. They inoculated plates containing basal medium and the test agents with suspensions of the test species and incubated them anaerobically at 35°C. They interpreted the MIC as the lowest concentration of the agent that completely inhibited the growth of the test species. The herbal mouthrinse was found to inhibit the growth of most of the 40 test species. The herbal mouthrinse exhibited significantly lower MICs for Actinomyces species, periodontal pathogens Eubacterium nodatum, Tannerella forsythia and Prevotella species, as well as the cariogenic pathogen S. mutans when compared with the essential oil mouthrinse. The CHX rinse had the lowest MICs compared with the essential oil rinse and the herbal rinse for all test species examined. Although less potent than CHX, the herbal rinse was more effective than the essential oil rinse in inhibiting the growth of oral bacteria *in vitro*.

Kshitish D, Laxman VK (2010)[39] evaluated the use of ozonated water and 0.2% CHX in the treatment of periodontitis patients aged 20-60 years. A randomized, double-blind, crossover split-mouth design was performed on 16 patients suffering from generalized chronic periodontitis. The study period of 18 days was divided into two time-intervals, baseline (0 days) to 7^{th} day, with a washout period of 4 days followed by a second time interval of 7 days. The use of ozone and CHX irrigation was randomized. The interpretation of clinical and microbial data was done from baseline to 7^{th} day. A higher percentage of plaque index (12%), gingival index (29%) and bleeding index (26%) reduction were observed using ozone irrigation as compared to CHX. The percentile reduction of Actinobacillus actinomycetemcomitans [(Aa) (25%)] using ozone was appreciable as compared to no change in Aa occurrence using CHX. By using ozone and CHX, there was no antibacterial effect on Porphyromonas gingivalis (Pg) and Tannerella forsythensis. The antifungal effect of ozone from baseline (37%) to 7^{th} day (12.5%) was more pronounced during the study period whereas CHX did not demonstrate any antifungal effect. Ozone could be considered as an alternative management strategy due to its powerful ability to inactivate microorganisms.

Agarwal P, Nagesh L, Murlikrishnan (2010)[2] assessed the antimicrobial activity of tulsi (Ocimum sanctum) against S. mutans and also assessed the concentration at which the maximum antimicrobial activity occurred (*invitro*). Preparation of ethanolic extract of tulsi was done by the cold extraction method. The extract was then diluted with an inert solvent, dimethyl formamide, to obtain 15 different concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7% 8%, 9%, 10%). 0.2% CHX was used as a positive control and dimethyl formamide was used as a negative control. The extract and controls were then subjected to microbiological investigation to determine which concentration gave a wider inhibition zone against S. mutans. The zones of inhibition were measured in millimeters using a vernier caliper. At the 4% concentration of tulsi extract, a 22 mm zone of inhibition was obtained. This was the widest zone of inhibition observed among all the 15 different concentrations of tulsi investigated. Tulsi extract demonstrated an antimicrobial property against S. mutans.

Agarwal P, Nagesh L (2011)[3] compared the effect of 0.2% CHX mouthrinse, Listerine mouthrinse and 4% tulsi extract mouth rinse on salivary S. mutans level. Forty-five school children aged 14 to 15 years were divided into three groups. The baseline unstimulated saliva samples were obtained from each group for assessing the S. mutans counts. The study was divided in to three phases, with each phase lasted for 8 days separated by a washout period of 15 days in between them. Groups A, B and C were treated with 0.2% CHX, Listerine and 4% tulsi extract mouth rinses respectively in the phase I. The subjects were instructed to use the assigned mouth rinse twice daily for 1 min for 7 days. On the 8th day, the subjects were instructed to use the mouthrinse only once in the morning. The follow up unstimulated saliva samples were collected 1 hour after the use of the assigned mouth rinse and assessed for salivary S. mutans counts. After phase I, mouth rinses were crossed over in phase II and III. All the three mouthrinses had individually shown a statistically significant reduction in the salivary S. mutans counts. There was no difference between the three mouth rinses. Tulsi was found to be as effective as CHX and Listerine in reducing the salivary S. mutans levels.

Malhotra R, Grover V, Kapoor A, Saxena D (2011)[48] compared the efficacy of a commercially available herbal mouthrinse (Herboral - neem pan, tulsi, mayphal, khadir chhal, pudina, lawang oil) CHX which the gold standard anti-plaque agent. A randomized, two-group, parallel study was carried out on 50 subjects aged 18 to 35 years (23 males and 27 females). At baseline, all participants received a professional prophylaxis and were randomly assigned to the test (herbal mouthrinse) and control (CHX) group. On the following three days, all subjects rinsed with 10 ml of the allocated mouthrinse twice daily for 1 minute. They were asked to refrain from the use of any other oral hygiene measures during the study period. At the end of the experimental period, plaque was assessed at six sites per tooth using the Quigley and Hein index and a questionnaire was filled by all subjects. CHX inhibited plaque growth significantly more than the herbal mouthrinse. The results of the questionnaire showed that Herboral was preferred by patients for its taste, taste duration (aftertaste) and its convenience of use. However, CHX was considered to be more effective in reducing plaque as compared to Herboral. Herbal mouthrinse was found to be a potent plaque inhibitor, though less effective than CHX. However, herbal mouthrinses can serve as a good alternative for patients with special needs as in the case of diabetics or xerostomias due to its lesser side effects.

Mehta S, Pesapathy S, Joseph M, Tiwari PK, Chawla S (2013)[50] compared the efficacy of a commercially available herbal mouthwash (Freshol - staphysagria, chamomilla, echinacea, plantago, ocium (tulsi) and cistus with CHX on plaque status, gingival status, and salivary S. mutans count. A total sample of 55 children, aged 8-14 years, were randomly divided into two groups. Group A (35) and Group B (20) were given 10 ml of test mouthwash Freshol and CHX respectively during phases 1 and 3 of the clinical trial, which was of 10 days each. Phase 2 was a 14 days washout period during which no mouthwash was given. Freshol was found to be better than CHX in reducing the salivary S. mutans count and equieffective to CHX in altering plaque and gingival scores. They concluded that herbal alternatives can prove to be an effective and safe alternative to conventional modes of treatment.

Biswas G, Anup N, Acharya S, Kumawat H, Vishnani P, Tambi S (2014)[15] evaluated the short-term clinical effects of a herbal mouthwash in the reduction of plaque and gingival inflammation in subjects with gingivitis on 50 subjects aged 20 to 40 years diagnosed with chronic generalized gingivitis. They were randomly divided into two groups: Group 1 - CHX mouthwash, Group 2 - herbal mouthwash (Herboral). Clinical evaluation was undertaken using the gingival index, the plaque index and bleeding on probing at baseline, 1 week, 2 weeks and 4 weeks. Both CHX and herbal mouthwash showed a significant reduction in plaque and gingival index scores from baseline to 1st week, 2nd week and at 4th week. However, the decrease in plaque (Turskey et al, 1970) and gingival (Loe and Sillness, 1963) index scores were better in CHX group than herbal mouthwash. Both the mouthwashes were equally effective in reducing bleeding on probing. Unlike CHX mouthwash, herbal mouthwash was not associated with any discoloration of teeth or unpleasant taste and was effective in reducing plaque accumulation and gingival inflammation.

Kaur RK, Singh MP, Chopra R, Bhatia A (2014)[35] evaluated the effectiveness of three herbal mouthwashes in the treatment of chronic gingivitis on 40 patients aged 18 to 30 years with chronic marginal gingivitis who were randomly divided into 4 groups of 10 patients each. Group A (control): rinsed with normal water, Group B: Neem mouthwash, Group C: Allfresh mouthwash (Aloe vera, tea tree, fennel, clove, cardamom, basil, peppermint and glycerine) and Group D: Hiora mouthwash (Terminalia bellirica, piper betle, salvadora persica, peppermint and cardamom) twice daily for 21 days. Clinical parameters such as Oral Hygiene Index-Simplified (OHI-S), Gingival Index (Loe and Silness, 1963) and Approximal Plaque Index (API), were assessed at baseline, 7 days and 21 days. At the end of study (21st day), all groups showed significant improvement in all clinical parameters while group D (Hiora) showed highly significant improvement in oral hygiene and gingival status as compared to all other groups. Herbal mouthrinse was found to be a potent plaque inhibitor when compared to the control group and could serve as an adjunct in treating chronic gingivitis.

Mishra R, Tandon S, Rathore M, Banerjee M (2014)[51] evaluated the antimicrobial and plaque inhibitory potential of herbal (Herboral) and probiotic rinses [probiotic mint tablet (Evora Plustm, Florida, USA, composed of Probiora 3) in 5 ml water] against Streptococcus viridans (S. viridans) with 0.2% CHX. A randomized clinical trial was conducted on 60 subjects aged 6 to 14 years and were divided into three groups comprising of 20 subjects in each group. Three oral rinses were administered twice daily for a period of a week. Estimation of plaque scores using plaque index (Silness and Loe, 1964) and S. viridans counts was done before and after intervention. Based on their observations herbal rinse was found to be as effective as 0.2% CHX in reducing S. viridans counts and plaque accumulation after 1 week of intervention, whereas probiotic rinse was found to be least effective.

Jaidka S, Somani R, Bajaj N, Jaidka R, Sharma S, Singh A (2015)[30] evaluated the efficacy of various mouthwashes on plaque accumulation, gingival inflammation and quantitatively with oral microflora on 40 children aged 7 to 14 years. They were randomly divided into 4 groups; Group A, Group B, Group C and Group D who rinsed with water, CHX mouthwash, xylitol mouthwash and herbal mouthwash (Hiora) respectively. Plaque index (Silness and Loe, 1964) and gingival index (Loe and Silness, 1963) were recorded at baseline and after 30 days. Salivary samples were collected for evaluation of total microbial colony count at baseline and after 30 days. Maximum reduction in plaque, gingival scores and microbial count after 30 days were shown in herbal group followed by xylitol group while minimum reduction was shown in CHX group.

Anumula L, Kumar KVS, Krishna CM, Lakshmi KS (2017)[8] evaluated the antibacterial efficacy of freshly prepared ozonated water, in proposing it as an alternative mouth rinse on mutan streptococcus (MS) in comparison to CHX. Subjects with high caries incidence and MS counts more than 10^5 colony forming unit (CFU) were selected and randomly divided into two groups of 23 subjects each. They were advised to use the respective mouth rinses consecutively for 14 days. Stimulated salivary samples were collected from the subjects on the first day, 7th and 14th day to analyze the changes in MS count during the course of use of oral rinses. Freshly prepared ozonated water showed a statistically significant reduction in MS count after an interval of 7 days and 14 days, when compared to CHX. Ozonated water when consecutively used as a mouth rinse resulted in a significant reduction of MS counts and can be used as an alternative for CHX.

Ahmed SI, Sekhara Reddy VC, Sudhir KM, Kumar RK, Srinivasulu G (2017)[4] evaluated effect of 4 % tulsi extract (obtained from courtyards that was dried and powdered finely. From that 200 g of tulsi was dissolved in 1 L of ethanol, 12 g of residue (extract) was obtained) and honey mouthrinses (10g was added in 100 ml distilled water) on S. mutans count in comparison with 0.2% of CHX. A randomized controlled trial was done on 45 individuals aged 15 years dividing them into 15 each for Group A (0.2% of CHX mouthrinse), Group B (honey mouthrinse) and Group C (tulsi extract mouthrinse). Oral hygiene status was assessed using OHI-S index and evaluation of S. mutans was done before and after using the mouthrinse in which the samples were streaked on mitis salivarious-bacitracin agar medium. There was a reduction in S. mutans in all the three groups with 0.2% CHX showing a maximum reduction. There was significant difference between Group A (0.2% CHX mouthrinse) and Group B (honey mouthrinse) and between Group A (0.2% CHX mouthrinse) and Group C (tulsi extract mouthrinse). They concluded that herbal mouthrinses containing tulsi and honey, though as not as effective as CHX in its antimicrobial property, has its own value and it could be effectively used in areas where people could not access to CHX.

Deshmukh MA, Dodamani AS, Karibasappa G, Khairnar MR, Naik RG, Jadhav HC (2017)[20] compared the efficacy of probiotic, herbal (Hiora) and CHX mouthwashes on gingival health of 45 subjects in the age group of 18 to 21 years. They received complete supragingival scaling at baseline and their OHI-S, Plaque Index (Silness and Loe, 1964) and Gingival Index (Loe and Silness, 1963) were recorded. Subjects were randomly divided into three groups with 15 in each and were randomly intervened with three different mouthwashes- Hiora mouthwash, CHX mouthwash and Probiotic mouthwash. Variables were recorded again on the 7th and 14th day after use of mouthwashes. There was no significant difference in the efficacy of CHX, Hiora and probiotic mouthwashes on plaque accumulation, gingival health and oral hygiene status. Herbal and probiotic mouthwashes could be effective alternatives to CHX with minimal side effects.

Somaraj V, Shenoy RP, Panchmal GS, Kumar V, Jodalli PS, and Sonde L (2017)[70] assessed and compared the effect of herbal and fluoride mouth rinses on Streptococcus mutans count (from the samples of unstimulated saliva (3 ml) that were collected at baseline, 6th month and 12th month), glucan synthesis by S. mutans (done by phenol sulphuric method at baseline, 6th month and 12th month) and dental caries (International Caries Detection and Assessment System- ICDAS for detection of caries on coronal tooth surfaces). It was a parallel group placebo controlled randomized trial conducted among 240 school children aged 12 to 15 years who were randomly divided into Group I (0.2% sodium fluoride mouthrinse), Group II (herbal mouthrinse- Freshol), and Group III (placebo - mint flavor in distilled water). All had received 10 ml of respective mouth rinses every fortnight for a period of one year. Intergroup and intragroup comparison were done for S. mutans count and glucan synthesis at baseline, 6th month and 12th month. S. mutans count showed a statistically significant difference between sodium fluoride group and placebo group and also between herbal group and placebo group. Glucan concentration levels showed a statistically significant difference between herbal group and placebo group at 12th month. The study showed that both herbal and fluoride mouth rinses when used fortnightly were equally effective and could be recommended for use in school-based health education program to control dental caries.

Parkar SM, Shah K, Darjee N, Sharma A (2017)[58] compared the efficacy of ozonated water and CHX mouth rinse against plaque and gingivitis. A randomized, double-blind clinical trial was conducted on 54 patients aged 18 to 30 years suffering from generalized chronic gingivitis. They were randomly assigned into 3 groups with 18 subjects in each- group 1: ozonated water, group 2- 0.2% CHX, group 3- water (placebo). The trial period of 15 days was divided into three time-intervals, baseline (0 day), 7th day and 15th day. The clinical parameters- plaque and gingival status were assessed using Turkesky-Gilmore-Glickman Modification of Quigley – Hein Plaque Index and Loe and Silness Gingival Index. The analysis was performed on 47 patients (attrition rate- 17%) at the end of 15th day. There was a highly significant difference when the mean plaque and gingival scores were compared between three different intervals of time. There was a significant difference when the mean plaque scores for CHX and water were compared. There was a significant difference when the mean gingival scores for ozonated water and CHX were compared, with CHX showing slightly lower mean score. There was a highly significant difference when the mean difference scores for ozonated water versus water and chlorhexidine versus water were compared. It was found that CHX mouthwash was more effective in reducing plaque as compared to ozonated water. However, ozonated water and CHX mouth rinse were equally effective in reducing gingivitis (Limitation of the study- as ozonated water was applied only for 1 min. It is known that activity of CHX is prolonged by its substantivity but same may not be true for ozone).

Yadav S, Kumar S, Srivastava P, Gupta KK, Gupta J, Khan YS (2018)[79] evaluated and compared the efficacy of three different mouthwashes containing CHX, essential oils and herbal extracts by using them as preprocedural rinsing agent in reducing the bacterial load of the aerosol produced by ultrasonic scaler on 40 subjects. They were randomly divided into four groups on the basis of agents used for preprocedural mouthrinsing - Group I: distilled water (control), Group II: 0.2% CHX, Group III: herbal extracts (Hiora) and Group IV: essential oils (Listerine). The aerosols were collected on three previously prepared and sterilized blood agar plates at three different positions in the operatory. After incubating the plates for 48 hours the colony forming units were counted. At all locations, the mean CFU was highest in distilled water group followed by herbal group, essential oils and CHX group. CHX was found to be most effective preprocedural mouthwash in reducing the bacterial load in the aerosol produced during ultrasonic scaling followed by essential oil and herbal mouthwash respectively.

METHODOLOGY

Armamentarium

Materials for screening

- Diagnostic instrument set – consisting of a mouth mirror, explorer and tweezer in kidney tray
- Sterile gloves
- Disposable mouth mask

Materials for testing the antimicrobial activity of the groups against *S. mutans* and microbial colony count

- Brain heart infusion agar [(BHI); Himedia]
- Muller Hinton agar (Himedia)
- Ruler
- Conical flask 250 ml
- Eppendorf tubes (1ml)
- Cotton swab
- Cork borer
- 1000 µl pipette
- 10 µl pipette
- 50 µl pipette
- Disposable micro tips
- Petri plates
- Glass rod
- Ethanol

- Plate rotator
- Laminar flow chamber (Sri Ganabathi Enterprises)
- Incubator (Narang scientific works PVT.)
- Automatic colony counter (Deep Vision)

Methods

A parallel multi-arm randomized controlled trial was conducted from the Department of Pediatric and Preventive Dentistry, K.S.R. Institute of Dental Science and Research (KSRIDSR), Tiruchengode, Tamil Nadu. The study design and protocol was analyzed and approved by the Institutional Review Board and Institutional Ethics Committee of KSRIDSR. The list of schools were obtained from the assistant elementary educational officer (AEEO). One school was randomly selected by lottery method. The study was conducted in a Government aided school, M.D.V. Government School in Tiruchengode. Permission of the AEEO was obtained to carry out the study. The purpose of the study was explained to the school authorities and their approval was obtained. A total of 206 children aged 10 to 12 years were screened.

Inclusion criteria

- School children aged 10 to 12 years
- DMFT/deft score ≤ 3

Exclusion criteria

- Children with systemic diseases
- Children with history of antibiotics usage in past 1 month
- Children undergoing orthodontic treatment

Based on these criteria a study population of 100 were selected. A written consent in mother tongue (Tamil) was also obtained from the parents of the children who participated in the study. The study was conducted between July and August 2018.

Sample size was estimated to be 20 per group based on previous similar studies. Anticipating an attrition of 25 %, 5 samples were added to each group. The power of test was set at 80% with p value < 0.05 considered significant.

Randomization

Hundred children were randomly divided into 4 groups with 25 children in each group using table of random numbers. Sequence generation and allocation was done by two dental residents who weren't involved in the study (allocation ratio 1:1:1:1). The recruited children were allocated to

HW: Herbal water

OW: Ozonated water

W: Water

CHX: Chlorhexidine

Blinding

In this study, the primary investigator was not blinded as the mouthrinses were prepared by him. He was aware of the odor and color of the mouthrinses prepared. The study population and the secondary investigators i.e. the lab technicians who did the microbiological assessment and the statistician were blinded.

Invitro study

Culture maintenance

Standard S. mutans (MTCC-890) was procured from, Microbial type culture collection (MTCC), Chandigarh. They were revived using BHI broth and were maintained actively in BHI agar for further studies

Dosage optimization of herbal water

Commercial herbal mouth wash reported to have antimicrobial activity was optimized for dosage determination using well diffusion method for intervention purpose. Muller Hinton agar was prepared and lawn with overnight culture of S. mutans (MTCC-890). Well was created at the center of the petriplate with 6 mm sterile cork borer. Each of the well was loaded with 50µl of herbal mouthwash prepared in different concentration (2 drops, 6 drops, 8 drops and 10 drops in 10 ml water). The plates were incubated at 38±2ºC room temperature (RT) for 48 hours. Triplicates were maintained for each concentrations.

Screening of antimicrobial activity

Screening of antimicrobial efficacy of herbal water, ozonated water, water and chlorohexidine was determined against S. mutans (MTCC-890) before the conduction of intervention. As described earlier Muller Hinton agar plate's lawn with overnight culture of S. mutans (MTCC-890) was loaded with 50µl of herbal water, ozonated water, water and chlorhexidine in the well created at the center of the plate. The plates were incubated at 38 ± 2ºC RT for 48 hours. Triplicates were maintained for all the three samples.

Invivo study

Measurement of oral health status

Oral hygiene index - simplified

Debris and calculus scores were recorded using Oral Hygiene Index- Simplified (OHI-S) index as described by John C. Greene and Jack R. Vermillion (1964) by using sterile mouth mirrors and explorers under natural light at school premise by the blinded secondary investigator.

Oral prophylaxis was performed for all children, 5 days prior to the intervention. Children were instructed to carry on with their routine oral hygiene practices.

Salivary Streptococcus mutans colony count

All children were instructed not to eat or drink 30 minutes prior to the saliva sample collection. Unstimulated salivary samples were collected at mid-morning using sterile uricups by the primary investigator. S. mutans count was evaluated using spread plate method. BHI agar medium was used for culturing S. mutans. The medium was autoclaved at 121°C, 15 lbs pressure for 15 minutes and then it was cooled at 55°C. Approximately 15 ml of molten cooled agar was then poured into the petri dish. Saliva samples of about 1 ml were serially diluted 5 times up to 10^5 dilution. 1 ml of diluted saliva was added in the center of sterile petri dish using a sterile pipette. After the solidification of the agar, 1 ml of the diluted saliva was plated by using a bent glass rod on the agar media and the plates were inverted and incubated at 37°C for 48 to 72 hours. The colonies were identified by colony morphological characteristics and confirmed using gram staining (minute spherical, translucent, pinpoint colonies that are gram positive cocci arranged in short chains or pairs). All the counts were established for 10^5 dilution. The results were tabulated and analyzed statistically.

Total microflora (CFU)

All the saliva samples were serially diluted using sterile water and the total microbial count was determined as CFU/ml by plating the diluted samples in BHI agar as given below.

$$CFU/ml = \frac{\text{Colonies in one quadrant of the plate x 4 x dilution factor } (10^5)}{\text{Volume of the sample (0.1ml)}}$$

All the counts were established for 10^5 dilution. The results were tabulated and analyzed statistically.

Preparation of herbal water (HW)

Herbal water was freshly prepared every day by adding 10 drops of Deltas Pancha tulasi drops (Manufactured by Delta Pharma, Mumbai, India) mixed in 10 ml of water and were given to the children in a container.

Preparation of ozonated water (OW)

Ozonated water was freshly prepared every day by ozonation of water by using ozone generator (Company- Ozone Engineers) and were given to the children in a container immediately.

Preparation of water (W)

Ten milliliter of normal water were given every day to the children in a container.

Preparation of chlorhexidine mouthwash (CHX)

Five milliliter of 0.12% CHX mouthwash (Chlorhex ICPA health products Ltd., India) was diluted in 5 ml of water every day and were given to the children in a container.

Intervention

All children were asked to rinse each day with the respective mouthrinses for 1 minute continuously in the morning for 15 days during school hours under the presence of the primary investigator. They were asked to refrain from eating 2 hours prior to the start of rinsing. OHI-S index was taken after 15 days and after 30 days. Saliva samples were collected at 3 time periods- baseline (T_1), after 15 days (T_2) and after 30 days (T_3) for the determination of S.mutans count and total microflora (CFU). Duration of the study was one month.

Statistical analysis

The statistical analysis was done using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. The statistical significance was set at $p \leq 0.05$. Intragroup comparison was done using Friedman test followed by post hoc analysis using Wilcoxon signed rank test. Intergroup comparison was done by One way ANOVA (Kruskall Wallis) test followed by post hoc analysis using Mann Whitney U test.

Flow chart methodology

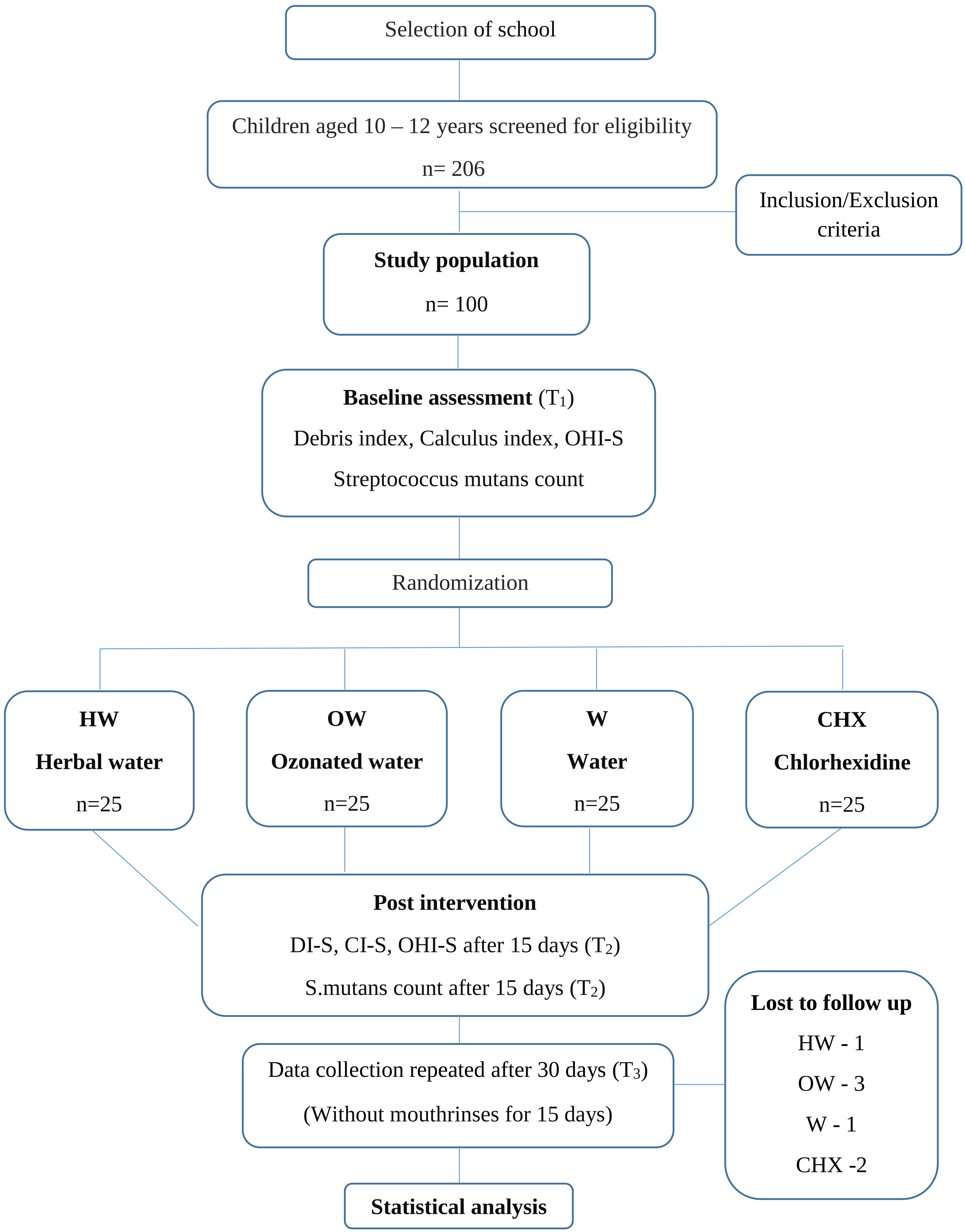


Figure 1. Armamentarium

Figure 2. Herbal product used for preparation of herbal water

(Deltas Pancha Tulasi drops)

Figure 3. Ozone generator for preparation of ozonated water

Figure 4. Screening children

Figure 5. Oral prophylaxis of the selected sample population

Figure 6. Measurement of OHI-S

Figure 7. Saliva sample collection

Figure 8. Saliva samples

Figure 9. Armamentarium for selective media

Figure 10. Preparation of selective media

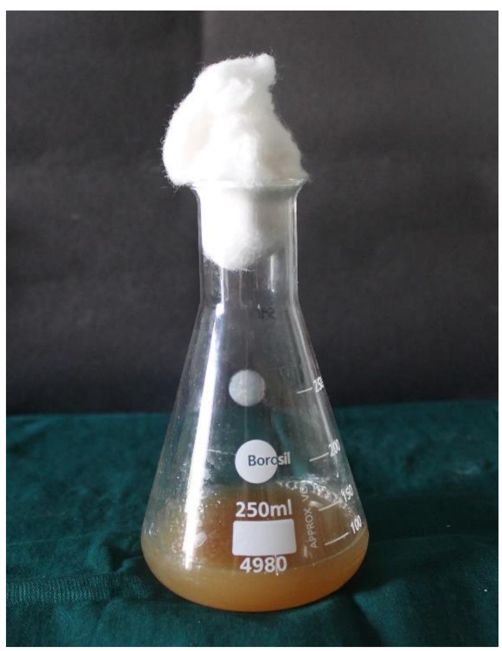


Figure 11. Prepared agar plate

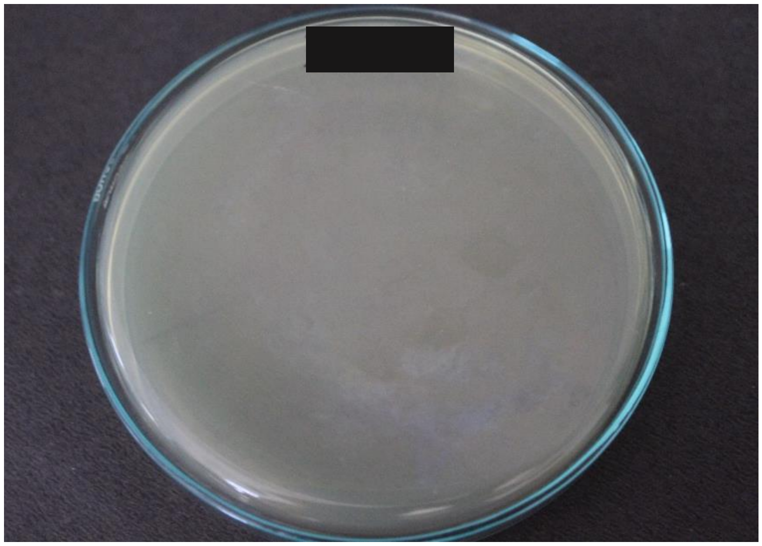


Figure 12. Distribution of mouthrinses

Figure 13. Dosage optimization of herbal water group showing antimicrobial activity against Streptococcus mutans

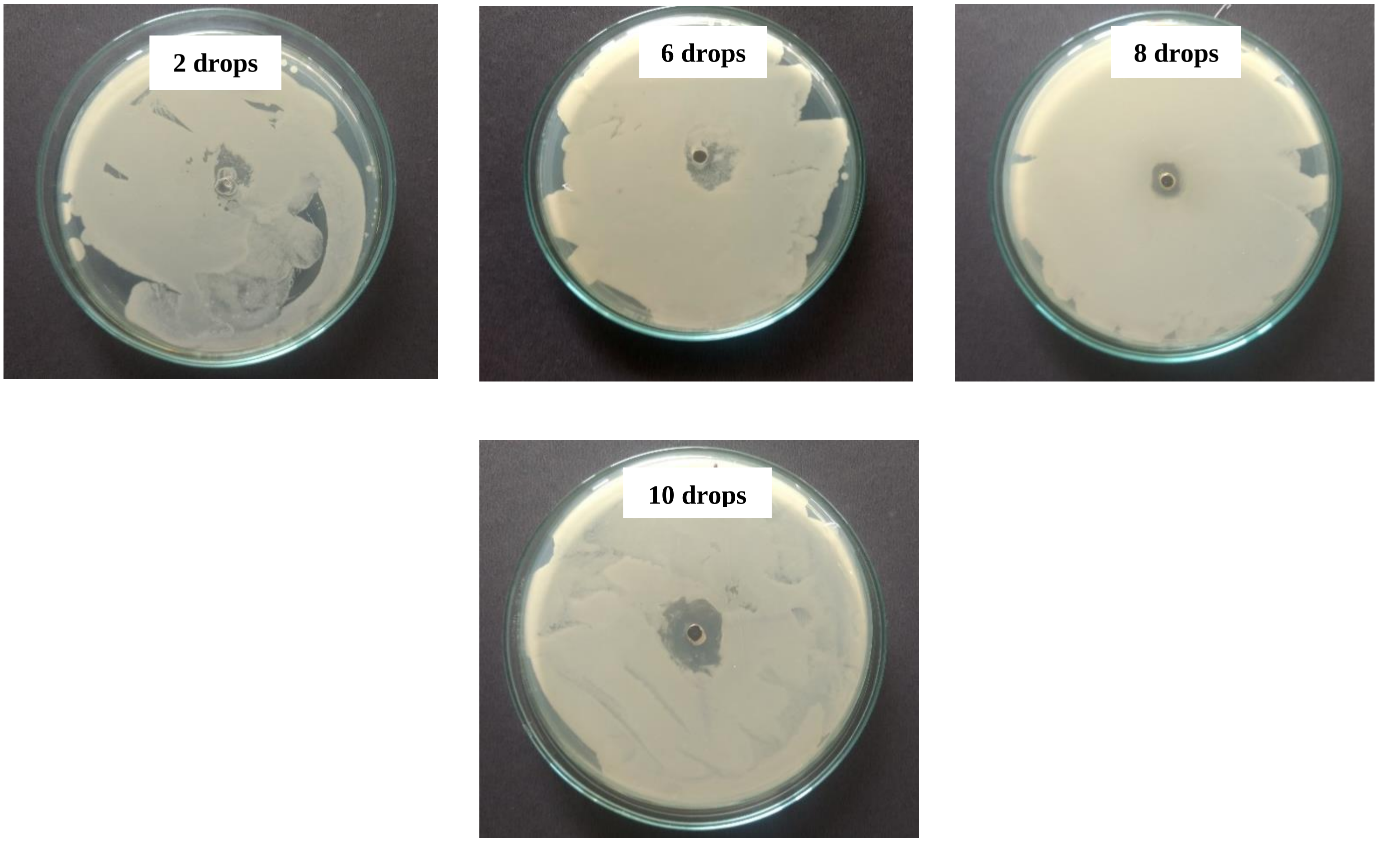


Figure 14. Antibacterial activity of the study groups (zone of inhibition) seen against Streptococcus mutans

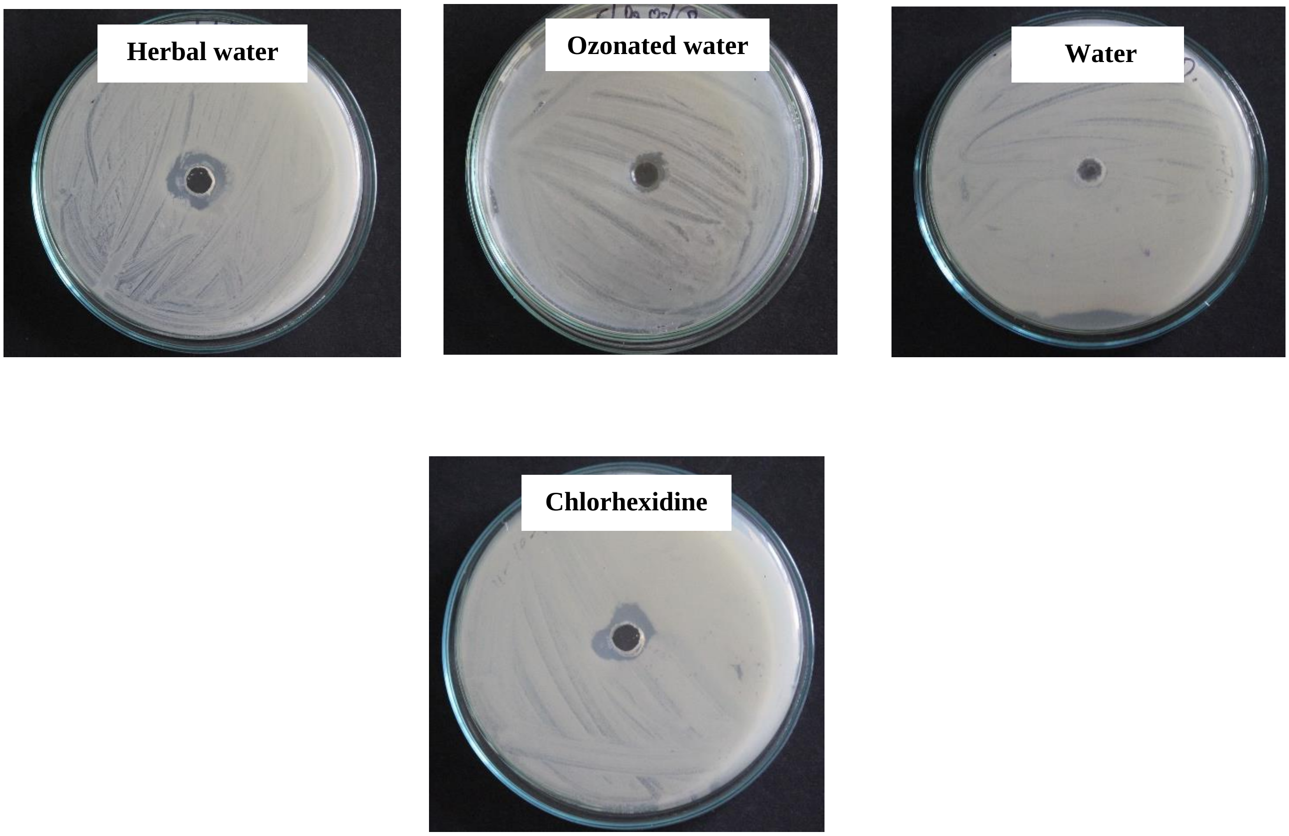


Figure 15. BHI agar showing growth of Streptococcus mutans

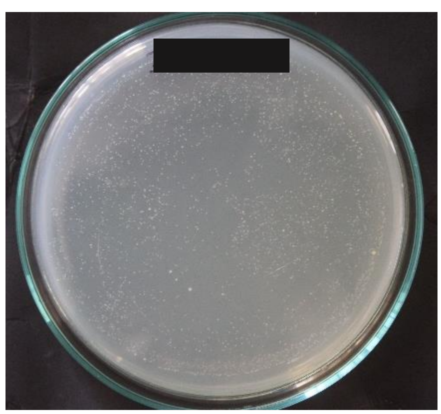
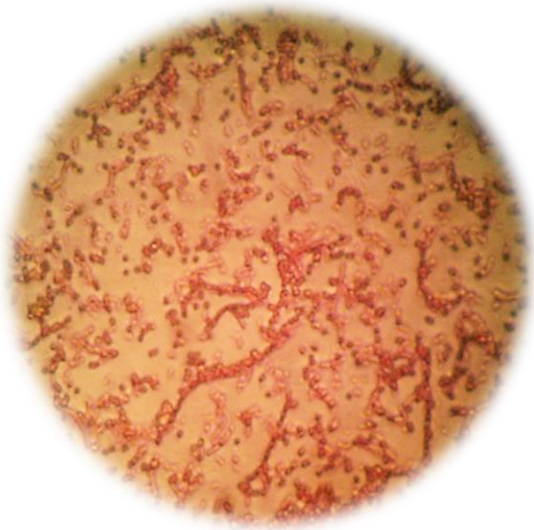


Figure 16. Gram staining of Streptococcus mutans



RESULTS

Table 1. Zone of inhibition against S. mutans

Groups	Zone of inhibition (mm) Mean ± SD
HW	30.33 ± 0.88
OW	16 ± 0.58
W	No zone observed
CHX	28.33 ± 0.33

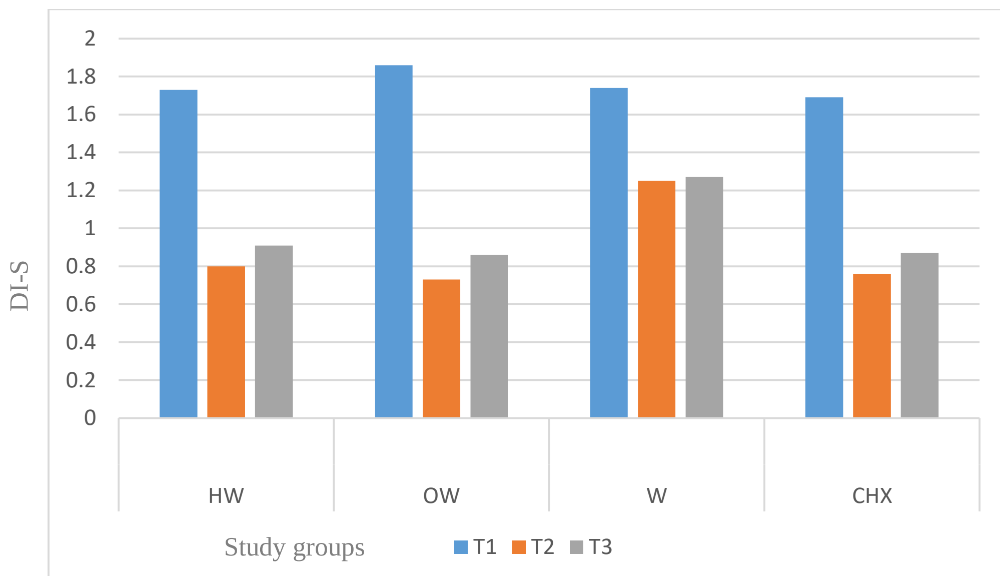
Table 1 shows zone of inhibition in the four groups against S. mutans. HW, OW and CHX groups showed zone of inhibition. HW showed the maximum zone of inhibition among the groups studied.

Table 2. Debris index-simplified score in the 4 groups at 3 time periods

Time	HW Mean ± SD	OW Mean ± SD	W Mean ± SD	CHX Mean ± SD
T_1	1.73 ± 0.48	1.86 ± 0.39	1.74 ± 0.62	1.69 ± 0.39
T_2	0.80 ± 0.37	0.73 ± 0.40	1.25 ± 0.39	0.76 ± 0.37
T_3	0.91 ± 0.31	0.86 ± 0.28	1.27 ± 0.39	0.87 ± 0.23

Table 2 shows the mean distribution of DI-S score in all the groups. All the groups showed reduction in DI-S score at T_2 period. All the groups showed a slight increase in DI-S score at T_3 period compared to T_2. OW showed minimum DI-S score among all the groups at T_2 and T_3.

Graph 1. Debris index-simplified score in the 4 groups at 3 time periods



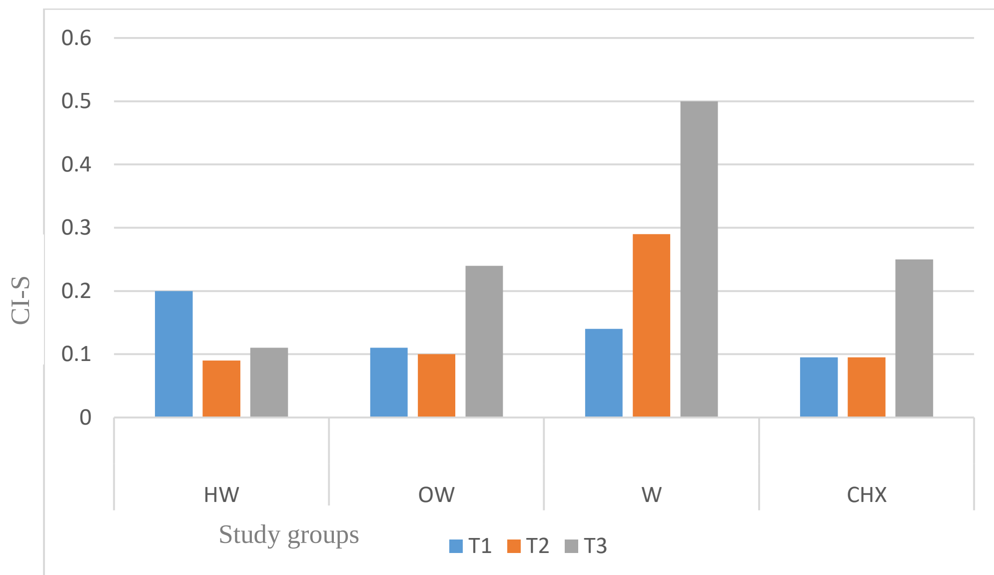
Graph 1 illustrates DI-S scores in the 4 groups at T_1, T_2 and T_3 period.

Table 3. Calculus index-simplified score in the 4 groups at 3 time periods

Time	HW Mean ± SD	OW Mean ± SD	W Mean ± SD	CHX Mean ± SD
T_1	0.20 ± 0.31	0.11 ± 0.11	0.14 ± 0.21	0.09 ± 0.16
T_2	0.09 ± 0.17	0.10 ± 0.14	0.29 ± 0.33	0.09 ± 0.14
T_3	0.11 ± 0.18	0.24 ± 0.24	0.50 ± 0.32	0.25 ± 0.24

Table 3 shows the mean distribution of CI-S scores in all the groups. HW has minimum CI-S score at T_2 period. W showed maximum increase in CI-S score at T_2 period. All the groups showed an increase in CI-S score at T_3 period when compared to T_2. HW has the minimum CI-S score at T_3 period when compared with other groups. All the groups except HW showed an increase in CI-S score at T_3 when compared with T_1.

Graph 2. Calculus index-simplified score in the 4 groups at 3 time periods



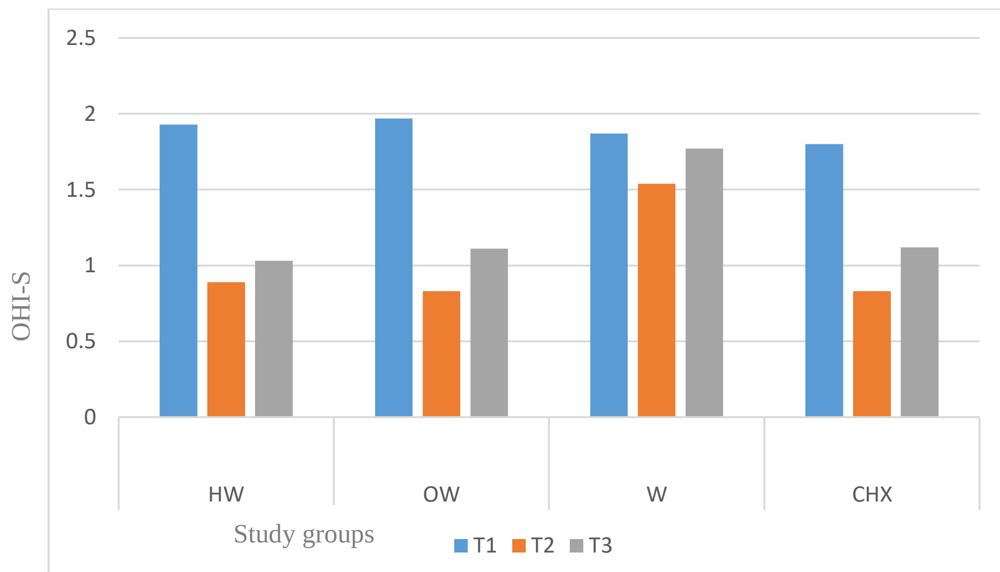
Graph 2 illustrates CI-S score in all groups at T_1, T_2 and T_3 period.

Table 4. Oral hygiene index-simplified score in the 4 groups at 3 time periods

Time	HW Mean ± SD	OW Mean ± SD	W Mean ± SD	CHX Mean ± SD
T_1	1.93 ± 0.7	1.97 ± 0.43	1.87 ± 0.76	1.80 ± 0.50
T_2	0.89 ± 0.43	0.83 ± 0.44	1.54 ± 0.61	0.83 ± 0.49
T_3	1.03 ± 0.36	1.11 ± 0.43	1.77 ± 0.63	1.12 ± 0.39

Table 4 shows the mean distribution of OHI-S score in all the groups. All the groups showed reduction in OHI-S score at T_2 period. OW showed maximum reduction in OHI-S score whereas W showed least reduction in OHI-S score at T_2 period. In all the groups there was an increase in OHI-S score at T_3 period when compared with T_2. HW showed least increase in OHI-S score at T_3 period when compared to T_2 period among others.

Graph 3. Oral hygiene index-simplified score in all the 4 groups at 3 time periods



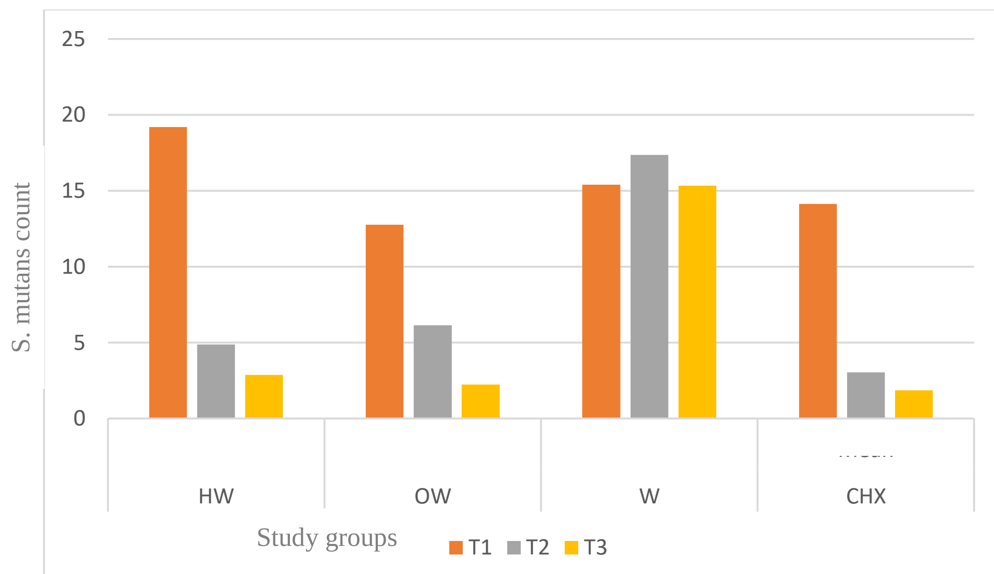
Graph 3 illustrates OHI-S score in all the groups at T_1, T_2 and T_3 period.

Table 5. Salivary streptococcus mutans count in the 4 groups at 3 time periods

Time	HW Mean ± SD (x 10^5 CFU/ml)	OW Mean ± SD (x 10^5 CFU/ml)	Water Mean ± SD (x 10^5 CFU/ml)	CHX Mean ± SD (x 10^5 CFU/ml)
T_1	19.20 ± 13.75	12.76 ± 9.7	15.41 ± 11.89	14.13 ± 12.88
T_2	4.87 ± 5	6.14 ± 4	17.37 ± 17.25	3.043 ± 1.91
T_3	2.87 ± 2	2.23 ± 0.76	15.33 ± 3	1.86 ± 1.74

Table 5 shows salivary S. mutans count in all the groups. HW, OW and CHX showed reduction in S. mutans count at T_2 period. CHX showed maximum reduction in S. mutans count at T_2 period. All the groups showed reduction in S. mutans count at T_3 period when compared to T_1 and T_2. CHX group showed maximum reduction in S. mutans count at T_3 period.

Graph 4. Streptococcus mutans count (x 10^5 /ml) in the 4 groups at 3 time periods



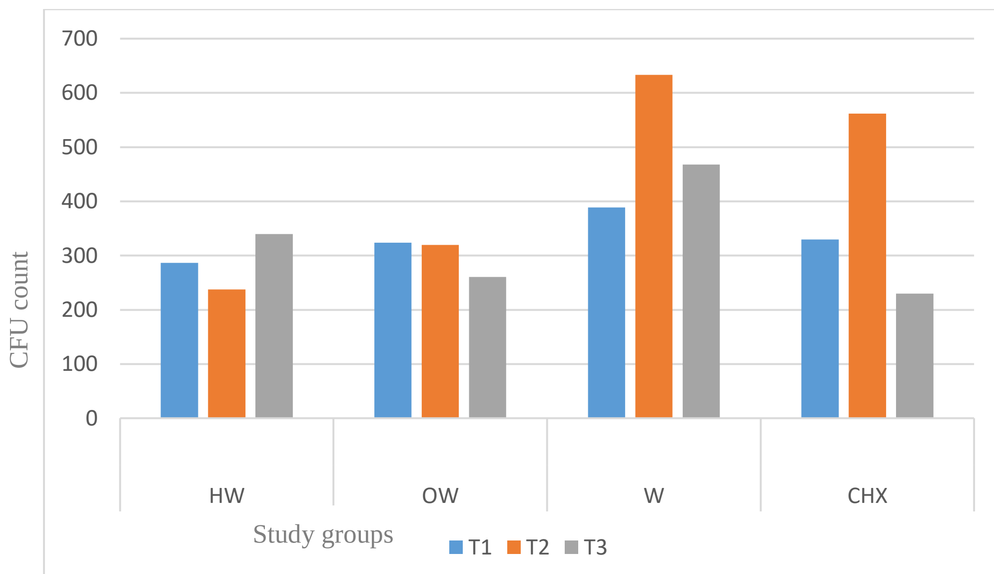
Graph 4 illustrates salivary S. mutans count in all groups at T_1, T_2 and T_3 period.

Table 6. Total microbial count in the 4 groups at 3 time periods

Time	HW Mean ± SD (x 10^5 CFU /ml)	OW Mean ± SD (x 10^5 CFU/ml)	W Mean ± SD (x 10^5 CFU/ml)	CHX Mean ± SD (x 10^5 CFU /ml)
T_1	286.5 ± 219.4	323.81 ± 192.5	388.96 ± 215.2	329.66 ± 238.4
T_2	237.33 ± 109.8	319.40 ± 192.1	633.12 ± 305.3	562 ± 246.3
T_3	339.58 ± 201.4	260.54 ± 183.2	467.84 ± 274.9	229.66 ± 156.3

Table 6 shows CFU count in all the groups. HW and OW showed reduction whereas CHX and W showed increase in CFU count at T_2 period. CHX showed maximum reduction in CFU count at T_3 period.

Graph 5. Colony forming unit bacterial count (CFU) in the 4 groups at 3 time periods



Graph 5 illustrates CFU count in all groups at T_1, T_2 and T_3 period.

Table 7. Intragroup comparison of debris index-simplified score at different time intervals

	T_1 Mean ± SD	T_2 Mean ± SD	T_3 Mean ± SD	p value*	T_1 vs T_2	T_1 vs T_3	T_2 vs T_3
HW	1.73 ± 0.48	0.80 ± 0.37	0.91 ± 0.31	**<0.001**	**0.001**	**0.001**	
OW	1.86 ± 0.39	0.73 ± 0.40	0.86 ±0.28	**<0.001**	**0.001**	**0.001**	
W	1.74 ± 0.62	1.25 ± 0.39	1.27 ± 0.39	**<0.001**	**0.001**	**0.001**	
CHX	1.69 ± 0.39	0.76 ± 0.37	0.87 ± 0.23	**<0.001**	**0.001**	**0.001**	

*Friedman test **post hoc Wilcoxon signed rank test

Table 7 shows intragroup comparison of the DI-S score at different time intervals studied. There was a significant reduction in DI-S score between T_1 and T_2 intervals and between T_1 and T_3 intervals in all the 4 groups. Maximum reduction in DI-S scores for all groups was seen between T_1 and T_2 intervals.

Table 8. Intragroup comparison of calculus index-simplified score at different time intervals

	T_1 Mean ± SD	T_2 Mean ± SD	T_3 Mean ± SD	p value*	T_1 vs T_2	T_1 vs T_3	T_2 vs T_3
HW	0.20 ± 0.31	0.09 ± 0.17	0.11 ±0.18	**0.04**	**0.01**		
OW	0.11 ± 0.19	0.10 ± 0.17	0.24 ± 0.24	**0.002**		**0.02**	**0.005**
W	0.14 ± 0.21	0.29 ± 0.33	0.50 ± 0.32	**0.005**		**0.001**	**0.001**
CHX	0.095 ± 0.50	0.095 ± 0.49	0.25 ± 0.39	<**0.001**		**0.004**	**0.001**

*Friedman test **post hoc Wilcoxon signed rank test

Table 8 shows intragroup comparison of the CI-S score at different time intervals. HW showed a significant reduction in CI-S score between T_1 and T_2 intervals. There was a significant increase in CI-S score between T_1 and T_3 intervals and T_2 and T_3 intervals in OW, CHX and W.

Table 9. Intragroup comparison of oral hygiene index-simplified score at different time intervals

	T_1 Mean ± SD	T_2 Mean ± SD	T_3 Mean ± SD	p value*	T_1 vs T_2	T_1 vs T_3	T_2 vs T_3
HW	1.93 ± 0.70	0.89 ± 0.43	1.02 ± 0.36	**0.001**	**0.001**	**0.001**	
OW	1.97 ± 0.43	0.83 ± 0.44	1.11 ± 0.43	**0.001**	**0.001**	**0.001**	**0.031**
W	1.87 ± 0.76	1.54 ± 0.61	1.77 ± 0.63	0.08			
CHX	1.80 ± 0.50	0.83 ± 0.49	1.125 ± 0.39	**0.001**	**0.001**	**0.001**	**0.003**

*Friedman test **post hoc Wilcoxon signed rank test

Table 9 shows intragroup comparison of the OHI-S score at different intervals. There was a significant reduction in OHI-S score between T_1 and T_2 intervals and T_1 and T_3 intervals in HW, OW and CHX. There was a significant increase in OHI-S score between T_2 and T_3 intervals in OW and CHX. Maximum reduction in OHI-S score in HW, OW and CHX was seen between T_1 and T_2 intervals.

Table 10. Intragroup comparison of salivary Streptococcus mutans count (x 10^5 CFU/ml) at different time intervals

	T_1 Mean ± SD	T_2 Mean ± SD	T_3 Mean ± SD	p value*	T_1 vs T_2	T_1 vs T_3	T_2 vs T_3
HW	19.20 ± 30.5	4.87 ± 3.5	2.87 ± 1.56	**<0.001**	**<0.001**	**<0.001**	**0.01**
OW	12.76 ± 9.7	6.14 ± 4.04	2.23 ± 0.76	**<0.001**	**0.003**	**<0.001**	**<0.001**
W	15.41 ± 11.89	17.37 ± 17.25	15.33 ± 3.0	0.42			
CHX	14.13 ± 12.8	3.043 ±1.9	1.86 ± 1.74	**<0.001**	**<0.001**	**<0.001**	**0.01**

*Friedman test **post hoc Wilcoxon signed rank test

Table 10 shows intragroup comparison of the salivary S. mutans count at different time intervals. There was a significant reduction in S. mutans count in HW, OW and CHX between T_1 and T_2 interval, T_1 and T_3 interval and T_2 and T_3 interval. HW, OW and CHX showed maximum reduction in S. mutans count between T_1 and T_3 interval.

Table 11. Intragroup comparison of the total microbial count (x 10^5 CFU/ml) at different time intervals

	T_1 Mean ± SD	T_2 Mean ± SD	T_3 Mean ± SD	p value*	T_1 vs T_2	T_1 vs T_3	T_2 vs T_3
HW	286.5 ± 219.4	237.33 ± 109.8	339.58 ± 201.42	0.204			**0.027**
OW	323.81 ± 192.54	319.40 ± 192.17	260.54 ± 183.20	0.41			
W	388.96 ± 215.25	633.12 ± 305.30	467.84 ± 274.90	**0.006**	**0.004**		**0.03**
CHX	329.66 ± 238.49	562 ± 246.38	229.66 ± 156.31	**<0.001**	**0.004**		**0.001**

*Friedman test **post hoc Wilcoxon signed rank test

Table 11 shows intragroup comparison of the total microbial count (CFU) at different time intervals. There was a significant increase in the CFU count in W and CHX between T_1 and T_2 intervals. CHX and W showed significant reduction whereas HW showed significant increase in CFU count between T_2 and T_3 intervals.

Table 12. Intergroup comparison of debris index-simplified score at different time intervals

Time	HW	OW	W	CHX	p value***	HW vs OW	HW vs W	HW vs CHX	OW vs W	OW vs CHX	W vs CHX
T_1	1.73 ± 0.48	1.86 ± 0.39	1.74 ± 0.62	1.69 ± 0.39	0.59						
T_2	0.80 ± 0.37	0.73 ± 0.40	1.25 ± 0.39	0.76 ± 0.37	**0.000**		**0.001**		**0.001**		**0.001**
T_3	0.91 ± 0.31	0.86 ± 0.28	1.27 ± 0.39	0.87 ± 0.23	**0.001**		**0.005**		**0.001**		**0.001**

***One way ANOVA (Kruskall Wallis) test ****post hoc Mann Whitney U test

Table 12 shows intergroup comparison of DI-S score at different time intervals. There was no significant difference in DI-S score between the groups at T_1 period. A significant difference was seen in all the groups at T_2 and T_3 periods. At T_2 and T_3 periods, there was a significant difference between HW and W, OW and W; W and CHX. OW had minimum DI-S score at T_2 and T_3 periods.

Table 13. Intergroup comparison of calculus index-simplified score at different time intervals

Time	HW	OW	W	CHX	p value***	HW vs OW	HW vs W	HW vs CHX	OW vs W	OW vs CHX	W vs CHX
T_1	0.20 ± 0.31	0.11 ± 0.11	0.14 ± 0.21	0.095 ± 0.16	0.65						
T_2	0.09 ± 0.17	0.10 ± 0.14	0.29 ± 0.33	0.095 ± 0.14	**0.009**		**0.001**		**0.001**		**0.01**
T_3	0.11 ± 0.18	0.24 ± 0.24	0.50 ± 0.32	0.25 ± 0.24	**0.000**		**0.002**	**0.03**	**0.001**		**0.001**

***One way ANOVA (Kruskall Wallis) test ****post hoc Mann Whitney U test

Table 13 shows intergroup comparison of CI-S score at different time intervals. There was no significant difference between the groups in CI-S score at T_1 period. A significant difference was seen at T_2 and T_3 periods. At T_2 period, there was a significant difference between HW and W, OW and W; W and CHX. At T_3 period, there was a significant difference between HW and W, H and CHX, OW and W; W and CHX. HW had the minimum CI-S score at T_2 and T_3 periods.

Table 14. Intergroup comparison of oral hygiene index-simplified score at different time intervals

Time	HW	OW	W	CHX	p value***	HW vs OW	HW vs W	HW vs CHX	OW vs W	OW vs CHX	W vs CHX
T_1	1.93 ± 0.7	1.97 ± 0.43	1.87 ± 0.76	1.80 ± 0.50	0.62						
T_2	0.89 ± 0.43	0.83 ± 0.44	1.54 ± 0.61	0.83 ± 0.49	**0.000**		**0.007**		**0.001**		**0.005**
T_3	1.028 ± 0.36	1.11 ± 0.43	1.77 ± 0.63	1.125 ± 0.39	**0.000**		**0.001**		**0.001**		**0.001**

***One way ANOVA (Kruskall Wallis) test ****post hoc Mann Whitney U test

Table 14 shows intergroup comparison of OHI-S score at different time intervals. There was no significant difference in OHI-S between the groups at T_1 period. At T_2 and T_3 periods, there was a significant difference seen in OHI-S score. At T_2 and T_3 periods, there was a significant difference between HW and W, OW and W; W and CHX. OW followed by CHX showed maximum reduction in OHI-S score at T_2 period. HW showed maximum reduction in OHI-S score at T_3 period.

Table 15. Intergroup comparison of salivary Streptococcus mutans count (x 10^5 CFU/ml) at different time intervals

Time	HW	OW	W	CHX	p value***	HW vs OW	HW vs W	HW vs CHX	OW vs W	OW vs CHX	W vs CHX
T_1	19.2 ± 13.75	12.76 ± 9.7	15.41 ± 11.89	14.13 ± 12.88	0.94						
T_2	4.87 ± 5	6.14 ± 4	17.37 ± 17.25	3.043 ± 1.91	**<0.001**		**<0.001**	**0.04**	**<0.001**	**0.006**	**<0.001**
T_3	2.87 ± 2	2.23 ± 0.76	15.33 ± 3	1.86 ± 1.74	**0.001**		**<0.001**	**0.002**	**<0.001**	**0.006**	**<0.001**

***One way ANOVA (Kruskall Wallis) test

****post hoc Mann Whitney U test

Table 15 shows intergroup comparison of salivary S. mutans count at different time intervals. There was no significant difference in S. mutans count between the groups at T_1 period. A significant difference was seen at T_2 and T_3 periods. At T_2 and T_3 periods, there was a significant difference in S. mutans count between HW and W, HW and CHX, OW and W; W and CHX. CHX had the minimum S. mutans count at T_2 and T_3 periods.

Table 16. Intergroup comparison of total microbial count (x 10^5 CFU/ml) at different time intervals

Time	HW	OW	W	CHX	p value	HW vs OW	HW vs W	HW vs CHX	OW vs W	OW vs CHX	W vs CHX
T_1	286.5 ± 219.4	323.81 ± 192.5	388.96 ± 215.2	329.66 ± 238.4	0.34						
T_2	237.33 ± 109.8	319.40 ± 192.1	633.12 ± 305.3	562 ± 246.3	**<0.001**		**0.001**	**0.001**	**0.001**	**0.001**	
T_3	339.58 ± 201.4	260.54 ± 183.2	467.84 ± 274.9	229.66 ± 156.3	**<0.001**			**0.03**	**0.003**		**0.001**

***One way ANOVA (Kruskall Wallis) test ****post hoc Mann Whitney U test

Table 16 shows intergroup comparison of CFU count at different time intervals. There was no significant difference in CFU count between the groups at T_1. A significant difference in CFU seen at T_2 and T_3 periods. At T_2 period, there was a significant difference between HW and W, HW and CHX, OW and W; OW and CHX. At T_3 period, there was a significant difference between HW and CHX, W and OW; W and CHX. HW showed maximum reduction in CFU count at T_2 period. Water group followed by CHX showed maximum increase in CFU count at T_2 period. CHX group showed maximum reduction and W showed maximum increase in CFU count at T_3 period.

DISCUSSION

Oral health is an integral part of general health and daily oral home care measures help in maintaining a healthy oral environment. However, it is also a well-known fact that children do not consistently perform home care procedures.[51] Poor oral hygiene practices can lead to plaque accumulation and calculus formation. Tooth-brushing, when accomplished properly, results in effective plaque control. However, mechanical plaque control methods have certain inherent limitations as it requires a minimum skill which might not be successful by a child alone unless under supervision.[20]

Dental plaque has been proved to be a paramount factor in initiation and progression of gingival and oral diseases hence dentists often recommend chemical adjuncts in addition to tooth brushing and flossing for routine home care, which provides unique and beneficial approach in the prevention of plaque formation and bacterial infection.[51] Among bacterial infections in the oral cavity, the most predominant bacteria that makes the teeth more vulnerable for the development of dental caries is Mutans Streptococci (MS).[44] Most of the studies have shown that it is difficult to eliminate MS from pits, fissures, and approximal surfaces by mechanical means alone. For effective caries control, these methods should be combined with the chemoprophylactic agents. These agents are generally delivered as mouth rinses, toothpastes, gels, and varnishes.[4,10] Among them, mouthwash can be effective in plaque control and reduction in MS. Mouthwash has been suggested as an additional therapeutic strategy to augment in improving oral hygiene.[7,20] Mouthrinses have the ability to deliver therapeutic ingredients and benefits to all accessible surfaces in the mouth including interproximal surfaces. They also remain effective for extended period of time depending on their substantivity.[55]

Commercially available mouthwashes containing synthetic and/or semi-synthetic active agents have several disadvantages like staining on the teeth, more alcohol content and irritation during its use. One such example is CHX which is till date considered to be the most effective anti-plaque agent, but with certain limitations.[20] To overcome its prolonged usage disadvantages naturally occurring antimicrobial herbs can be used individually or in combination.[35] Among them, tulsi, scientifically known as Ocimum sanctum, is a time-tested premier medicinal herb. It is bestowed with enormous antimicrobial substances and is used to treat a variety of illnesses ranging from diabetes mellitus, arthritis, bronchitis, skin diseases.[62,76] Literature review reveals that the antimicrobial property of tulsi has been tested against a variety of microorganisms like Candida albicans, Staphyloccus aureus, enteric pathogens, Klebisella, Escherichia coli and Proteus.[2,26]. Recent studies have also demonstrated its significant anticancer properties.[46]

Ozonated water could be a good alternative as a mouthrinse which is very effective in inhibiting bacterial growth in biofilms. Treatment of experimental plaque with ozonated water has shown a significant reduction in the number of viable S. mutans.[55] Mere thirty seconds rinse of ozonated water can reduce the dental plaque bacterial load to almost half.[64] Its biocompatibility with oral epithelial cells has also been established.[29] The cost-effectiveness and ease of preparation of ozonated water can make it a very useful adjunct to tooth brushing and flossing.

Hence, this study was conducted to evaluate and compare the antibacterial effect of herbal water, ozonated water and CHX on salivary S. mutans level in children. The study was a double blinded randomized controlled trial conducted over a period of 30 days. Age group of 10-12 years were selected because they belong to the concrete operational stage of Jean Piaget's cognitive theory. Hence, they accept the rules and they follow the instructions given to them.[59] This age group of children can easily rinse the mouthwash without swallowing it and hence side effects of mouthrinses can be avoided.[4,78]. Oral prophylaxis was carried out for all the study subjects to maintain homogeneity in baseline data between the four groups.

Herbal water

Natural herbs when used in mouthwashes, have shown significant advantages over the chemical ones.[5,36] Side effects of herbal agents have been reported rarely till age. Balappanavar AY et al. reported that herbal mouthwash (0.5% tea and 2% neem) had more efficacy than 0.2% CHX for maintaining the oral health.[11]

Tulsi, the “Queen of Herbs” is considered as the most sacred herb of India. It contains alkaloids, glycosides, tannins and volatile oil (eugenol, thymol, urosolic acid). Tannins act as an antioxidant and scavenger against reactive oxygen species and free radicals. Urosolic acid helps in increasing leukocyte count and significantly protects mast cell membrane; thus, preventing cell degradation and histamine release. Linolenic acid present in tulsi has the capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism and hence responsible for its anti-inflammatory activity.[15,33,69] The leaves contain an essential oil which has antibacterial, antifungal, and antiviral properties.[66,71]. Agrawal P et al. reported that tulsi extract had demonstrated a significant antimicrobial potential against S. mutans.[2]

In our study, we used Deltas Pancha tulasi drops (Manufactured by Delta Pharma, Mumbai, India) which is in liquid form and is chemical and alcohol free. Its composition is - Ocimum sanctum (60%), Ocimum gratissimum (5%), Ocimum canum (5%), Ocimum basilicum (5%), Ocimum citriodorum (5%). According to the manufacturer it can be used as a natural water purifier, antibacterial, antimicrobial, anti-infective, immune modulator, powerful antioxidant and natural adjuvant in the management of cough and cold. Zone of inhibition action against S. mutans showed that the dosage optimization of this product was 10 drops in 10 ml of water. Zone of inhibition of 4 % tulsi extract obtained by Agrawal P et al was 22 mm. In an *invitro* study conducted by Sreekumar S et al., a zone of inhibition of 11 ± 6.35 mm for commercially available tulsi plant extract of 50% concentration was obtained.[2,71] Vijayaalakshmi LG and Geetha RV compared the antibacterial effect of herbal mouthrinse (Clove, peppermint satva, eucalyptus globulus, cinnamon zeylanicum, myristica fragrans, ocimum basilicum) with CHX *invitro* by tube dilution method for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) and the results showed that herbal mouthwash was as equivalent to CHX in use.[75] In our study, we found herbal water with a mean zone of inhibition of 30 ± 0.88mm, which was almost equivalent to CHX with a zone of inhibition of 28.3 ± 0.33 mm.

Ozonated water

Ozone is currently being discussed in dentistry as a possible alternative antiseptic agent.[58] Both gaseous as well as aqueous forms of ozone are equally effective as anti-microbial agents against oral pathogens.[9,14,28]. Ozone was discovered by CF Schonbein in 1839.[63] It was first suggested as a disinfectant for drinking water in view of its powerful ability to inactivate microorganisms (against bacteria, fungi, protozoa, and viruses). The aqueous form is easy to handle and is a potent microbicidal solution suitable as a soaking solution for medical and dental instruments.[8] Nagayoshi M et al. studied the effect of ozonated water on oral microorganisms and dental plaque *invitro* and found almost no microorganism detected after being treated with ozonated water for 10 seconds. They concluded ozonated water might be especially useful for killing oral infectious microorganisms.[55] Huth KC et al. in an *invitro* study evaluated whether gaseous or aqueous ozone has any toxic effect on human oral epithelial and gingival fibroblast cells in comparison with established antiseptics (CHX 2% and 0.2%; sodium hypochlorite 5.25% and 2.25%; and hydrogen peroxide 3%) during a 1 minute time period.[29] The investigators found ozone gas to be toxic to the cell lines and aqueous ozone was more biocompatible than gaseous ozone. In our study we used aqueous ozone which was freshly prepared each day by ozonation of water for 1 minute by using ozone generator. For the production of the ozonated water, 250 ml of normal water was placed in the system with a glass tube coupled to the ozone generator. Next, triatomic form of oxygen (O_3) was bubbled through the water for 1 minute, thereby producing O_3 at a concentration of 2.4 mg/l per minute (>2ppm) similar to the study by Katti SS et al.[34] Around 10ml of ozonated water was given to the children immediately after preparation as mouthrinse, to rinse for 1 minute. The rinsing time is in accordance with study by Anumula et al. who found ozonated water to be as effective as CHX for using them for 14 days in the age group of 16 to 30 years.[8] Currently, in literature search, there are no studies conducted using ozonated water as mouthrinse in children. In our *invitro* trial, zone of inhibition was 16 ± 0.58 mm. Zone of inhibition against S. mutans was least in ozonated water when compared to herbal water and CHX. This might be due to the fact that ozonated water has a short half-life as ozone lasts at full strength in water for as little as 20 minutes. This was in contrast with an *invitro* study by Anand SK et al. where ozonated water showed complete inhibition of S. mutans and E. feacalis.[6]

Culture media

The most commonly used selective media for S. mutans is Mitis Salivarius Bacitracin agar, which was modified by Kimmel and Tinanoff as Mitis salivarius agar (MSB) with kanamycin sulfate, sorbitol, potassium tellurite and bacitracin (MSKB).[37] Even though MSB is considered as one of the reliable medium to identify S. mutans, it is quite expensive compared to other medias. Also, Yoo SY et al. concluded that MSB medium cannot be specific for selecting streptococci of the mutans group, suggesting that a new selective medium is required for reliable isolation of mutans streptococci.[80] Llena MC et al added 5% potassium tellurite and 64 µg/ml fluconazol to MSKB.[41] Many other medias such as Trypticase soy with sucrose and bacitracin (TSY 20B), Thioglycolate sucrose blood agar[68], Staat and glucose-sucrose-tellurite-bacitracin (GSTB) Tanzer are available[72]. The culture media used for this study was Brain Heart Infusion agar (BHI) which is cost effective and most commonly used for culture maintenance of S. mutans in laboratories. According to an *invitro* study done by Murchison H et al, 1982, S. mutans appear as smooth or slightly rough, convex, round colony with an erose margin in BHI agar.[53]

Oral health status

Debris index simplified score

All the groups including water group showed a reduction of DI-S score after 15 days. These values slightly increased after follow up for another 15 days without using mouthrinses but was significantly lesser than the baseline scores. All groups showed maximum reduction in DI-S score during the use of mouthrinses. From this study, it was clear than even normal rinsing with water can remove debris. Ozonated water was comparatively better in reducing debris after 15 days of usage of mouthrinses and at the end of the study.

Calculus index simplified score

The reduction in CI-S score was seen only in herbal water group, whereas there was no change in calculus status in ozonated water and CHX groups after using mouthrinses for 15 days. Supragingival calculus is essentially a mineralized plaque. Immature calculus formation would be more accelerated due to other factors like poor brushing, diet or any medications, as mouthrinse is just an adjunct that can lead to early mineralization. Moreover, there is a higher tendency to calculus formation in lingual aspects of lower anterior and buccal aspects of upper posterior tooth surfaces due to the location of the submandibular and parotid ducts location. In these areas, the abundant supply of urea from the saliva and the high salivary film velocity tend to promote base formation to plaque and calcium phosphate precipitation.[19] Hence, these locations may be more susceptible to calculus formation because of the low sucrose concentration in saliva with a high saliva film velocity promoting clearance of salivary sugar and acid from plaque, and the higher plaque pH associated to better access to salivary urea.[42] This can lead to calculus formation in a faster stage. Clearly, herbal water was the only mouthrinse that was found to be better in reducing calculus when compared to others after 30 days. A probable explanation might be that herbal water was successful in reducing the immature calculus formation. After follow up for 15 days without mouthrinses, CI-S score increased significantly more than the baseline value in all the groups except herbal water group. This was another conclusive finding in our study, that after cessation of mouthrinses use, there was more calculus formation than when with use of mouthrinses. In water group, CI-S score increased throughout the study period in both with/without rinsing. Water rinsing might not be effective in preventing further calculus formation.

Oral hygiene index simplified score

There was a reduction in OHI-S score in herbal water, ozonated water and CHX groups after 15 days. Ozonated water and CHX showed a significant increase in OHI-S after 15 days without mouthrinses but did not reach upto their previous baseline values. Maximum reduction of OHI-S score was during the use of mouthrinse. Though water had a slight decrease in OHI-S score it wasn't clinically significant. Ozonated water was more effective in reducing overall OHI-S score followed by CHX after 15 days of use of mouthrinses. But, by the end of the study, herbal water was found to be effective in having the least OHI-S score. This was in contrast with the study conducted by Ahmed SI et al., he found CHX was more effective than tulsi extract (herbal) mouthrinse in the age group of 15 years in reducing OHI-S score. But their study had a short duration of 2 weeks.[4] Our study period was 30 days which involved a follow up of 15 days without mouthrinses.

Salivary S. mutans and total microbial count

There was a reduction in S. mutans count in all the groups except water after usage of mouthrinses for 15 days. Interestingly, these counts further decreased in all the groups except water at the end of 30 days. CHX was found to be more effective in reducing S. mutans count followed by herbal water and ozonated water. CHX indirectly affects the enzymatic function of dehydrogenase and adenosine triphosphatase present in the cell wall of bacteria resulting in the disruption of cell membrane.[4,43] Various studies in the literature have showed the superiority of chlorhexidine when compared with other ingredients in reducing S. mutans.[4] Effectiveness of herbal water may be attributed to single active component (tulsi) or combination of other herbal ingredient traces acting against S. mutans and more over dosage optimization of herbal water was obtained *invitro* from its zone of inhibition against S. mutans to know its antimicrobial activity. Ozonated water had significantly influenced the S. mutans population under *invivo* condition rather than *invitro* studies against S. mutans. The oxidation potential of ozonated water that destructs the cell wall and cytoplasmic membranes of bacteria and fungi. Ozone when dissociates into oxygen, creates an oxygen-rich environment, thus disturbing the normal ecosystem of the plaque. The enzymatic control system of the cell is blocked as ozone inhibits glycoproteins, glycolipids, and other amino acids the outcome of which is the functional cessation and death of the microorganism.[16] Ozonated water has also been proposed to promote remineralisation of incipient carious lesion by causing decarboxylation of the pyruvic acid produced by acidogenic bacteria to acetic acid during cariogenesis.[49] Anumula et al. compared the antibacterial efficacy of freshly prepared ozonated water with CHX against S. mutans for 14 days and concluded that ozonated water showed significant reduction of MS count and could be used as an alternative to CHX.[8] Though CHX was found to be superior to herbal water and ozonated water one cannot rule out that it has short comings such as mucosal desquamation, impaired wound healing and fibroblast attachment to the tooth surfaces.[8,17]

After 15 days of use of mouthrinses there was an increase in total microflora count (CFU) in water group and suprisingly, in CHX group. By the end of the study, there was a decrease in CFU count in CHX group. This study showed a strong negative correlation between the total microflora and S. mutans in CHX and water groups. The increased CFU with reduction in S. mutans in CHX group, suggest the ability of the beneficial microflora, probiotics and biofilm forming microbes on S. mutans population reduction. The increased CFU with increase in S. mutans in water group, suggest increase in pathogenic organism and early colonizers that gives further environment for S. mutans to grow and this hypothesis can be correlated to the oral hygiene status of children from their OHI-S score. In the intestine, bacteria do not adhere to the mucosal layer directly because the thick mucin layer acts as a reservoir of microbes. In contrast, oral epithelial tissue is coated with a thin mucosal layer, which consists of mucin from the saliva in the oral cavity. Oral microbes can adhere directly to oral epithelial tissue or teeth via this mucosal layer. Therefore, saliva can function as a reservoir of harmful and beneficial oral microbes via their adherence to tissue.[74] These beneficial bacteria like probiotics adhere to dental tissues as a part of biofilm, acting as a protective lining in oral tissue against oral diseases.[18,21]

In the present study, herbal water showed slight reduction in CFU count when compared to others during use of mouthrinses whereas there was not much change in CFU count in ozonated water group. Ozone effects on the microbes might not be a direct action which is confirmed by the population of total microflora and hence forth reduction in oral beneficial microbes that act as health promoter. This might result in provoking the opportunity for pathogenic microbes in turn causing caries. This is in accordance with an *invitro* study by Baysan et al., assessing the antimicrobial effect of the application of ozone gas on the microflora of infected dentin associated with non-cavitated occlusal carious lesions. They concluded that ozone failed to reduce the number of viable bacteria in the underlying infected dentin suggesting the mechanism of action of ozone on bacteria occurs in an indirect manner.[12] They further speculated that there might be a diffusion with reaction and the interaction between ozone gas and the micro-organism should be so strong to reduce the effective diffusion gradient of ozone within the tissue. It requires higher concentration to kill all the viable microorganisms. This indirect action and lower concentration of ozone might be the cause in reducing S mutans count but not CFU. But, this discrepancy needs further studies for a specific explanation. Sadatullah S et al. measured the supragingival plaque micro-organisms (total CFU) before and after rinsing once with 0.1 ppm of ozonated water and unveiled a reduction in the microbial load including S. mutans.[64] At the end of 30 days, CHX and ozonated water were found to be effective in reducing the CFU count.

Advantages of ozonated water

- Ozonated water is cost effective as the ozone gas is an one time investment in buying the ozone generator. For purifying water with a lower power generators in cheaper rates are also available in the market
- The participants who used ozonated water were more willingful to use it as its appearance did no differ much from the normal water other than in slight odor and taste.

Disadvantage of ozonated water

- The only shortcoming is that O_3 molecule is unstable and ozonated water should be prepared immediately before use. Its oxidizing property gradually decreases with time.[8]

Advantages of herbal water

- Literature search showed no ill effects of tulsi on human beings, one can confidently recommend the use of tulsi as a mouthrinse, especially for rural population which has an easy access to tulsi.
- Highly cost-effective.

Disadvantage of herbal water

- The few participants in herbal water group initially had a slight unacceptance towards the bitter taste of tulsi.

Limitations of the study

- There is a need to study the long-term effects of the mouthrinses on oral health.
- The role of these mouthrinses needs to be assessed with emphasis on individuals with poor oral hygiene.
- Larger sample of children with different caries status could have been assessed, to get better picture of the antibacterial properties of these mouthrinses.
- Further studies are required to find the optimum ozonation time and concentration as required for mouthrinse.

SUMMARY AND CONCLUSION

The present randomized controlled trial was conducted from the Department of Pediatric and Preventive Dentistry, K.S.R. Institute of Dental Science and Research (KSRIDSR), Tiruchengode, Tamil Nadu. The study was planned and organized in association with a Government aided school in Tiruchengode to determine Debris index-simplified (DI-S), Calculus index-simplified (CI-S) and Oral hygiene index-simplified (OHI-S) score, S. mutans and CFU count after usage of various mouthrinses in children. Hundred children aged 10-12 years with DMFT/dmft ≤ 3 were included in the study. They were randomly divided into 4 equal groups (n=25): HW- Herbal water, OW- Ozonated water, W- Water and CHX- Chlorhexidine. They were instructed to use their respective mouthrinses for 15 days. Children were followed up another 15 days without using any mouthrinses and hence the study period was 30 days. DI-S, CI-S, OHI-S, S. mutans and CFU count were estimated at baseline, after 15 days and after 30 days. Saliva samples were used to evaluate S. mutans and CFU counts that were seen in BHI agar media. The colonies were identified by their morphological characteristics and confirmed using gram staining. All the counts were established for 10^5 dilution. *Invitro* evaluation of mouthrinses by measuring the zone of inhibition against S. mutans were done in Muller Hinton agar prior to the study. Results were tabulated and analyzed statistically.

The following findings can be inferred from the study:

- Herbal water showed the maximum zone of inhibition against S. mutans followed by CHX.
- Herbal water, ozonated water and CHX showed significant reduction in DI-S, CI-S, OHI-S score and S. mutans count after 15 days.
- Though, there was increase in DI-S and OHI-S score in all the groups at the end of the study, these values were significantly lesser than baseline values.

- Herbal water, ozonated water and CHX showed significant reduction in S. mutans after 30 days.
- Ozonated water was more effective in reducing debris followed by herbal water and CHX.
- Herbal water was the only mouthrinse that was found to be effective in reducing calculus.
- Ozonated water showed maximum reduction in overall OHI-S score after 15 days of mouthrinses, but after follow up for another 15 days without muthrinses herbal water was more effective in having least OHI-S score.
- Chlorhexidine was found to be most effective in reducing S. mutans count when compared with other mouthrinses studied.
- Herbal water was found to be effective in reducing CFU count during the use of mouthrinses whereas CHX and ozonated water was effective in reducing CFU at the end of 30 days.
- From this study, it is clear that herbal water and ozonated water can be used as an alternative to CHX.

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APPENDIX I

INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH

KSR Kalvi Nagar, Tiruchengode-637 215, Tamilnadu.
Phone : 04288-274981, Fax : 04288-274761,
email : ksrdentalcollege@yahoo.com

Chairman
Dr. PHILIP ROBINSON, Ph.D
Prof. & Head Dept. of Biotechnology
KSR College of Technology,
KSR Kalvi Nagar, Tiruchengode.

Member Secretary
Dr. G.S. KUMAR, MDS.,
Principal,
KSR Institute of Dental Science & Research,
KSR Kalvi Nagar, Tiruchengode.

Members

Dr.G.Ayppadasan,Ph.D.,
Biotechnologist

Mr.A.Thirumoorthi,M.A.B.L.,
Human Activist

Dr.R.Renuka,M.D.S.,(Perio),M.Sc.,
Family Counsellor

Dr.M.Rajmohan,MDS,(Oral Path)

Dr.R.Prakash, MDS,(PHD)

Dr.Suman, M.D.S.,(OMDR)

Dr.Sharath Ashokan,MDS.,(Pedo)

Dr.G.Rajeswari,Ph.D.,(Biochemistry)

Dr.K.Karthick,MDS.,(Cons.Dent.)

Mr.V.Mohan,M.Sc.,M.Phil.,(Physicist)

Mr.A.P.S.Raja,B.A.,
(Layperson)

Ref.: 165/KSRIDSR/EC/2016 **Date : 19.12.2016**

To

Dr.Jijo Mon,
Postgraduate Student,
Dept. of Peadodontics,
KSR Institute of Dental Science & Research,

*****

Your dissertational study titled "EFFECT OF HERBAL WATER, OZONATED WATER, WATER AND CHLORHEXIDINE MOUTHRINSES ON ORAL HEALTH STATUS OF CHILDREN – A RANDOMIZED CONTROLLED TRIAL" presented before the ethical committee on 16th Dec. 2016 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary
(Dr.G.S.Kumar)

APPENDIX II

URKUND

Urkund Analysis Result

Analysed Document:	Plagarism thesis.pdf (D46641717)
Submitted:	1/10/2019 5:55:00 PM
Submitted By:	contact_drjijz@yahoo.com
Significance:	12 %

Sources included in the report:

EFFECT OF BAEL, CINNAMON AND NEEM EXTRACT MOUTHWASHES ON DENTAL PLAQUE, GINGIVAL HEALTH AND SALIV.pdf (D46342331)
Mendeley Editing- Pre Final 26.12.2018.docx (D46379847)
https://www.science.gov/topicpages/c/chlorhexidine+digluconate+chx
http://ircmj.com/en/articles/60576.html
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https://www.science.gov/topicpages/c/chlorhexidine+mouth+rinse
https://www.science.gov/topicpages/f/fluoride+mouth+rinse.html
http://www.jcsjournal.org/article.asp?issn=2468-6859;year=2017;volume=14;issue=2;spage=81;epage=85;aulast=Parkar
https://worldwidescience.org/topicpages/c/chlorhexidine.html
http://www.ijds.in/article.asp?issn=0976-4003;year=2018;volume=10;issue=1;spage=6;epage=10;aulast=Yadav;type=0

Instances where selected sources appear:

39

APPENDIX III

CONSENT FORM

KSR INSTITUTE OF DENTAL SCIENCE AND RESEARCH

DEPARTMENT OF PEDODONTICS AND PREVENTIVE DENTISTRY

I ...., as legally responsible parent/guardian give my consent for the participation of my child ...., standard........... in the study titled “Effect of herbal water, ozonated water, water and chlorhexidine mouthrinses on oral health status of children- A randomized controlled trial”.

Dr. Jijo Mon discussed with me to my satisfaction, the procedures, possible discomforts and the benefits of the study. I have read this consent and have clearly understood the procedures to be performed on my child. The information obtained will be kept strictly confidential. I understand that the participation is voluntary and no payment will be provided. I also understand that I have the right to withdraw my child at any point during the study without any penalty.

Legally responsible parent/guardian: .... Date: ....

Address: ....

....

Contact number:

I certify that I explained the above information to the parent/guardian, before requesting his or her signature.

Signature of the dentist: .... Date: ....

APPENDIX IV

குழந்தைகள் நல பல் மருத்துவப்பிரிவு

தகவலறிந்த ஒப்புதல் படிவம்

பெயர் ________________________ வயது ________ ஆண்/பெண் ஆகிய என்னிடம் என் மகனை/மகளை _______________________ ,வயது ___ வகுப்பு _____ **மருத்துவர் Dr. ஜிஜோ மோன்** அவர்களின் "**வெவ்வேறு வாய் கழுவும் மருந்துகளால் வாய் சுகாதாரத்தில் ஏற்படும் விளைவுகள்**" என்கிற ஆராய்ச்சிக்கு உட்படுத்த அனுமதி கோரப்பட்டுள்ளது.

Dr. ஜிஜோ மோன் இவ்வாராய்ச்சியின் செயற்பாடுகளையும், அதன் சாதகபாதகங்களையும் நன்கு விளக்கினார். இவ்வாராய்ச்சியில் பெறப்படும் தகவல்கள் இரகசியமாக வைக்கப்படும் எனவும் பங்கேற்பாளர்களின் பெயர்கள் எவ்விடத்திலும் உபயோகப்படுத்த மாட்டாது எனவும் தெரிவித்தார். இவ்வாராய்ச்சியில் பங்கேற்பதற்கு எவ்வித கட்டணமும் இல்லை என்றும் இதில் பங்கெடுப்பது அவரவர் தனிப்பட்ட விருப்பத்தை சார்ந்தது என்றும் தெரிந்துகொண்டேன். அதேபோல் எந்த நேரத்திலும் விலகிக்கொள்ளவும், எனது மகன்/மகள் சம்மந்தப்பட்ட தகவல்களை அழிக்க கோரவும் எனக்கு உரிமை உண்டு என அறிந்துகொண்டேன். எனவே இவ்வாராய்ச்சியில் எனது மகன்/மகள் பங்கெடுக்க நான் முழுமனதுடன் சம்மதிக்கிறேன்.

பெற்றோர்/பாதுகாவலர்: ______________________________________

தேதி:______________

APPENDIX V

CERTIFICATE - II

This is to certify that this dissertation work title **"Effect of herbal water, ozonated water, water and chlorhexidine mouthrinses on oral health status of children- A randomized controlled trial "** of the candidate **Dr. Jijo Mon** with registration number.......................for the award of "**Master of Dental Surgery"** in the branch **of Pedodontics and Preventive Dentistry**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **12%** percentage of plagiarism in the dissertation.

PROFESSOR AND HOD
DEPARTMENT OF PAEDODONTIA
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR POST,
TIRUCHENGODE - 637 215

APPENDIX VI

ABBREVIATIONS

S No.	Abbreviation	Expansion
1.	S. mutans	Streptococcus mutans
2.	CHX	Chlorhexidine
3.	HW	Herbal water
4.	OW	Ozonated water
5.	W	Water
6.	MIC	Minimum inhibitory concentration
7.	MBC	Minimum bactericidal concentration
8.	DI-S	Debris index simplified
9.	CI-S	Calculus index simplified
10.	OHI-S	Oral hygiene index simplified
11.	API	Approximal plaque index
12.	S. viridans	Streptococcus viridans
13.	CFU	Colony forming units
14.	MS	Mutans streptococcus
15.	BHI	Brain heart infusion agar
16.	RT	Room temperature
17.	T_1	Baseline
18.	T_2	After 15 days
19.	T_3	After 30 days

20.	O_3	Trioxygen (ozone)
21.	TSY 20B	Trypticase soy with sucrose and bacitracin
22.	GSTB	Glucose-sucrose-tellurite-bacitracin

APPENDIX –VII

DECAYED-MISSING-FILLED INDEX (DMF) - Klein, Palmer and Knutson in 1938

- DMF teeth index (DMFT) which measures the prevalence of dental caries/Teeth.

D component

Used to describe (Decayed teeth) which include:

1. Carious tooth.
2. Filled tooth with recurrent decay.
3. Only the root is left.
4. Defect filling with caries.
5. Temporary filling.
6. Filled tooth surface with other surface decayed.

M component

Used to describe (Missing teeth due to caries) other cases should be excluded

1. Tooth that extracted for reasons other than caries should be excluded, which include:
    a- Orthodontic treatment.
    b- Impaction.
    c- Periodontal disease.
2. Unerupted teeth.
3. Congenitally missing.
4. Avulsion teeth due to trauma or accident.

F component

Used to describe (Filled teeth due to caries). Teeth were considered filled without decay when one or more permanent restorations were present and there was no secondary (recurrent) caries or other area of the tooth with primary caries. A tooth with a crown placed because of previous decay was recorded in this category. Teeth stored for reason other than dental caries should be excluded, which include:

1. Trauma (fracture).
2. Hypoplasia (cosmetic purposes).
3. Bridge abutment (retention).
4. Seal a root canal due to trauma.
5. Fissure sealant.
6. Preventive filling.

WHO modification of DMF index, 1987

- All third molars included
- Initial white spot lesion – counted as decay
- Above 30 years of age, tooth lost due to any reason – counted as missing

***def in*dex - Gruebbel, Morris and Knutson - 1943**.

The initials of the index were definedas follows:

- *d* = decayed primary teeth indicated for filling,
- *e* = decayed primary teeth indicated for extraction,
- *f* = filled primary teeth.

APPENDIX VIII

SIMPLIFIED ORAL HYGIENE INDEX (OHI-S)

OHI-S was developed in 1964 by John C. Greene and Jack R. Vermillion.

Teeth to be examined	
16- Upper right first molar	Buccal
11- Upper right central incisors	Labial
26- Upper left first molar	Buccal
36- Lower left first molar	Lingual
31- Lower left central incisor	Labial
46- Lower right first molar	Lingual

Substitution of tooth (in case of missing)	
16	17, 18 (if 17 missing)
11	21
26	27, 28 (if 27 missing)
36	37, 38 (if 37 missing)
31	41
46	47, 48 (if 47 missing)

Exclusion: Natural teeth with full crown restorations and surfaces reduced in height by caries or trauma are not scored

Instruments used: Mouth mirror and No. 23 explorer

Examination methods and scoring

OHI-S has two components- Simplified Debris Index (DI-S) and the Simplified Calculus Index (CI-S).

Debris Index- Simplified (DI-S)

Surface area covered by debris is estimated by running the side of an explorer along tooth surface being examined. The occlusal or incisal extent of debris is noted as it is removed.

Scoring criteria for DI-S

Score	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 Simplified (CI-S)

1) Supragingival calculus- denotes deposits, usually white to yellowish brown in color, occlusal to the free gimgival margin
2) Subgingival calculus- denotes deposits usually light brown to black in color, apical to the free gingival margin.

Scoring criteria for CI-S

Score	Criteria
0	No calculus present
1	Supragingival calculus covering not more than one 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 thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both

Calculation of the Index

For each individual, the debris and calculus scores are totaled and divided by the number of tooth surfaces scored.

$$\text{Calculation of DI-S} = \frac{\text{Total score}}{\text{No. of surfaces examined}}$$

$$\text{Calculation of CI-S} = \frac{\text{Total score}}{\text{No. of surfaces examined}}$$

OHI-S = DI-S + CI-S

Interpretations:

For the DI-S and CI-S score,

Good- 0.0 to 0.6

Fair- 0.7 to 1.8

Poor- 1.9 to 3.0

For the OHI-S score,

Good- 0.0 to 1.2

Fair- 1.3 to 3.0

Poor- 3.1 to 6.0

APPENDIX IX

OHI-S data form

Patient name: **Age/Sex:** **Class:**

Baseline:

DI-S index

16	11	26
46	31	36

Score Good/Fair/Poor

CI-S index

16	11	26
46	31	36

Score Good/Fair/Poor

OHI-S= DI-S+CI-S

Score Good/Fair/Poor

Post OP- 2 weeks:

DI-S index

16	11	26
46	31	36

Score Good/Fair/Poor

CI-S index

16	11	26
46	31	36

Score Good/Fair/Poor

OHI-S= DI-S+CI-S

Score Good/Fair/Poor

Post OP- 1 month:

DI-S index

16	11	26
46	31	36

Score Good/Fair/Poor

CI-S index

16	11	26
46	31	36

Score Good/Fair/Poor

OHI-S= DI-S+CI-S

Score Good/Fair/Poor 42244