



Research Paper

## Effectness of Azadirachta Indica Mouth Wash on Plaque and Gingival Health- An Interventional Study

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### ABSTRACT

**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. 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. **METHODOLOGY:** A Parallel, multi arm, randomized controlled trial was done to evaluate the effectiveness of Azadirachta Indica (neem extract) mouth rinse on plaque and gingival health in the Department of Public Health Dentistry of Babu Banarasi Das College of Dental Science, Lucknow. A simple random sampling by fish bowl method was done to select the participants. **RESULT:** No side effect was noted during the study and as neem was found to be as effective as gold standard Chlorhexidine it can be prescribed to patient as no staining was observed in Neem. SPSS version 22 was used for data analysis.

**KEY WORDS:** Azadirachta Indica, Plaque, Chlorhexidine, Periodontal disease,

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### I. 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,<sup>1,2</sup> 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). Oral diseases disproportionately affect the poor and socially-disadvantaged members of society.<sup>3</sup> 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. 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.<sup>4-7</sup> 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. Hence the present study aims at evaluating effectiveness of azadirachta indica (neem extract) mouth rinse on plaque and gingival health. The use of Azadirachta 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 Azadirachta Indica mouth rinse so that it can benefit the common people both economically as well as through its anti-bacterial properties.<sup>8-11</sup> The aim of the study was to assess the effectiveness of neem mouth rinse on plaque and gingival health.

## **II. METHODOLOGY:**

A Parallel, multi arm, randomized controlled trial was done to evaluate the effectiveness of Azadirachta Indica (neem extract) mouth rinse on plaque and gingival health.

### **ETHICAL CLEARANCE:**

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.

### **BLINDING:**

A double 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 participants, examiner were blinded to th groups.

The allocation ratio of the study was 1:1

### **ELIGIBILITY CRITERIA:**

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

B. A mean plaque index (PII) 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.

### **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**

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.

**TYPE OF RANDOMIZATION:**

Simple randomization was done and there were no restrictions.

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.

**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.

**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.

**III. RESULT**

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

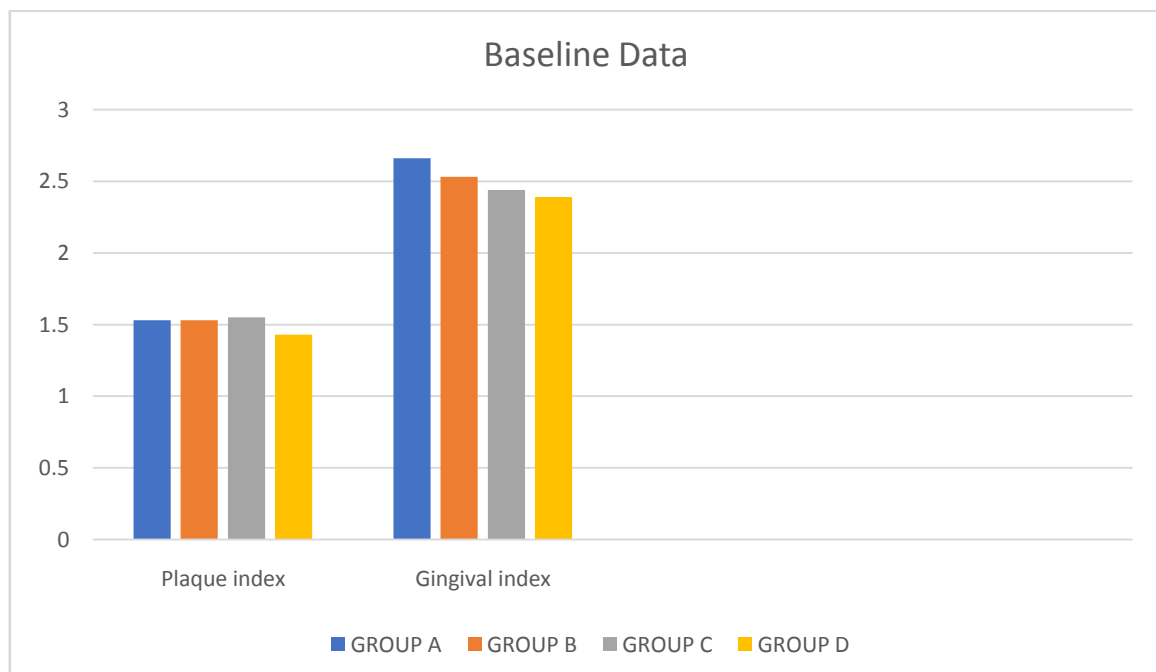
Table 1: The mean age of the participants was 39 years.

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

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

Table 2: 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

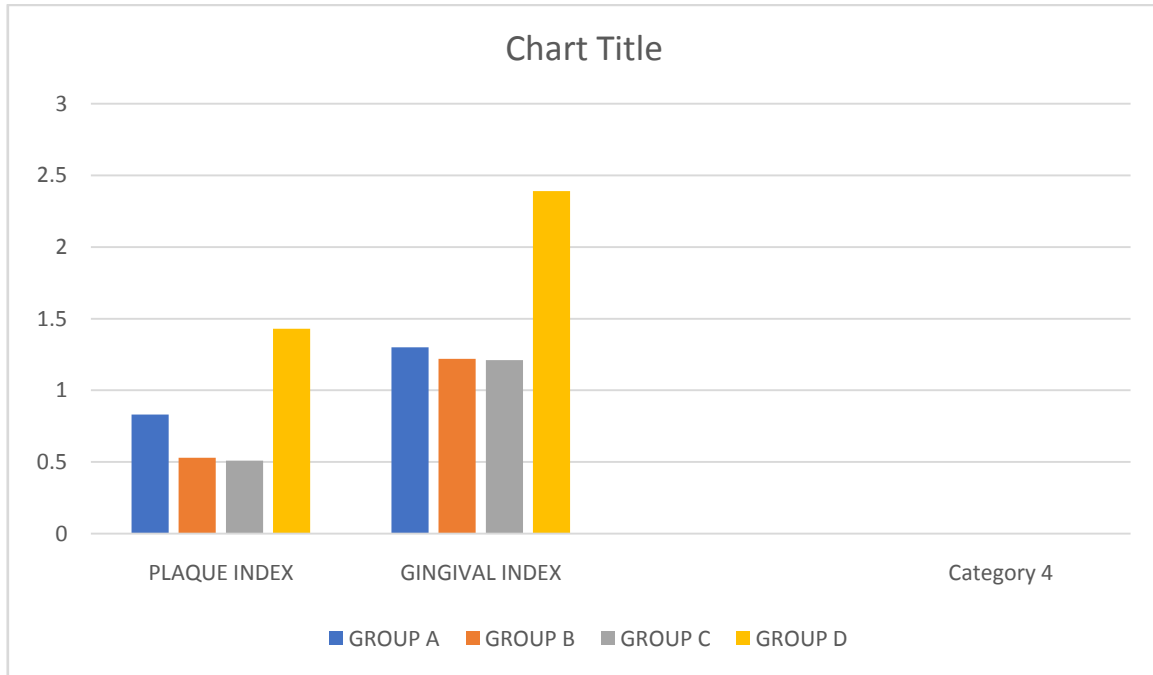


Graph 1: distribution and comparison of baseline of subjects. ANOVA applied.

Table 2 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 2 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+-0.073	1.21+-0.7	2.39+-0.075	0.671

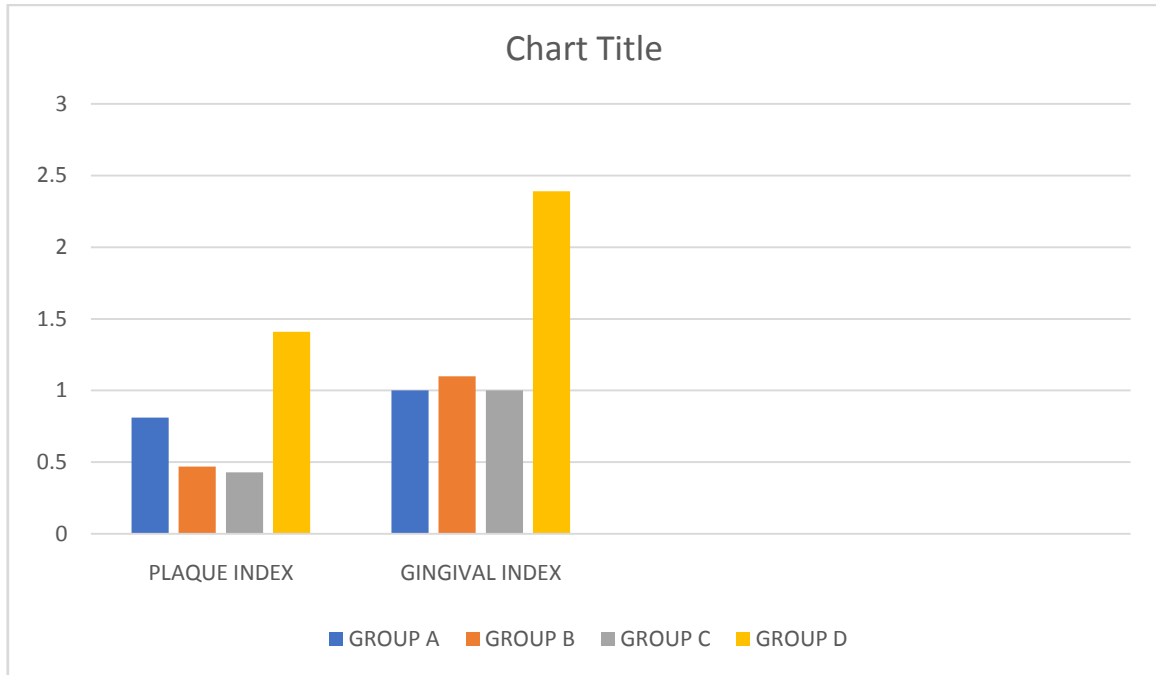


Graph 2: shows distribution and comparison of Plaque Index and Gingival Index of subjects after 15 days

Table 3 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 3: 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+-0.075	0.039



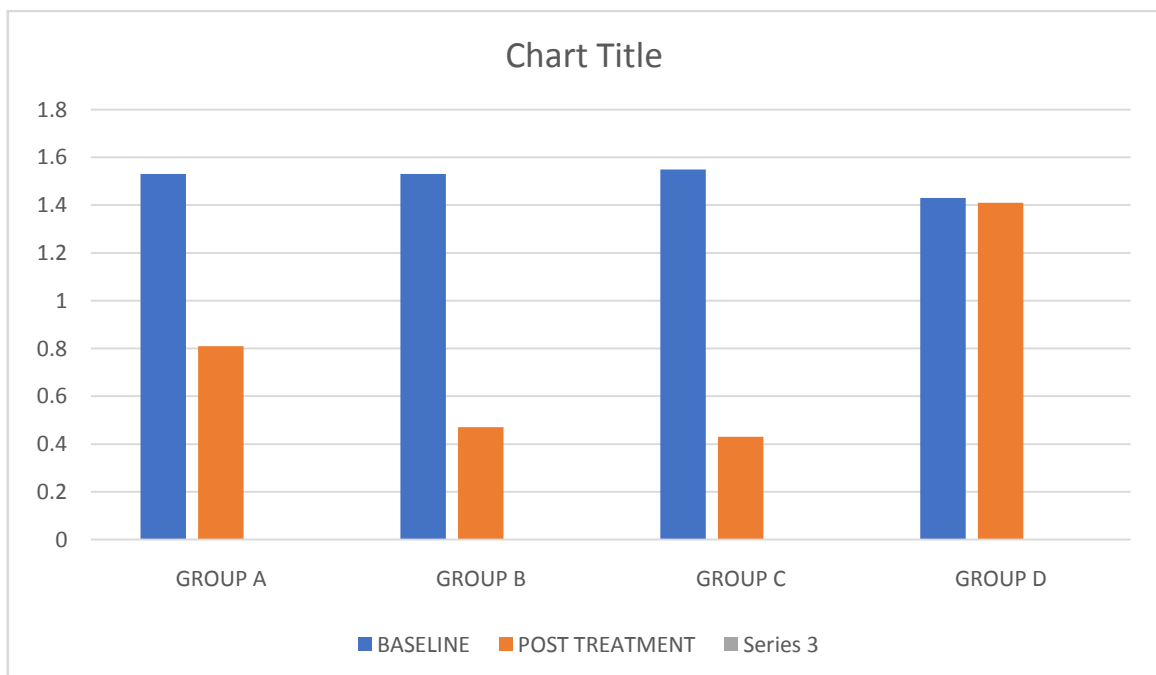
Graph 3: comparison and distribution of Plaque index and Gingival Index of Group A 30 days.

Table 4 shows comparison between of plaque index at base line and post treatment.

Table 4: 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 5 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 5: 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

#### IV. DISCUSSION

The present randomized controlled 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 limitations 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 it can be used as an alternative to Chlorhexidine.<sup>12</sup>

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 Chatterjee et al<sup>13</sup>, Jalaludin et al<sup>14</sup>, Nikita et al<sup>15</sup>, Balppanavar et al<sup>16</sup>, Hegazy and Awad<sup>17</sup>, Rathika<sup>18</sup> et al. None of the studies present in the literature show 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 Chatterjee et al<sup>13</sup> in 2011 on 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 7<sup>th</sup> and 21<sup>st</sup> day as compared to baseline except in saline group (p=0.91). It showed a gingival index score at baseline as well as 7<sup>th</sup> and 21<sup>st</sup> 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 baseline 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).<sup>14</sup>

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 effectively against Gram-positive bacteria and weaker against Gram-negative ones. CHX is also active against fungi and viruses.<sup>19</sup> Chlorhexidine is not lethal to acid-fast organisms. It is not sporicidal, however may be sporicidal at elevated temperatures. Some bacteria, e.g. strains of Proteus and Providencia, may be highly resistant to the CHX. Chlorhexidine as an antiseptic shows comparable activity against Staphylococcus aureus strains susceptible to methicillin (MSSA) and strains resistant to methicillin (MRSA). Also in the case of both resistant to vancomycin strains of enterococci (VRE) and sensitive to vancomycin showed a comparable sensitivity to chlorhexidine. In ex vivo studies have been shown effectiveness of CHX solution against Actinomyces israelii and Enterococcus faecalis<sup>42-44</sup> in infected root canal systems.<sup>20</sup> Vianna 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<sup>21</sup>. 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, while the studies between herbal mouthwashes and placebos could have a longer follow-up up to 24 weeks.<sup>22</sup>

All similar studies 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.

## V. 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.

## VI. 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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