EFFECTIVENESS OF AZARDIRACHTA INDICA (NEEM EXTRACT) MOUTH RINSE ON PLAQUE AND GINGIVAL HEALTH- AN INTERVENTIONAL STUDY

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In

PUBLIC HEALTH DENTISTRY

By

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

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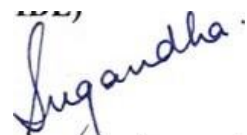Date :   05.07.2021

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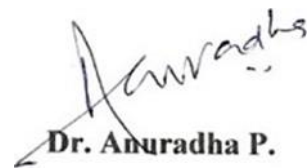
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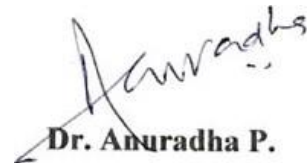
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I dedicate all my work and the knowledge that I have gained from different spheres to my mother, who has and will always be the constant support in my life.

Above all I bow my head in gratitude to ALMIGHTY GOD for bestowing his blessings on me.

DR. SIDDHARTH JOEL DAVID

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LIST OF ABBREVIATION

PI	Plaque Index	
GI	Gingival Index	
p	Level of significance	
CHX	Chlorhexidine	

ABSTRACT

Azadirachta indica otherwise called neem has been utilized in India and South Asia for millennia as an ideal tool for maintaining healthy periodontium. Neem has been for some time considered to have an astringent, antiseptic, insecticidal, antiulcer and for medicinal properties. It is utilized for periodontitis and other dental diseases. The antibacterial effects of neem has been assessed and known from ancient times. The efficacy of Azadirachta indica leaf extract against plaque formation was analysed. Almost all parts of the tree ware utilized in indigenous systems of medicine for the treatment of an assortment of human infirmities, especially against infections of bacterial and parasitic beginning. Consequently, new formulatios with comparative or predominant adequacy and conceivable few long impact should be examined.

Chlorhexidine is certainly the most broadly contemplated and effective antimicrobial agent for the chemical control of dental biofilm, and is viewed as the gold standard with which all anti-biofilm agents should be compared. Both alcohol containing and alcohol free chlorhexidine prepartions are sold around the world. Ethyl liquor is utilized in oral mouth washes as a solvent and antiseptic agent.

METHODOLOGY

A randomized controlled trial was done on 120 participants by the method of simple random sampling. The study was conducted during March 2019. Patient who had A mean plaque index (P1I) of 1.0 and A mean gingival index of 1.0 was selected for the study based on simple random sampling. Double blinding was conducted to reduce bias. Chlorhexidine, water based neem mouth wash, alcohol based neem mouth wash and water was used. Participants were instructed to rinse

with the mouth wash daily. Data were collected on baseline, 15 days and 30 days. MANOVA and paired t test applied using SPSS version 22 keeping level of significance p<0.05.

RESULT

A mean difference between Plaque Index and Gingival Index was observed Chlorhexidine mouth wash, water based neem mouthwash and alcohol based neem mouthwash, which was found to be significant. A significant difference was observed between pre and post treatment amongst the group.

CONCLUSION

In the present study there was a decrease in the Plaque Index and Gingival Index in Chlorhexidine, water based neem mouthwash, Alcohol based neem mouthwash. The results show a significant reduction in gingival bleeding, and plaque indices in the three groups over a period of 30 day as compared to the negative control group. Neem mouthwash was found to be as effective as Chlorhexidine mouthwash.

INTRODUCTION

Oral health is a key indicator of overall health, well-being and quality of life. It encompasses a range of diseases and conditions that include dental caries, Periodontal disease, Tooth loss, Oral cancer, oral manifestations of HIV infection, oro-dental trauma, and birth defects such as cleft lip and palate. Most oral diseases and conditions share modifiable risk factors with the leading NCDs (cardiovascular diseases, cancer, chronic respiratory diseases and diabetes). These risk factors include tobacco use, alcohol consumption and unhealthy diets high in free sugars, all of which are increasing at the global level. The psychosocial impact of many oral diseases significantly reduces the quality of life. While the global burden of untreated dental caries for primary and permanent dentition has remained relatively unchanged over the past 30 years, the overall burden of oral conditions on services is likely to keep increasing because of population growth and ageing. Oral diseases disproportionally affect the poor and socially-disadvantaged members of society. There is a very strong and consistent association between socioeconomic status (income, occupation and educational level) and the prevalence and severity of oral diseases. This association remains across the life course, from early childhood to older age, and across populations in high-, middle- and low-income countries. Oral care treatment is costly, averaging 20% of out-of-pocket health expenditure in most high-income countries. The demand for oral health care is beyond the capacity of health care systems in most low- and middle-income countries.

The periodontium is the specialized tissues that both surrounds and supports the teeth, maintaining them in the maxillary and mandibular odontologic spaces. The word comes from the Greek terms περί peri-, meaning "around" and -odont, meaning "tooth". Literally taken, it means that which is "around the tooth". It provides the support, anchorage, and nutrition necessary to maintain teeth in

function. It consists of four principal components, namely Gingiva, Periodontal ligament (PDL), Cementum, Alveolar bone. Each of these components is different in location, architecture, and biochemical properties, which adapt during the life of the structure. The periodontal ligament in itself is an area of high turnover that allows the tooth not only to be suspended in the alveolar bone but also to respond to the forces. [3]

Periodontal disease, also known as gingival disease, is a set of inflammatory conditions affecting the tissues surrounding the teeth. In its early stage, called gingivitis, the gingiva become swollen, red, and may bleed. In its more serious form, called periodontitis, the gingiva can pull away from the tooth, bone can be lost, and the teeth may loosen.[4] As dental plaque or biofilm accumulates on the teeth near and below the gums there is some dysbiosis of the normal oral microbiome. Research in 2004 indicated three gram negative anaerobic species: Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythus and Eikenella corrodens most commonly found in biofilm. Plaque may be soft and uncalcified, hard and calcified, or both. For plaque that are on teeth the calcium comes from saliva for plaque below the free gingival sulcus, it comes from blood via oozing of inflamed gums. The damage to teeth and gingiva comes from the immune system as it attempts to destroy the microbes that are disrupting the normal symbiosis between the oral tissues and the oral microbe community. As in other tissues, Langerhans cells in the epithelium take up antigens from the microbes, and present them to the immune system, leading to movement of white blood cells into the affected tissues. This process in turn activates osteoclasts which begin to destroy bone, and it activates matrix metalloproteinases that destroy ligaments.[4]

India represents almost 17.31% of the world's population. About 72.2% of the population live in approximately 638,000 villages and the remaining 27.8% in approximately 5,480 towns and urban agglomerations. India reveals a lot of

disparities in terms of ratios, one of which is the field of the oral health. The dentist-to-population ratio in the rural areas is dismally low with less than 2% dentists being available for 72% of the rural population. Statistics present the grim reality that 95% of the population in India suffer from periodontal disease, only 50% use a toothbrush, and just 2% visit the dentist.[5]

Azadirachta indica also known as neem has been used in India and South Asia for thousands of years as a perfect tool for maintaining healthy periodontium. Neem has been long considered to have an astringent, antiseptic, insecticidal, antiulcer and for medical properties. It is used for periodontitis and other dental diseases. The antibacterial activity of neem has been evaluated and known from ancient times. the effectiveness of Azadirachta indica leaf extract against plaque formation was assessed. Almost every part of the tree was used in indigenous systems of medicine for the treatment of a variety of human ailments, particularly against diseases of bacterial and fungal origin. Therefore, new formulation with similar or superior efficacy and possible few long effect need to be investigated.

Chlorhexidine is certainly the most widely studied and efficient antimicrobial agent for the chemical control of dental biofilm, and is considered the gold standard with which all anti-biofilm agents should be compared. Both alcohol-containing and alcohol-free chlorhexidine solutions are sold worldwide. Ethyl alcohol is used in oral mouth rinses as a solvent and antiseptic agent.

Alcohol is important to ensure product stability and activity, since it may prevent contamination of the solution. It is also known for ots antiseptic properties

Hence the present study aims at evaluating effectiveness of azardirachta indica (neem extract) mouth rinse on plaque and gingival health. The use of Azardirachta Indica as an antibacterial agent has been anciently proved in Ayurveda. Hence the scope of the study is to evidently establish the efficacy of

the Azardirachta Indica mouth rinse so that it can benefit the common people both economically as well as through its anti-bacterial properties.

AIM-

To assess the effectiveness of neem mouth rinse on plaque and gingival health.

OBJECTIVES-

To assess the effectiveness of Chlorhexidine on plaque and gingival health.

To assess the effectiveness of Neem mouth rinse on plaque and gingival health.

To compare the efficacy between Chlorhexidine and Neem mouth rinse in reducing plaque and improving gingival health.

To assess the effectiveness of alcohol based neem mouth rinse

REVIEW OF LITERATURE

Quirynen M et al in **2001**[32] conducted a study to compare the plaque and gingivitis inhibiting effect of commercial products containing 0.2%, 0.12% and 0.06%chlorhexidine in a modified experimental gingivitis model.Methods:In three groups of healthy volunteers, experimental gingivitis was induced and monitored over 21 daysand simultaneously treated with the commercial solutions containing 0.2%, 0.12% and 0.06% chlorhexidine. Themaxillary right quadrant of each individual received mouthwash only, whereas the maxillary left quadrant wassubject to both rinsing and mechanical oral hygiene. Compliance and side effects were monitored at days 7, 14,and 21. Plaque and gingivitis scores were obtained at baseline and day 21.Results:The commercial mouthwash containing 0.2% chlorhexidine resulted in statistically significantly lower plaquescores than the 0.12 and 0.06% mouthwashes after 21 days use, whereas no statistically significant difference wasfound between the effects of the two latter.Conclusion:A commercially available mouthwash containing 0.2% chlorhexidine had statistically significant bettereffect in preventing dental plaque than the 0.12% and 0.06% solutions.[16]

Pizzo G in the year **2006**[40] conducted a study at evaluating the impact of neem-containing mouthwash on plaque and gingivitis. This randomized, double-blinded, crossover clinical trial included 40 participants aged 18 to 35 years with washout period of 1 week between the crossover phases. A total of 20 participants, each randomly allocated into groups I and II, wherein in the first phase, group I was provided with 0.2% chlorhexidine gluconate and group II with 2% neem mouthwash. After the scores were recorded, 1-week time period was given to the participants to carry over the effects of the mouthwashes and then the second phase of the test was performed. The participants were instructed to

use the other mouthwash through the second test phase. There was a slight reduction of plaque level in the first phase as well as in the second phase. In the study, it has been concluded that neem mouthwash can be used as an alternative to chlorhexidine mouthwash based on the reduced scores in both the groups. [15]

Botelho MA in **2008**[20] did a study to compare the short-term efficacy and safety of a Azadirachta indica mouthrinse on gingival inflammation and microbial plaque, compared to 0.12% chlorhexidine. A double-masked, randomized, parallel armed study was carried out to assess the efficacy of an oral mouthrinse based on leaves of the neem tree reducing gingivitis. Study subjects were recruited from a slum in Brazil. Fifty-four subjects were enrolled and randomly assigned in two groups (26 neem group, 28 chlorhexidine control). Interventions consisted of a seven day therapy of the A. indica-based mouth rinse and chlorhexidine 0.12%, respectively. Plaque index, gingival index and gingival bleeding index were obtained at baseline, as well as after one and four weeks. Additionally, the count of cariogenic bacteria (Streptococcus mutans) in the saliva was assessed before and after treatment. All clinical index scores were reduced in both groups seven and 30 days after treatment. There was no statistically significant difference between groups in clinical and microbiological parameters. Adverse events were mild and of transient nature. This short-term study demonstrated that A. indica-based mouth rinse is highly efficacious and that it may be used as an alternative therapy in the treatment of periodontal disease. [49]

Neto CAF, Parolo CC, Rosing CK, Maltz M.in **2008** did A crossover, randomized, double-blind clinical trial to evaluate the effect of two chlorhexidine solutions against supra- and subgingival biofilm formation (NCT#02656251). Thirty-five participants were randomized and asked to rinse twice daily with 15 ml of an alcohol-containing 0.12% chlorhexidine solution, an alcohol-free 0.12% chlorhexidine solution, or placebo. The study was conducted in three experimental periods of 4 days each, with a 10-day washout between the periods. All the experimental periods followed the same protocol, except that the solutions were switched. Biofilm distribution was evaluated every 24 hours by the Plaque-Free Zone Index, during 96 hours. Adverse events were self-reported and sensory evaluation was performed using a hedonic scale. Compared to the placebo, the chlorhexidine solutions resulted in a significantly higher number of surfaces free of plaque over 96 hours ($p < 0.01$), and were able to prevent subgingival biofilm formation ($p < 0.01$). The alcohol-free chlorhexidine solution was associated with a lower incidence of adverse events, compared with alcohol-containing chlorhexidine ($p < 0.05$); it also received better sensory evaluation and acceptance by trial participants, compared with the alcohol-containing chlorhexidine , and had a similar inhibitory effect on the formation of supra- and subgingival biofilms.

Chatterjee A, Saluja M, Singh N, Kandwal A in **2011**[13] aimed to evaluate the effect of an herbal toothpaste containing neem leaves extract against gingivitis. A total of 40 subjects were divided into two groups (n=20 each): Experimental and control. Those in the test group were instructed to brush their teeth twice a day for 7 days with neem leaves extract toothpaste, whereas the control group subjects were asked to continue with the non-herbal paste they used for tooth brushing. Plaque index (PI) and papillary bleeding index (PBI) were measured on

day 0 and day 7. Significant reductions in PI and PBI were noted between the test and control groups. The findings of this clinical study provide evidence that neem leaf extracts are effective in reducing gingivitis.

Elavarasu S, et al in **2012**[43] did a study to compare the short-term efficacy and safety of a Azadirachta indica mouthrinse on gingival inflammation and microbial plaque, compared to 0.12% chlorhexidine. A double-masked, randomized, parallel armed study was carried out to assess the efficacy of an oral mouthrinse based on leaves of the neem tree reducing gingivitis. Study subjects were recruited from a slum in Brazil. Fifty-four subjects were enrolled and randomly assigned in two groups (26 neem group, 28 chlorhexidine control). Interventions consisted of a seven day therapy of the A. indica-based mouth rinse and chlorhexidine 0.12%, respectively. Plaque index, gingival index and gingival bleeding index were obtained at baseline, as well as after one and four weeks. Additionally, the count of cariogenic bacteria (Streptococcus mutans) in the saliva was assessed before and after treatment. All clinical index scores were reduced in both groups seven and 30 days after treatment. There was no statistically significant difference between groups in clinical and microbiological parameters. Adverse events were mild and of transient nature. This short-term study demonstrated that A. indica-based mouth rinse is highly efficacious and that it may be used as an alternative therapy in the treatment of periodontal disease.

Balappanavar AY, Sardana V, Singh M.in 2013[16] conducted a study to compare and to analyze the antimicrobial efficacy of green tea mouthwash, neem mouthwash, homeopathic (Frezyderm) mouthwash, rexidine mouthwash on dental plaque.This is a double-blinded, random controlled research study conducted in the Department of Periodontics. A total of 200 patients were selected randomly, comprising of four groups (Group-I:green tea, group-II-:neem sticks, Group-III: homeopathic (Frezyderm) mouthwash and group-IV: rexidine mouthwash) with gingival index score II. Saliva samples were collected on at baseline and on day 10 and 20 and microbial colony count was done. Microbial colonies were reduced better in Group IV on the day 10, whereas on day 20, greater reduction was observed in Group II with the difference was statistically significant. Neem mouthwash formulation had better effects as compared to other three mouthwashes and it could overcome the disadvantages of other mouthwashes as well.[55]

Shashi Prabha Tyagi[31] in the year 2013 did a study on anti-microbial efficacy of 2.5% sodium hypochlorite (SHC) and 0.2% chlorhexidine gluconate werecompared with an experimental irrigant formulated from the Neem tree, Azadirachta indica A sample of 36 single rooted anterior teeth with periapical radiolucency and absence ofresponse to vitality tests that required root canal treatment were selected for this study. The test irrigants and their combinations were assigned to five different groups and saline served as the control. Access cavities were preparedusing an aseptic technique and samples collected for both anaerobic culture and Gram stained smears, followed byirrigation and sample collection again. The number of organisms were expressed in colony forming units/ml after 72 hof incubation; the smears were analyzed for their microbial loads and tissue clearance and assessed as per defined criteria.Our results found the maximum reduction in microbial loads, when analyzed by culture method,

with acombination of SHC and the experimental neem irrigant. Maximum tissue clearance on the Gram Stained smears wasalso found with the same combination. Neem irrigant has anti-microbial efficacy and can be considered for endodontic use.[38]

Vatne JF, Gjermo P, Sandvik L, Preus HR[37] in 2015 conducted study to compare the effect that garlic juice and Chlorhexidine mouthwash has on oral pathogens. Fresh garlic bulbs were used to extract the antibacterial juice. The yellow juice was separated from the pulpwith Whatman filter paper. After filtration the liquid was freeze dried and stored at 4°C until required. The bacteria that were tested were: Streptococcus mutans, S. sanguis, S. salivarius and Lactobacillus casei. After cultivation of the bacteria, the Minimal Inhibitory Concentration (MIC) of the garlic juice and Chlorhexidine were measured using the E-test method, then the Minimal Bactericidal Concentration (MBC) of the Chlorhexidine and garlic juice were measured by tube test. The lowest MIC of garlic juice was for S. mutans 0.25 µg/mL and the highest was for L. casei 2. 5 µg/mL. The MIC of Chlorhexidine for these two bacteria was 0.62 µg/mL and 5 µg/mL respectively. The MBC of Chlorhexidine and garlic for S. mutans had the lowest concentration compared with the other tested bacteria. The MBC of Chlorhexidine and garlic for S. mutanswas 0.35 µg/mL and 0.3 µg/mL respectively. The highest MBC of Chlorhexidine was for S. sali-varius 10 µg/mL. The MBC of garlic for S. sanguis was similar at 10. 4 µg/mL. The efficacy of garlic juice was higher than Chlorhexidine against target bacte-ria and could be used as an effective mouthwash, but its side effects need to be investigated.[40]

R. Rodan, F. Khlaifat, L. Smadi, R. Azab, and A. Abdalmohdi[39] in 2015 The purpose of this study was to determine and com-pare anti-bacterial effects of the chlorhexidine gluconate and herbal mouthwashes in intensive care unit patients.In this double blind randomized clinical trial, 60 patients who were admitted in ICU were divided into two equal 30 persons groups. In the fi rst group

Chlorhexidine gluconate 2 % mouthwash and in the second group herbal mouth wash was used. Just before and again after 6 min of oral rinsing, salivary samples were obtained without any stimulation in order to culture Staphylococcus aureus and Streptococcus pneumoniae. The data were processed in SPSS16 software and were analyzed by appropriate statistical tests. Matrica® and chlorhexidine mouthwashes have significant antibacterial effects against Streptococcus pneumoniae and Staphylococcus aureus. Decreasing of the number of bacteria in samples after oral rinsing was signifi cant in both groups ($p < 0.001$) but chlorhexidine was significantly more effective than herbal mouth wash in reducing the number of colonies ($p < 0.001$). Conclusion: The herbal mouth wash has signifi cant antibacterial effects against Staphylococcus aureus and Streptococcus pneumoniae, but its effi cacy is less than chlorhexidine, so after further investigation, it would be considered as an alternative to chlorhexidine in ICUs[42]

Rao DV, Singh I, Chopra P, Chhabra PC , Ramanujalu G[51] in 2016 conducted a study to assess the efficacy of neem based mouth rinse regarding itsantigingivitis effect. Forty five subjects with plaque induced gingivitis were selected for the study. They were equallydivided into three groups. Group I patients were asked to rinse with 15 ml of neem mouthwash twicedaily, group II with 15 ml of chlorhexidine mouthwash twice daily, and group III with 15 ml of salinetwice daily. The three groups were asked to perform the routine oral hygiene procedures thought outthe study period. Bleeding on probing and gingivitis were evaluated by Muhlemann and Son's Sulcusbleeding index (1971) and Loe and Sillness gingival index (1963), respectively, at base line, after everyweek till one month. result showed that an A. indica mouthrinse is equally effective in reducing periodontal indices asChlorhexidine. The results demonstrated a significant reduction of gingival, bleeding, and plaqueindices in

both groups over a period of 21 days as compared to placebo. A. indica-based mouth rinse is equally efficacious with fewer side effects as compared to chlorhexidineand may be used as an adjunct therapy in treating plaque induced gingivitis.[43]

Datta PS, Chand GP, Sashidhar RK, Sunil KC, Melpati H, Swamy R[53] in 2016 did The study to formulate 2% neem and 2% liquorice mouthwashes and to compare theantimicrobial efficacy of these mouthwashes with the standard 0.2% chlorhexidine mouthwash.Alcoholic solution was prepared and added to neem mixture and liquorice mixture separately andmade up to a volume of 16000 ml with purified water. Nine dilutions of each drug were done withBrain heart infusion broth (BHI) for MIC. Culture suspension was added in each serially diluted tubeof 200 µl. The tubes were incubated for 24 hours and observed for turbidity. Minimum inhibitoryconcentration (MIC) of 2% neem, 2% liquorice and 0.2% chlorhexidine against Lactobacillus,Actinomyces naeslundii, Streptococcus sanguis, Streptococcus mutans is determined by serialdilution analysis. Streptococcus mutans shows sensitivity to all three mouthwashes at aconcentration starting from 0.2 µg/ml. Lactobacillus shows sensitivity to neem and chlorhexidine mouthwashes at a concentration starting from 1.6 µg/ml, whereas liquorice is effective at aconcentration starting from 3.125 µg/ml. Streptococcus sanguis shows sensitivity to chlorhexidineand liquorice mouthwashes at a concentration starting from 25 µg/ml, whereas it shows sensitivityto neem at a concentration starting from 50 µg/ml. Actinomyces naeslundii shows sensitivity tochlorhexidine and neem mouthwashes at a concentration starting from 1.6 µg/ml, whereas it showssensitivity to liquorice at

a concentration starting from 3.125µg/ml. Analysis showed an inhibition ofall the four strains by the mouthwashes. The MIC for the studied mouthwashes was found to besimilar to that of 0.2% chlorhexidine.

Jalaluddin M, Rajasekaran UB, Paul S, Dhanya RS, Sudeep CB, Adarsh VJ in 2017[14] aimed at evaluating the impact of neem-containing mouthwash on plaque and gingivitis. This randomized, double-blinded, crossover clinical trial included 40 participants aged 18 to 35 years with washout period of 1 week between the crossover phases. A total of 20 participants, each randomly allocated into groups I and II, wherein in the first phase, group I was provided with 0.2% chlorhexidine gluconate and group II with 2% neem mouthwash. After the scores were recorded, 1-week time period was given to the participants to carry over the effects of the mouthwashes and then the second phase of the test was performed. The participants were instructed to use the other mouthwash through the second test phase.There was a slight reduction of plaque level in the first phase as well as in the second phase. When comparison was made between the groups, no statistically significant difference was seen. Both the groups showed reduction in the gingival index (GI) scores in the first phase, and there was a statistically significant difference in both groups at baseline and after intervention (0.005 and 0.01 respectively). In the second phase, GI scores were reduced in both groups, but there was a statistically significant difference between the groups only at baseline scores (0.01).In the present study, it has been concluded that neem mouthwash can be used as an alternative to chlorhexidine mouthwash based on the reduced scores in both the groups.Using neem mouthwash in maintaining oral hygiene might have a better impact in prevention as well as pervasiveness of oral diseases as it is cost-effective and easily available.

Vinod K S et al[19] in **2018** The aim of the present study was to compare the effect of two broad categories of mouth washes namely chlorhexidine and herbal mouth washes. Eleven randomized control studies were pooled in for the meta-analysis.

The search was done from the Pub Med Central listed studies with the use keywords with Boolean operators (chlorhexidine, herbal, mouth wash, randomized control trials). The fixed effects model was used for analysis. results: This meta-analysis brings to light, the fact that a wide range of newer herbal products are now available. As with a plethora of herbal mouthwashes available it is the need of the hour to validate their potential use and recommendation. This study found that only two studies favor the use of herbal products and four studies favor the use of chlorhexidine, of the 11 studies that were analyzed.

METHODOLOGY

Effectiveness of Azardirachta Indica (neem extract) mouth rinse on plaque and gingival health- an interventional study.

STUDY AREA:

•   Lucknow is the capital city of the Indian state of Uttar Pradesh.

•   The city stands at an elevation of approximately 123 meters (404 ft) above sea level. Lucknow district covers an area of 2,528 square kilometers (976 sq mi)

•   Bounded on the east by Barabanki, on the west by Unnao, on the south by Raebareli and in the north by Sitapur and Hardoi, Lucknow sits on the northwestern shore of the Gomti River.

•   According to the provisional report of 2011 Census of India, Lucknow city had a population of 2,815,601, of which 1,470,133 were men and 1,345,468 women.

STUDY DESIGN:

 A Parallel, multi arm, randomized controlled trial was done to evaluate the

effectiveness of Azardirachta Indica (neem extract) mouth rinse on plaque and gingival health.

Allocation ratio:

The allocation ratio of the study was 1:1

The randomized control trial (RCT) is a trial in which subjects are randomly assigned to one of two groups: one (the experimental group) receiving the intervention that is being tested, and the other (the comparison group or control) receiving an alternative (conventional) treatment. The two groups are then followed up to see if there are any differences between them in outcome. The results and subsequent analysis of the trial are used to assess the effectiveness of the intervention, which is the extent to which a treatment, procedure, or service does patients better than harm. RCTs are the most stringent way of determining whether a cause-effect relation exists between the intervention and the outcome.

ELIGIBILITY CRITERIA:

Inclusion criteria- A. Patients with a minimum 20 sound natural teeth.

B. A mean plaque index (P1I) of 1.05

C. A mean gingival index of 1.0

Exclusion Criteria- A. Patients not willing to complete the treatment protocol.

B. Pregnant women

C. Subjects under any type of antibacterial mouth rinse within 4 weeks of recruitment in the study.

PILOT STUDY:

Pilot study consisting 30 participants of the age group 18-55 years were done. The result of the pilot study was not included in the final result of the research.

Examiner calibration was done at the same time.

A pilot study saves precious time, identify potential difficulties and prompt modifications that may be necessary before the actual survey is initiated.

SAMPLING METHOD:

A simple random sampling by fish bowl method was done to select the participants.

SAMPLE SIZE:

A total sample size of 120 participants was calculated using G Power analysis software. The power of the study was kept to a moderate range of 80% with effect size of 3 for medium range using Cohens effect size with a B error of 20% and alpha error of 5%. The confidence interval was set to 95% with margin of error of 5%.

LOCATION OF THE STUDY:

The study was a single centre trial conducted in the Department of Public Health Dentistry of Babu Banarasi Das College of Dental Science.

METHOD OF RECRUITMENT:

The participants were selected from the the OPD of the department.

SCHEDULE:

The study was carried for 4 months

PREPARATION OF NEEM AQUEOUS EXTRACT-

Neem aqueous extract was prepared by mixing 15.0 g of dry powder of neem leaves with 100 ml of sterile distilled water in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth for

coarse residue and finally through Whatman NO. 1 filter paper and kept in an airtight amber coloured container.

PREPARATION OF NEEM ETHANOLIC EXTRACT-

Neem extract was prepared by macerating 15.0 g of dry powder of neem leaves with 100 ml of 70% (w/v) ethyl alcohol for a week in a round bottom flask with ocassiona shaking. The flask was kept in the dark to avoid effect of light on the active ingredients of the neem. The exrract was then filtered through a muslin cloth for coarse residue and finally through Whatman NO.1 filter paper and kept in an airtight amber coloured container.

METHOD

A randomized controlled, parallel group clinical trial was undertaken for 4 months to assess the effect of neem based mouth rinse on gingival health. After obtaining informed consent from the study participants, eligible patients was randomly assigned into 4 groups.

GROUP 1- patients using aqueous based neem mouth rinse.

GROUP 2- patients using alcohol based neem mouth rinse.

GROUP 3- patients using chlorhexidine mouth rinse who served as positive controls.

GROUP 4- patients using normal saline as mouth rinse who served as negative controls.

The preparation of neem mouth wash rinse was carried out at the Dept. of Pharmacy of BBDU. The appearance, color and smell of all the mouth rinses were kept identical as far as possible. Eligible participants were given instructions regarding oral hygiene procedures in order to ensure standardization. This was

followed by a baseline examination which included recording of gingival index and plaque index. The same was continued till 4 months at monthly intervals. The gingival index and the plaque index readings of all the time periods was entered into a master chart.

ADMINISTRATION OF MOUTHWASH

Patient were allocated to four groups accordingly— CHX, neem, neem and alcohol and water negative control group. Before using the mouth wash patients were asked to refrain from eating, drinking, or performing oral hygiene procedures for at least 30 min. Patients were requested to rinse with 5ml of their mouth with the allocated mouth-rinse for 30 s regularly for 15 days and recalled at regular intervals. Patient were asked to refrain from eating or drinking half an hour after using the moth wash.

ETHICAL ISSUES:

Ethical clearance was given by the Institutional Ethical committee of Babu Banarasi Das College Of Dental Sciences.

CONSENT:

Informed consent was obtained from the participants.

Patients' informed consent is a legal regulation and a moral principle. It represents patients' rights to take part in the

BLINDING:

A triple blinding method was done to reduce the bias of the study. The blinding was done by providing both the treatment and placebo group with identical bottles. The bottles were coded as A,B,C &D. the examiner as well as the statician were blinded to th grups.

ALLOCATION CONCEALMENT:

The allocation concealment for the participants was done by envelope method. The groups were coded as A,B,C & D. the participants were given the envelops with the codes and assigned to the group.

IMPLEMENTATION:

The allocation concealment, enrollment of participnats and assigning them to intervention group was done by the organizing clerk.

RANDOMIZATION:

The unit of randomization were participants with periodontal health.

TYPE OF RANDOMIZATION:

Simple randomization was done and there were no restrictions.

ANY INTERIM ANALYSES AND STOPPING GUIDELINES:

The participants were asked to discontinue the research if they develop any allergic reaction due to the mouthwash.

None of the participants developed any reaction so there were no missing data.

CALIBRATION AND TRAINING

The calibration of the principal investigator was done by the research head who had conducted various clinical trials study and has thorough knowledge of the subject.

Research head who was trained in accordance with the recommended methods for basic oral hygiene survey was appointed to act as a validator for the survey team.

The calibrator examined at least 25 subjects who was also examined by the investigator

INTRA-EXAMINER REPRODUCIBILITY

Examiner first practiced the examination on a group of 10 subjects with a periodontal health

These subjects were pre-selected so that they collectively represent the full range of conditions expected in periodontal health.

By comparing the results of the two examinations, the examiner can obtain an estimate of the extent and nature of their diagnostic variability.

DUPLICATE EXAMINATION

To allow detection and correction of this tendency, the examiner performed duplicate examinations on 5–10% of the sample to assess the plaque index.

RECORDING CLERK

Examiner was assisted by an alert and cooperative recording clerk who was seated close to the examiner, followed instructions precisely and neatly note down numbers and letters.

Clear instruction was given to the clerk clear about recording the data on the assessment form. The meaning of the terms that will be used in the proforma was explained to the clerk and she were instructed in the coding systems so that, with practice, she will be able to recognize obvious mistakes or omissions made by the examiner.

Before the survey begins, the clerk practiced recording the findings of a few preliminary examinations.

Special instructions were given and additional practice was undertaken as the clerk was not familiar with the alphabetical or numerical symbols used on the assessment form.

Failure to ensure that the recording clerk is making clear entries may result in confusion between codes later on in the process.

ORGANIZING CLERK

Organizing clerk was to maintain a constant flow of subjects to the examiner(s) and to enter general descriptive information on the record forms.

The organizing clerk also checked the finished records for accuracy and completeness, so that missing information may be obtained before the survey team moves to another location.

This person was also be responsible for ensuring that the examiners have an adequate supply of sterile instruments.

INSTRUMENTS AND SUPPLIES

The quantity and weight of instruments and supplies used in the survey was kept to a minimum; however, sufficient numbers of instruments were available to avoid the need to temporarily stopping examinations while used ones are being sterilized.

A table or platform to hold the dental instruments and basins should be within easy reach of the examiner.

The following instruments and supplies were used

• instruments for oral examination: plane mouth mirrors; metallic periodontal probes (Community Periodontal Index (CPI) probe) that conform to WHO specifications, i.e. 0.5 mm ball tip; a black band between 3.5 and 5.5 mm and rings at 8.5 and 11.5 mm from the ball tip; and several pairs of tweezers;

• containers (one for used instruments and one for disinfecting or sterilizing instruments) and concentrated disinfecting solution in sufficient quantity;

• rubber gloves;

- wash basin for either water and soap or disinfectant solution;

- cloth or paper hand towels; and

- gauze.

Used instruments was placed in disinfectant solution, then washed and drained well before sterilization.

INFECTION CONTROL

The investigator and all personnel participating in the survey were made aware of the possibility of cross-infection when conducting examinations or handling contaminated instruments

Current national recommendations were responsibly followed for both infection control and waste disposal.

EXAMINATION AREA

The participants were examined in a full dental set up with proper lighting. ADA specification type II clinical examination was used .

The participnts were seated on dental chair upright.

SURVEY FORMS

An adequate supply of assessment forms, hardboard bases and clips, sharpened pencils, erasers and copies of the recording instructions, coding lists and measurement criteria was readily available

PRIMARY OUTCOME:

gingival index

plaque index

SECONDARY OUTCOME:

oral hygiene index

ANY SIDE EFFECTS:

No Side effects were noted during the study

DATA ANALYSIS

Descriptive statistics were used for the demographic data.

Paired t test was used to compare the means within the group

Multivariate ANOVA was used on continuous data to compare the means between the group.

Post hoc test was use to determine the effectiveness of the groups.

PREPARATION OF THE AZARDIRACHTA INDICA (NEEM) MOUTHWASH

AIM: The aim is to prepare an Anti-bacterial Herbal Mouthwash from the extract of leaves obtained from Azadirachta Indica.

COLLECTION OF PLANT LEAVES: Leaves of Azadirachta Indica (Neem) were randomly collected from mature plants.

EXTRACTION PROCESS: The leaves were washed with sterile water, shadow dried, pulverized and stored in air-tight bottles.

The Aqueous extracts were prepared by soaking the powdered leaves in sterile distilled water and maintained in Incubator at 37°C for 72 h and were filtered using Whattmann filter paper.

EQUIPMENTS: Sterile Petriplates, Test tubes, Conical flask, Whattmann filter paper, Incubator, Autoclave, Laminar air flow, Pippetting device, Hotair-oven.

Aqueous extracts of Leaves by Shadow drying technique

(i)   The leaves of mature plants were collected and washed 3-5 times with tap water to remove dust and dirt.

(ii)   The leaves were allowed to soak in already boiled water bath at 30-40ºC for 10- 15 mins to kill microbes present in the surface of the leaves.

(iii)   The leaves undergo Shadow drying technique in which

 the leaves were spread in sterile container trays and kept at ambient temperature for 5 days.

(iv)   After 5 days, the dried leaves were taken and powdered by using sterile mixer under aseptic condition.

(v)   The pulverized leaves are transferred to air-tight sterile container jars.

(vi)   100ml of sterile distilled water was taken in 4 conical flasks (250 ml), the pulverised leaves were weighed and suspended in distilled water under sterile condition.

(vii)   The preparation was heat sterilised at 40°C for 5-10 mins and was kept for incubation at 37°C for 72h.

(viii)   After incubation, the extracts were filtered with the help of a sterile Whattmann filter paper no: 1 and a funnel under lab condition.

(ix)   The filtered extracts were boiled vigorously again to kill the bacterial spores, which will prevent contamination.

(x)   The extracts after heating is ready to use for the formulation of Mouth wash.

RESULT

The study comprised of 120 individuals, of which 60 were male and 60 were female.

The mean age of the participants was 39 years.

GROUP A	Chlorhexidiene (positive control)
GROUP B	Neem
GROUP C	Neem + Alcohol
GROUP D	Distilled water (negative control)

Group A comprised of 30 individuals,15 males and 15 females. The baseline data was collected at the beginning of the study. The mean plaque index was 1.53+/-0.06.The gingival index at baseline was 2.66+/- 1.00 which was more compared to the plaque index. as depicted in table 2.

Table 2: baseline data of group A

GROUP A	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	1.53+-0.06	2.66+/-1.00

Group B comprised of 30 individuals, the baseline data was collected at the beginning of the study. The mean plaque index was 1.53+/- 0.06. (TABLE 3). The gingival index at baseline was 2.53+/- 0.73.The plaque Index of Group B was less compared to Gingival Index.

TABLE 3: baseline data of group B for Plaque Index and Gingival index

GROUP B	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	1.53+-0.06	2.53+/-0.73

Group C comprised of 30 individuals, the baseline data was collected at the beginning of the study. The mean plaque index was 1.55+/- 0.06.

The gingival index at baseline was 2.44+/- 0.71 (TABLE 4)

Table 4: baseline data for plaque and gingival index for group C

GROUP C	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	1.55+-0.06	2.44+/-.071

Group D comprised of 30 individuals, the baseline data was collected at the beginning of the study. The mean plaque index was 1.43+/- 0.03

The gingival index at baseline was 2.39+/- 0.75 (TABLE 5)

Table 5: baseline data for plaque and gingival index for group D

GROUP D	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	1.43+-0.03	2.39+/-0.75

Table 6 shows distribution and comparison of baseline of subjects and was found to be non significant.

Table 6: distribution and comparison of baseline of subjects. ANOVA applied.

	GROUP A	GROUP B	GROUP C	GROUP D	p value
MEAN P.I	1.53+-0.06	1.53+-0.06	1.55+-0.6	1.43+-0.3	0.543
MEAN G.I	2.66+/-1.00	2.53+/-0.73	2.44+/-0.71	2.39+/-0.75	0.671
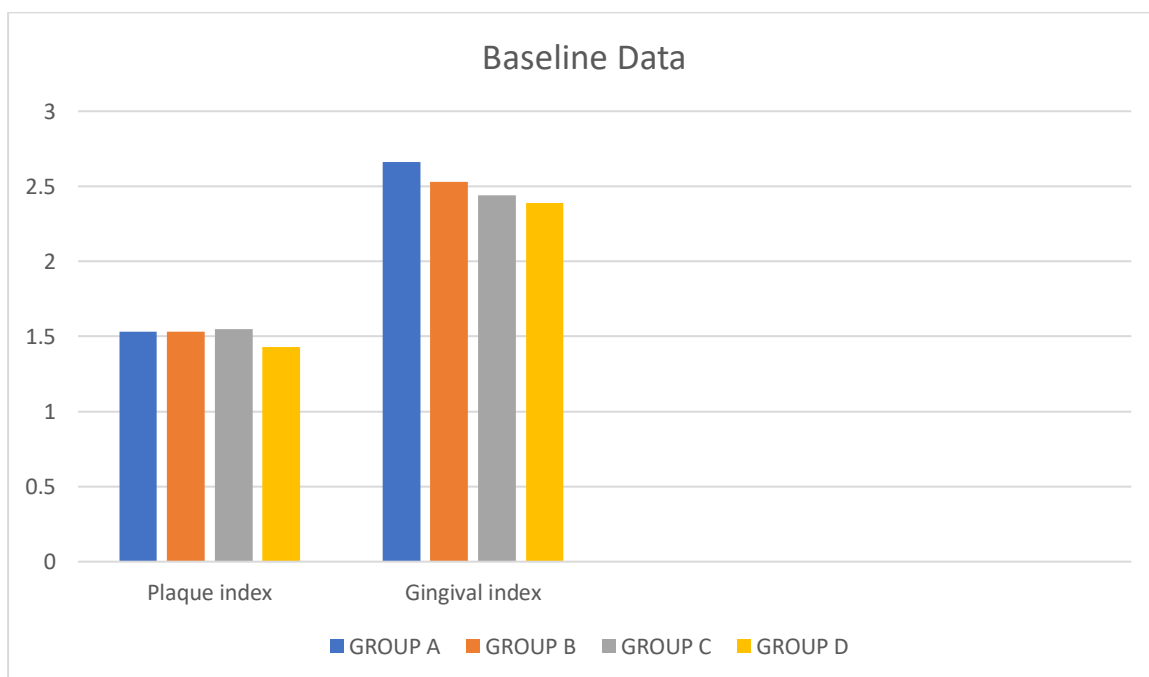
Graph 1: distribution and comparison of baseline of subjects. ANOVA applied.

Table 7 shows the change in plaque and gingival index after use of 15 days. Mean was found to be 0.83+/-0.43 and the gingival index was found to be 1.3+/- 0.43

Table 7: Plaque index and Gingival Index of Group A after 15 days.

GROUP A	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	0.83+/- 0.43	1.3+/-0.43

Table 8 shows the change in plaque and gingival index after use of 15 days. Mean was found to be 0.53+/-0.55 and the gingival index was found to be 1.22+/- 0.71

Table 8: Plaque index and Gingival Index of Group B after 15 days.

GROUP B	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	0.53+/- 0.55	1.22+/-0.71

Table9 shows the change in plaque and gingival index after use of 15 days. Mean was found to be 0.51+/-0.49 and the gingival index was found to be 1.21+/- 0.7

Table 9: Plaque index and Gingival Index of Group C after 15 days.

GROUP C	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	0.51+/- 0.49	1.21+/-0.7

Table 10 shows the change in plaque and gingival index after use of 15 days. Mean was found to be 1.43+/-0.03 and the gingival index was found to be 2.39+/-0.75
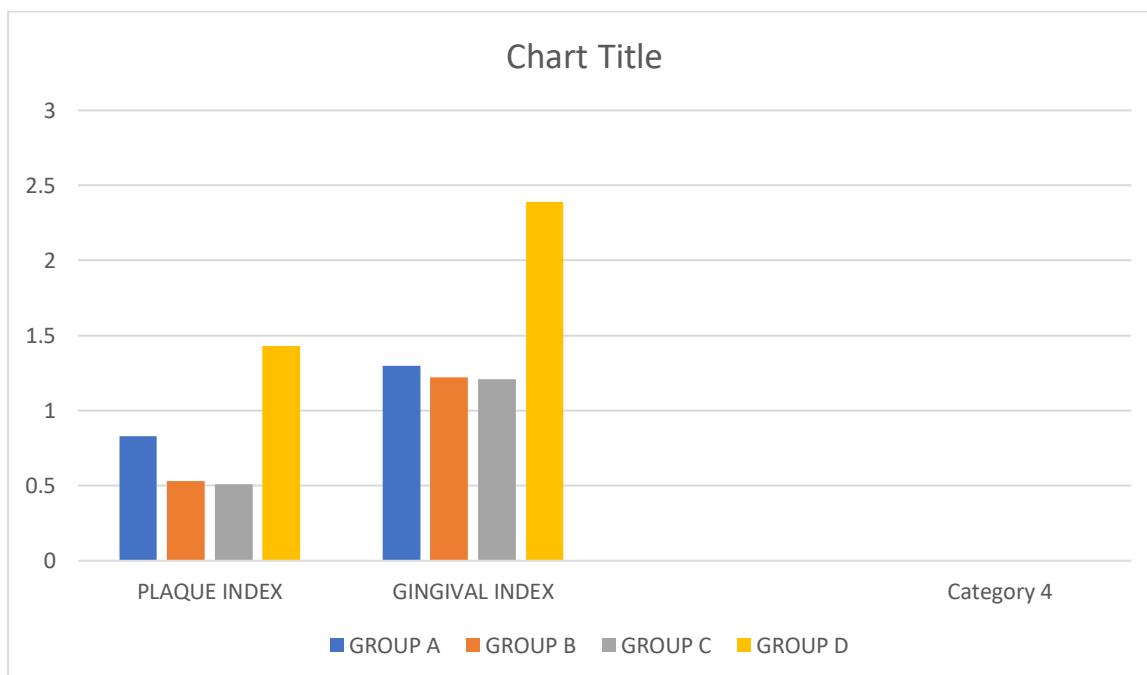
Table 10: Plaque index and Gingival Index of Group A after 15 days.

GROUP D	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	1.43+-0.03	2.39+/-0.75

Table 11 shows distribution and comparison of Plaque Index and Gingival Index of subjects after 15 days. A difference in mean was observed in between group comparison which was found to be non significant

Table 11: distribution and comparison of Plaque Index and Gingival Index of subjects after 15 days

	GROUP A	GROUP B	GROUP C	GROUP D	p value
P.I	0.83+-0.43	0.53+-0.55	0.51+-0.49	1.43+-0.03	0.543
G.I	1.3+/-0.43	1.22+/-.073	1.21+/-0.7	2.39+/-.075	0.671

Graph 2: shows distribution and comparison of Plaque Index and Gingival Index of subjects after 15 days

Table 12 shows the change in plaque and gingival index after use of 30 days. Mean was found to be 0.83+/-0.44 and the gingival index was found to be 1.3+/- 0.43

Table 12: Plaque index and Gingival Index of Group A after 30 days.

GROUP A	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	0.81+-0.41	1.1+/-0.75

Table 13 shows the change in plaque and gingival index after use of 15 days. Mean was found to be 0.47+/-0.6 and the gingival index was found to be 1.1+/-0.75

Table 13: Plaque index and Gingival Index of Group B after 15 days.

GROUP B	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	0.47+-0.6	1.1+/-0.75

Table 14 shows the change in plaque and gingival index after use of 30 days. Mean was found to be 0.43+/-0.53 and the gingival index was found to be 1.0+/-0.5
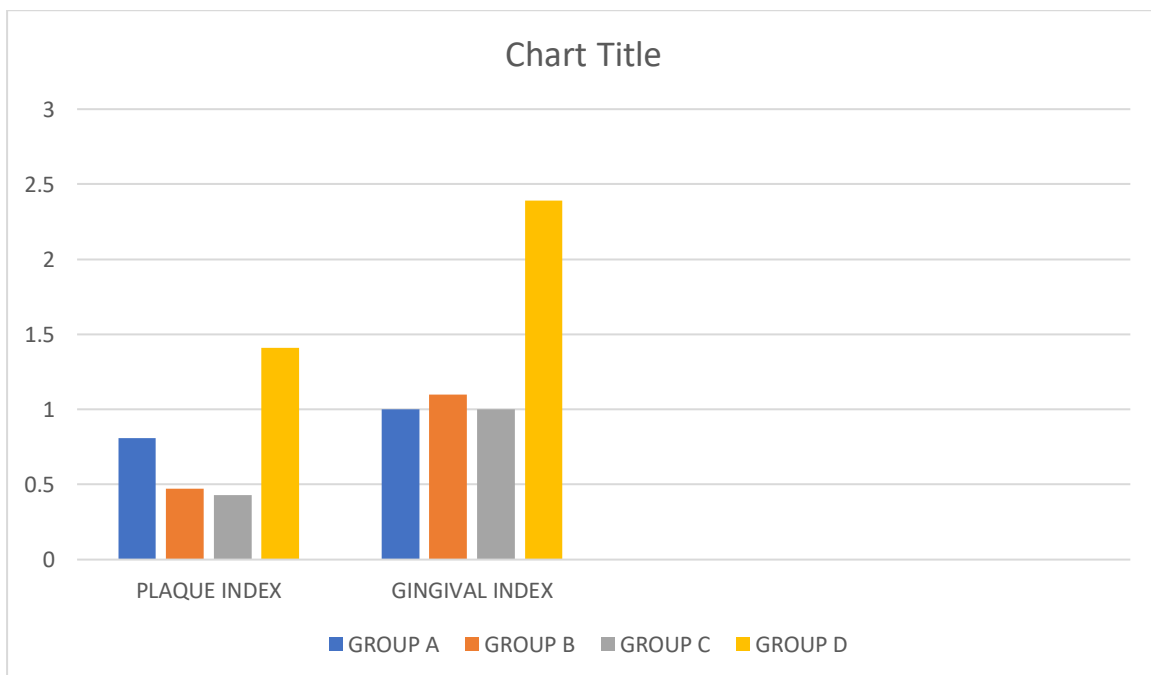
Table 14: Plaque index and Gingival Index of Group C after 30 days.

GROUP C	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	0.43+-0.53	1.0+/-0.5

Table 15 shows the change in plaque and gingival index after use of 30 days. Mean was found to be 1.43+/-0.03 and the gingival index was found to be 2.39+/-0.75

Table 15: Plaque index and Gingival Index of Group D after 30 days.

GROUP D	MEAN PLAQUE INDEX	MEAN GINGIVAL INDEX
	1.43+-0.03	2.39+/-0.75

Table 16 shows distribution and comparison of baseline of subjects and was found to be non significant.the mean difference for plaque index was found to be highest in group D i,e water which was a negative control as compare to other groups. For gingival index it was lowest for chlorhexidine and the mean difference was found to be statistically SIGNIFICANT.

Table 16: comparison and distribution of Plaque index and Gingival Index of Group A 30 days.

	GROUP A	GROUP B	GROUP C	GROUP D	p value
MEAN P.I	0.81+-0.43	0.47+-0.3	0.43+-0.53	1.41+-0.03	0.031
MEAN G.I	1.0+/-0.75	1.1+/-0.75	1.0+/-0.5	2.39+/-.075	0.039

Graph 3: comparison and distribution of Plaque index and Gingival Index of Group A 30 days.
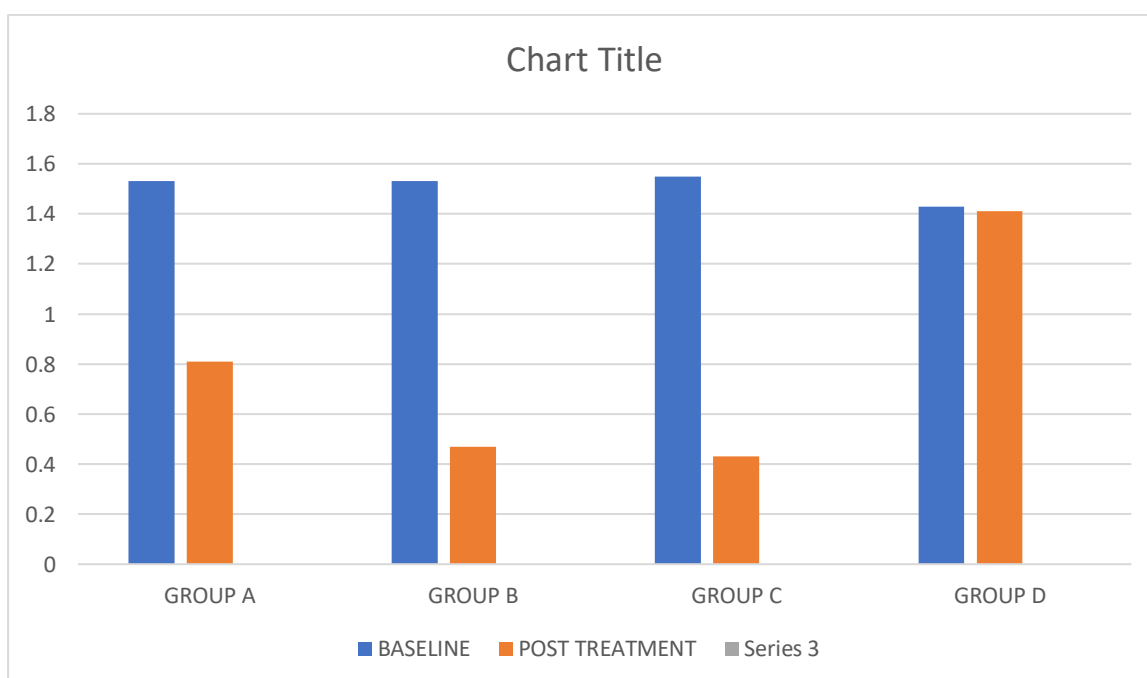
Table 17 shows comparison between of plaque index at base line and post treatment. Group D was found to be non significant, while group A, B and C was found to be significant. A maen difference was found within the groups, neem and alcohol showed the largest difference followed by neem and chlorhexidine, a non significant difference was found in water.

Table 17: comparison and distribution of mean difference within groups

| GROUPS	BASELINE	POST TREATMENT	MEAN DIFFERENCE	p value
GROUP A	1.53+/-0.6	0.81+/-0.43	0.72+/-0.17	0.0231
GROUP B	1.53+/-0.6	0.47+/-0.60	1.06+/-0.3	0.0417
GROUP C	1.55+/-0.6	0.43+/-0.53	1.12+/-0.7	0.0391
GROUP D	1.43+/-0.6	1.41+/-0.3	0.02+/-0.3	0.3112

Paired t test applied; $p < 0.05$

Graph 4: comparison and distribution of mean difference within groups

Table 18 shows comparison of gingival index at base line and post treatment. Group D was found to be non significant, while group A, B and C was found to be significant.

Table 18: comparison and distribution of mean difference within groups

GROUPS	BASELINE	POST TREATMENT	MEAN DIFFERENCE	p value
GROUP A	2.66+/-1.0	1.0+/-0.75	1.66+/-0.25	0.012
GROUP B	2.53+/-0.73	1.1+/-0.75	1.43+/-0.02	0.029
GROUP C	2.44+/-0.71	1.0+/-0.5	1.44+/-0.21	0.013
GROUP D	2.31+/-0.69	2.39+/-0.75	0.08+/-0.06	0.6713

Paired t test applied; $p < 0.05$
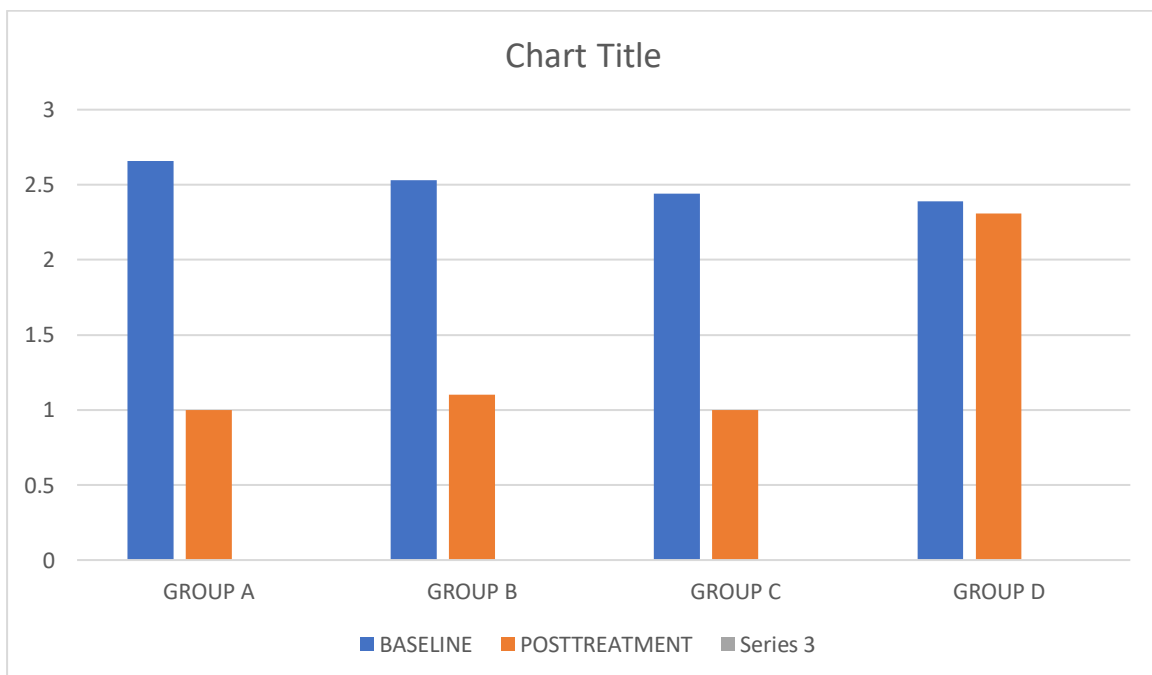
Table 19. Statistical results of the One-way analysis of varience (ANOVA)

Source	Ss	Df	Ms	F	P
Between Groups	12.568	5	51.114 49.874		0.001
Within Groups	33.267	48		0.038 – –	
Total	68983.000	53		–	– –

DISCUSSION

The present randomized controlld trial was done to check the efficiency of neem, a herbal mouth wash as compared to Chlorhexidine a gold standard. There are 4 groups of mouthwash used in the study: Chlorhexidine, Neem, Neem with alcohol and water.

Chlorhexidine is a gold standard with a proven efficiency of reducing in periodontal health but the prolonged use of Chlorhexidine has few limitation like staining of teeth and alteration in taste buds. Hence a herbal mouthwash was tested to see if the efficacy of neem is equal to that of gold standard and if can be used as an alternative to Chlorhexidine.

Neem plant (Azadirachta indica L.) has been considered as one of the most important medicinal plants worldwide.It acts on both gram positive and gram negative bacteria. In India the importance of neem is not unknown. Neem leaf extract has been used as anti-inflammatory, anti-hyperglycemic, anti-ulcer, immunomodulatory, antiviral, anti-fungal, anti-bacterial, nematicidal, anti-malarial, insecticidal, anti-mutagenic and anti-oxidant properties.Neem leaves have the ability to reduce the the tooth and within gingival margins. Gingivitis is also influenced by number of *Streptococcus mutans*, a common plaque-forming bacteria several factors that are divided into two main categories, local factors found in the oral cavity.[10,11]

Alcohol base neem mouth wash provides stability, preservative property and to the mouthwash . it has been known for its antiseptic property Hence, it has been used to see if this can be as effective as water based neem mouth wash.[12]

In our study a significant result was found with the use of Neem mouthwash which rejects the null hypothesis proving Neem to be as effective as Chlorhexidine. Neem with Alcohol was also found to be equally effective.

This result was found to be similar to many studies done by Chaterjee at al[13,] Jalaludin et al[14],Nikita et al[15] Balppanavar aet al[16], Hegazy and Awad[17] Rathika[18] et al. None of the studies present in the literature sgiws any negative result.

The baseline data of the study shows mean plaque index of 1.53+/- 0.06.The gingival index at baseline was 2.66+/- 1.00 which showed significant result after 30 days. In a study done by Chaterjee et al [13] in 2011on 45 participants Within subjects data demonstrated a statistically significant decrease in mean bleeding, for both Chlorhexidine (p=0.03) and Neem(p=0.211) group at 7th and 21st day as compared to baseline except in saline group(p=0.91). It showed a gingival index scores at baseline as well as 7th and 21st day after treatment and the data demonstrated a significant decrease in mean gingival index in Chlorhexidine (p-0.43) Neem (p=0.31) as compared to base line except in saline group (0.61). this result was similar to our study where gingival index for Chlorhexidine was (p=0.012), Neem (p=0.029) and Water was (p=0.673). In the same study by Chaterjee et al shows a plaque index scores at baseline, as well as 7th and 21st day after treatment and data demonstrated same decrease in mean plaque index as compared to baseline except in saline group which was similar to our study.

The result of our study was in accordance with similar study done by Jalaluddin et al [14] A total of 40 participants were involved in the study. a slight reduction of plaque level in chlorhexidine group (0.565 ± 0.258) compared with neem mouthwash group (0.730 ± 0.359). However, no significant difference was seen statistically between the groups. The GI recordings were reduced in both the groups and there was a statistically significant difference in both groups at baseline and after intervention (0.005 and 0.01 respectively). There was a slight reduction of plaque level in neem group (0.670 ± 0.301)

compared with chlorhexidine mouthwash group (0.750 ± 0.209). However, no statistically significant difference was seen between the groups. The GI recordings were reduced in both the groups, but statistically significant difference was seen between the groups only at baseline scores (0.01).

In a similar study done by Nikita et [15] al on 30 participants shows that there was a highly significant reduction between base line and post treatment in Chlorhexidine and Neem groups with significant differences between them (P<0.001). The intra group mean gingival score reduction from baseline to 21 days in Chlorhexidine was (5.39+/-0.10) and Neem (0.577+/_0.112) in the groups. It shows that there was a highly significant reduction in all groups with significant differences between them (P<0.001) which was similar to the current study (p=0.012) for Chlorhexidine and for neem it was (p=0.029) there was a highly significant difference in reduction of the mean plaque scores among neem, CHX groups (P<0.05). However, the maximum mean reduction was shown by neem (mean=0.336) group followed by CHX (mean=0.130) (P<0.001).

These results of the present study is in accordance with the study done by Balappanavar et al, [16] and Hegazy and Awad [17].

The result of study done by Rathika et al [18] showed a statistically significant difference between the plaque scores of mango and neem mouthwashes at 21 days ($P = 0.036$), and at 1 month ($P \leq 0.001$) and 2 months ($P = 0.027$). Mango and chlorhexidine also differed significantly at 1 month ($P = 0.36$) and 2 months ($P = 0.01$). There was a statistically significant difference between the gingival scores neem mouthwashes at 21 days ($P < 0.001$) and at 1 month ($P \leq 0.001$). Chlorhexidine also differed significantly at 21 days ($P < 0.001$), and at 1 month ($P = 0.001$) and 2 months ($P = 0.001$). Gingival scores of neem and chlorhexidine differed significantly at 2 months ($P = 0.001$).

Vinod K [19] at al did a study on Comparison between herbal and CHX group using revealed that, in relation to the baseline microbial colony count (day 0), to day 7, was found reduced in both the mouthwash groups, but the difference was not statistically significant (P = 0.35 and P = 0.74 for herbal and CHX respectively), and on day 14, microbial count was reduced in both the groups and was statistically significant (P< 0.001) with greater reduction noted in the herbal group

According to Botelho et al, [20] A. indica-based mouthrinse has high efficacy and it can be used as an alternative treatment for periodontal diseases, which is in accordance with our current study. A reduction in the probing pocket depth and gain in the clinical attachment level by the use of neem extract were reported by Patel and Venkatakrishna-Bhatt.[21]

The results of this study were in accordance with the results of a study conducted by Pai et al,[22]*A. indica* extract showed a significant reduction in the PI

Wolinsky et alwho [23]stated that there was a marked reduction in the bacterial aggregation, growth, adhesion to hydroxyapatite, and production of insoluble glucan that affects the formation of *in vitro* plaque by the use of aqueous extracts of neem, derived from the bark-containing sticks (neem stick) of *A. indica*.

In another study conducted by Nayak A *et al* [24] observed inhibition of *E. faecalis, S. mutans, C.albicans* by alcoholic neem extract at 1.88%, 7.5% and 3.75% respectively and the aqueous neem extract at 7.5%. Maragathavalli S. *et al* [25] demonstrated inhibition of *Bacillus pumillus, Pseudomonas aeruginosa* and *Staphylococcus aureus* by the methanol and ethanol extracts of neem. Widowati[26] in their study concluded that the neem stick extract had a higher antibacterial effect on *Streptococcus mutans* than the neem leaf extract. Chloroform extracts of neem were identified to inhibit *Streptococcus mutans, Streptococcus salivarious* and *Fusobacteriumnucleatum* by Packialakshmi *et al* [27] The minimum

inhibitory concentration of acetonic extract of neem for *Streptococcus sobrinus* was observed to be 0.05% (w/v) by M Bhuiyan *et al* [57] In a study by Prashant *et al* [28] on the effect of neem extract on Streptococcus count a maximum zone of inhibition on *Streptococcus mutans* was observed at 50% concentration of neem extract with minimal effect on *Streptococcus mitis, Streptococcus salivarius* and *Streptococcus sanguis.*

Currently, toothbrushing is the most popular self-performed oral hygiene method to mechanically remove dental plaque. However, this mechanical approach by most individuals is often not sufficiently effective, [29]suggesting that a chemical plaque control by mouthwashes could be beneficial as a supplement to daily oral care [30]. Chlorhexidine (CHX) is a bis-biguanide with documented bacteriostatic and bactericidal effects, on both Gram positive and - negative bacteria 23, fungi and some lipophilic viruses [31]. In the 1970's CHX was studied and recommended by researchers as part of the prevention and therapy of periodontal diseases because of its plaque inhibitory effect.[32-34] Besides its proven immediate bactericidal effect, chlorhexidine binds to the oral mucosa from which it is slowly released, prolonging its antibacterial effect.[35] a broad-spectrum antiseptic, is considered as the gold standard for chemical dental plaque control,[36] but CHX mouthwashes can lead to the staining of teeth and tongue, taste disturbance, and adverse effects on the oral mucosa after prolonged use.[37,38] Those undesirable side effects limit the long-term use and the patient acceptability of CHX mouthwashes. Thus, the search for alternatives continues, and the focus shifted toward biogenic agents.The antimicrobial effect of chlorhexidine is dose-dependent. Chlorhexidine at low concentrations (0.02%-0.06%) has bacteriostatic activity, whereas at higher concentrations (> 0.12%) acts more effective against Gram-positive bacteria and weaker against Gram-negative ones.33 CHX is also active against fungi and viruses5,6. Chlorhexidine is not lethal to acid-fast organisms.[39] It is not sporicidal, however may be sporicidal at elevated

temperatures.[40,41] Some bacteria, e.g. strains of Proteus and Providencia, may be highly resistant to the CHX.[42-44] Chlorhexidine as an antiseptic shows comparable activity against Staphylococcus aureus strains susceptible to methicillin (MSSA) and strains resistant to methicillin (MRSA)[45,46]. Also in the case of both resistant to vancomycin strains of enterococci (VRE) and sensitive to vancomycin showed a comparable sensitivity to chlorhexidine [47]. In ex vivo studies have been shown effectiveness of CHX solution against Actinomyces israelii and Enterococcus faecalis42-44 in infected root canal systems. Vianna [48] et al have investigated in vitro the antimicrobial activity of CHX against endodontic pathogens: Enterococcus faecalis, Staphylococcus aureus and Candida albicans. CHX eliminated also anaerobic periopathogens: Porphyromonas endodontalis, Porphyromonas gingivalis, and Prevotella intermedia. Agents containing chlorhexidine gluconate are effective against Propionibacterium, Selenomonas and Serratia marcescens [49,50].As the taste should not be a hindrance for its use with maximal inhibition of bacteria and plaque, 2% of neem was used in this study. For reducing periodontal that registers as chlorhexidine, neem mouthwash was very effective. The results assured an outstanding decrease in GI scores in Chlorhexidine, Neem, Alcohol based neem mouthwash during the 30 days of analysis.Follow-ups within studies between herbal and CHX were always less than four weeks as the long-term use of CHX mouthwashes is not recommended due to its side effects,[52] while the studies between herbal mouthwashes and placebos could have a longer follow-up up to 24 weeks[53]

All similar studied showed significant plaque reduction in Chlorhexidine and Neem. The Gingival reduction has reduced between baseline and post treatment. Neem was found to be equally efficient as Chlorhexidine, no studies were found for Neem with alcohol. Hence further investigation is needed in that area.

LIMITATION

The limitation of the study was its small sample size. Hawthorne's effect can be seen in patients due to over brushing and maintenance of oral health as they were aware of being included in the study. No articles were found for neem with Alcohol which limits the finding in respect to the group.

CONCLUSION

In the present study there was a decrease in the Plaque Index and Gingival Index in Chlorhexidine, water based neem mouthwash, Alcohol based neem mouthwash. The results show a significant reduction in gingival bleeding, and plaque indices in the three groups over a period of 30 day as compared to the negative control group. Neem mouthwash was found to be as effective as Chlorhexidine mouthwash. No side effect was noted during the period of trial. Thus, making waterbased neem mouthwash a better alternative to chemical mouthwash. A herbal mouthwash is more acceptable and of greater importance to Public Health. Water based Neem mouthwash is cost effective hence can serve its purpose for lower socioeconomic status. Comparing the effectiveness of waterbased neem mouth wash and alcohol based neem mouthwash to be equal to Chlorhexidine it can be effectively used to improve oral health of the community.

RECOMMENDATION

Studies with larger sample size should be conducted. No side effect was noted during the study and as neem was found to be as effective as Chlorhexidine it can be prescribed to patient as no staining was observed in Neem. A herbal mouthwash is more acceptable by the people and is also cost effective which makes neem a good choice for patients to improve oral health.

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Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 15

BBDCODS/01/2019

Title of the Project: Effectiveness of Azardirachta Indica (Neem Extract) Mouth Rinse on Plaque and Gingival Health- An Interventional Study.

Principal Investigator: Dr. Siddharth Joel David

Department: Public Health Dentistry

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Siddharth Joel David,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 10th January 2019.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Member-Secretary
(Dr. Lakshmi Bala)
Member-Secretary
IEC
Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
PRINCIPAL
Babu Banarasi Das College of Dental Sciences
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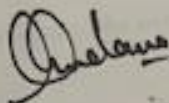BBD City, Faizabad Road, Lucknow-226028

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

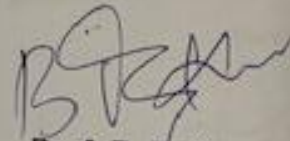## INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled "Effectiveness of Azardirachta Indica (Neem Extract) Mouth Rinse on Plaque and Gingival Health- An Interventional Study." submitted by **Dr Siddharth Joel David** Post graduate student from the **Department of Public Health Dentistry** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **26th November 2018** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

CASE RECORDING PROFORMA

ASSOCIATION BETWEEN DENTAL CARIES AND BODY MASS INDEX AMONG 12- AND 15-YEARS SCHOOL GOING CHILDREN IN LUCKNOW CITY, UTTAR PRADESH

Sl. No.

Date:

Name:

Age/ Gender:

Address:

RECORDING FORMAT FOR PLAQUE INDEX

Score = ☐ Excellent/Good/Fair/Poor

Recording format

Score = ☐