COMPARATIVE CLINICAL EVALUATION OF EFFECTS OF VARIOUS HERBAL MOUTHWASHES ON PERIODONTAL DISEASES

Dissertation

Submitted to

BABU BANARASI DAS UNIVERSITY,

LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirements for the degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTICS

By

Dr. Sugandha Anand

Under the guidance of

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES

LUCKNOW

(Faculty of Babu Banarasi Das University)

BATCH: 2015-2018

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Dr. Sugandha Anand

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CONTENTS

LIST OF APPENDICES

ABBREVIATIONS

GI	GINGIVAL INDEX
PI	PLAQUE INDEX
PPD	POCKET PROBING DEPTH
CHX	CHLORHEXIDINE GLUCONATE
SRP	SCALING AND ROOT PLANING
OHI-S	ORAL HYGIENE INDEX- SIMPLIFIED

ABSTRACT

Periodontal diseases are among the most common infectious diseases affecting human kind and can lead to destruction of the periodontal ligament, cementum, gingiva and alveolar bone. Plaque is the primary etiological factor in gingival inflammation. Thus, control of dental plaque holds the key to halt the progression of periodontal disease. 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. Chlorhexidine has been prescribed by dentists for decades and accepted as the gold standard in reducing dental plaque as it has profound antiplaque and antibacterial properties. However, it has few undesirable adverse effects primarily brown staining of the teeth and transient impairment of taste sensation.

Numerous studies have been conducted to verify the enormous wealth of medicinal plants. These herbal mouthwashes are gaining popularity as they contain naturally occurring ingredients called as Phytochemicals that achieve the desired antimicrobial and anti-inflammatory effects. Herbal formulations may be more appealing because they work without alcohol, artificial preservatives, flavors or colors.

Considering the limitations in present assessment, an attempt was made to evaluate three common medicinal plants from Indian flora representatives for assessment of their use in periodontics. These herbs are Aloe vera, Neem and Curcumin. The purpose for taking them as representatives is their vast utility as medicinal plants in traditional Indian medicine. Therefore, the present study was undertaken to evaluate the clinical effects of various herbal mouthwashes containing Aloe vera, Neem and Curcumin and comparing it with CHX mouthwash on periodontal diseases.

Aloe vera, curcumin and neem mouthwash (test group) are prepared in collaboration with CIMAP Lucknow. Furthermore, the CHX mouthwash used in (control group) is commercially available under the trade mark HEXIDINE with concentration 0.2%. The formulations formed were divided into 4 groups according to the concentrations of herbal extracts with Aloe vera juice base Group A (0.5gm neem and curcumin), Group B (1gm neem and curcumin), Group C (1.5gm neem and curcumin) and Group D (2gm neem and curcumin).

A total of 50 subjects were taken for the study aged 18-35 suffering from chronic gingivitis. The subjects were divided into 5 groups Group CHX, A, B, C and Group D 10 subjects in each group. Clinical parameters PI, GI and PPD at baseline were recorded. All patients underwent scaling and root planing, polishing and oral hygiene instructions were given. Patients were instructed to rinse with their assigned mouthwash (10ml) twice daily for 30 seconds over a period of 28 days. They were recalled for re-evaluation on 7^{th}, 14^{th}, 21^{st} and 28^{th} day and all clinical parameters were recorded and plaque control measures were reinforced.

Upon Inter-group comparison of the mean PI and GI between Group A and C, Group A and CHX, Group B and C, Group B and CHX, Group C and D and Group D and CHX difference was statistically significant at 21 and 28 day interval, which showed that Group C and CHX showed reduced plaque and gingival index.

In Intra-group comparison of the mean PI and GI Group C and Group CHX showed a reduction in PI and GI from baseline to 28 day the reduction was statistically significant.

This study thus showed that the herbal mouthwashes containing Aloe vera, neem and curcumin had antiplaque and antigingivitis property. However, concentration of 1.5 gm was found to be statistically significant and showed results comparable to 0.2% chlorhexidine. The use of natural herbal preparations in oral healthcare continues to be popular, and these herbal extracts may be a useful substitute.

Dental plaque is an adherent bacterial biofilm that forms on hard and soft tissues intra-orally. Plaque control and prevention of gingivitis is the main goal of prevention of periodontal diseases, affecting more than 90% of the population, regardless of age, sex or race.[1, 2] Mouth rinses generally considered as adjuncts to oral hygiene and widely used in delivery of active agents to the teeth and gums. Such agents have been frequently prescribed as adjuvant in the prevention treatment of oral diseases because they have inhibited bacterial colonization, growth and metabolism and consequently interrupt the formation of mature bio-film, changing it at biochemical and ecological levels.[3, 4, 5]

Chlorhexidine

The most frequently used compound for plaque control is Chlorhexidine (CHX) which is a broad spectrum antibiotic with pronounced anti-microbial effects on both gram-positive and gram-negative bacteria as well as on fungi and some viruses.[6] Side effects include: transient impairment of taste sensation or taste perturbation where salt tastes appear to be preferentially affected.[7] Parotid swelling is a rare unwanted effect of CHX mouthrinse. Over vigorous mouth rinsing may predispose patient to such condition as it may create negative pressure in the duct and aspiration of CHX. Occasionally reported cases are case of burning sensation and painful desquammative lesion on oral mucosa.[8] There may also be increased supragingival calculus formation due to use of CHX mouthrinse as CHX causes precipitation of salivary protein on the tooth surface thereby increasing pellicle thickness and precipitation of inorganic salts on the pellicle layer. The brownish discoloration of teeth is due to disintegration of the bacterial membrane leading to denaturation of the bacterial protein. It should not be used for more than 2 weeks because of its side-effects.[9]. Hence, there is need to develop a naturally occurring indigenous and having no side- affects oral hygiene aid. Such aid could be in form of Aloe Vera, curcumin and neem extract.

Aloe Vera (*Barbadensis miller*)

It a medicinal plant that has been used as a traditional remedy for a variety of conditions like burns, hair loss, skin infections, hemorrhoids, gastrointestinal pain (GI). It is also a wound healer for bruises, x-ray burns, insect bites. Aloe Vera can be used as a moisturizing agent; it has been used for various skin diseases including radio dermatitis, frostbite, psoriasis and genital herpes

infection. Vitamin C present in Aloe Vera is involved in collagen synthesis and increases the concentration of oxygen at the wound site because of the dilation of blood vessel.[10] Plaque reduction is seen with Aloe Vera over a period of 28 days, when compared to chlorhexidine.[11] Aloe Vera may therefore, be used as good, easily available and with minimal or no adverse effects in comparison to CHX.

Neem (*Azadirachta indica*)

It is popularly known as Indian neem (margosa tree) or Indian lilac. Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine.[12] Given its immunostimulant, antiulcerative, antifungal, antibacterial, antiviral and antioxidant activity as well as its varying degrees of effect on central nervous system, Neem has been tried as an excellent antimicrobial agent, pain reliever and tissue protector in Periodontics. Today, neem extracts are used to treat various skin diseases, as an antiseptic substance, against endo and ectoparasites or simply as an herbal mouthwash[13] .Neem extract has also an excellent effect as a non-toxic repellent, insecticide and pesticide. Neem has been shown to have significant effects on both gram-positive and gram-negative organisms and other bacteria that cause a wide array of human and animal diseases including E. coli, streptococcus and salmonella. Some of the more recent work has focused on oral care, a critical issue in both developing countries where professional dental care is limited and in developed nations where populations are aging. Extracts from neem sticks or bark have been shown to inhibit the growth of Streptococcus mutans.

Curcumin (*Curcuma Longa*)

Turmeric or *Curcuma longa*, a perennial herb, is a member of the family Zingiberaceae (ginger). It is extensively used for the treatment of sprains and swelling caused by injury.[14] In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, hepatic disorders, rheumatism and sinusitis. Curcuma longa is used for diseases associated with abdominal pains.[15] Current research has focused on turmeric's antioxidant,

hepatoprotective, anti-inflammatory, anti-carcinogenic and antimicrobial properties, in addition to its use in gastric ulcer (also can cause ulcer at high doses), cardiovascular disease and gastrointestinal disorders, antioxidant and wound healing. Massaging the aching teeth with roasted ground turmeric eliminates pain and swelling. Local drug delivery system containing 2% turmeric gel can be used to adjunct to scaling and root planing.[16]

The clinical disadvantages of CHX warrants the need of safe herbal compounds, which has led to explore vistas of herbal extracts used in alternative therapy. To the best of the authors knowledge no study has been conducted so far that evaluates a combination of three herbal components namely Aloe vera, curcumin and neem as a mouthwash.

Hence, this study has been undertaken to evaluate clinically the effect of Aloe vera, neem and curcumin mouthwash in treatment of periodontal diseases.

AIM

Comparative clinical evaluation of effects of various herbal mouthwashes on periodontal diseases.

OBJECTIVES

1. To assess the effect of different concentration of neem and curcumin extracts on PI, GI and PPD after 28 days.
2. To compare the results with CHX.

CHLORHEXIDINE:-

Some of the empirical studies on Chlorhexidine are being enumerated below. An attempt has been made to include the contemporary evidence based literature:

Goldschmidt P, Cogen R, Taubman S (1977)[17] conducted a study on cytopathologic effects of CHX on human cells. This study concluded that exposure of human cells in culture to CHX at concentrations equal to or greater than 0.004% resulted in impaired cellular function and/or cell death. Release of membrane bound 51Cr, inhibition of protein synthesis as measured by incorporation of 3H-leucine into protein-like material, and staining by trypan blue were seen as sequellae to exposure to 0.006% CHX for 3 hours. Lower doses were capable of inhibiting protein synthesis and releasing 51Cr, but did not result in staining of cells by trypan blue. Exposure of cells to 0.2% CHX for 30 seconds produced maximal suppression of protein synthesis and release of 51Cr.

Bassetti C, Kallenberger A (1980)[18] conducted a study on influence of CHX rinsing on the healing of oral mucosa and osseous lesions. This study was done using standerdised open mucosal-osseous wounds in the left side of the palate in Wistar rats. In five test groups, each containing 10 rats, rinsing was performed twice daily for 30 sec with 0.1, 0.2 and 0.5% CHX solution, CHX solution vehicle, and Ringer solution. A sixth test group (control) was not rinsed at all. Seven days postoperatively, wound healing was evaluated clinically (size of the defect) and histomorphometrically (percent composition of mature connective tissue, immature connective tissue, granulation tissue, fibrin with granulocytic infiltrate). Clinically it was clear that wound healing was best in those animals that rinsed with Ringer solution, and worst in those that rinsed with 0.5% CHX solution. Increasing concentration of CHX caused a delay in wound healing, which in the following cases resulted in significant differences: rinsing with Ringer solution and vehicle versus all concentrations of CHX, no rinsing versus 0.5% CHX. Intensive rinsing with high concentrations of CHX may, after oral surgical operations, especially surgery in which bone is exposed, result in delay and disturbance of wound healing in humans.

Hefti AF, Huber B (1987)[19] conducted a study to investigate the effectiveness of mouthwashes containing hexeitidine/zinc (HZA) or tin (ASF) in inhibiting plaque formation and gingivitis in

human. 24 dental students and assistant participated in the study, they rinsed twice daily for 1 min with formulations: HZA = 750 ppm hexetidine/750 ppm zinc acetate, ASF= 100 ppm aminefluorid/310 ppm stannous fluoride, CHX = 0.1% and M = negative control. Plaque accumulation was determined planimetrically and gravimetrically. Gingivitis was evaluated with the papillary bleeding index. The result showed that HZA and CHX completely inhibited plaque accumulation and gingivitis. ASF was left effective than HZA and CHX.

Brightman JL et al (1991)[20] conducted a study to analysis the effects of 0.12% CHX mouthrinse on orthodontic patients aged 11 through 17 with established gingivitis. In this study 34 subjects were divided into 2 groups (CHX and Placebo) 17 students in each group, they were evaluated at baseline, 6 week, and 12 weeks. GI, PI, Eastman interproximal bleeding index was recorded. The result showed that a significant reduction in plaque accumulation, gingival inflammation, and gingival bleeding could be attained with CHX mouthrinse was being used. Staining caused with CHX was mild to moderate and were removed with oral prophylaxis.

Joyston SB, Hernaman N (1993)[21] studies the effect of mouthrinse containing CHX and fluoride on plaque and gingival bleeding, 47 adults with > 20 teeth and a CPITN score > 1 but < 4 were randomised into test and control groups. After baseline assessments for plaque, bleeding and stain, teeth were professionally cleaned. Subjects were asked to rinse for 30 s with 10 ml of the respective test or placebo rinse after normal oral hygiene for 8 weeks. 39 subjects completed the study. Study concluded that, as an adjunct to normal oral hygiene, the CHX/fluoride rinse had a significant inhibitory effect on plaque and bleeding but its effect on staining is uncertain.

Quirynen M et al (2000)[22] conducted a study on the the rôle of chlorhexidine in the one-stage full-mouth disinfection treatment of patients with advanced adult periodontitis. In the study 3 groups of 12 patients each with advanced periodontitis were followed, both from a clinical and microbiological point of view, over a period of 8 months. The patients from the control group were scaled and root planed, quadrant per quadrant, at two-week intervals. The 2 other groups underwent a one stage full-mouth scaling and root planing (all pockets within 24 h) with (Fdis) or without (FRp=full-mouth root planing) the adjunctive use of chlorhexidine. At baseline and after 1, 2, 4 and 8 months, the following clinical parameters were recorded: plaque and gingivitis indices, probing depth, bleeding on probing and clinical attachment level. Microbiological

samples were taken from different intra-oral niches (tongue, mucosa, saliva and pooled samples from single- and multi-rooted teeth). The samples were cultured on selective and non-selective media in order to evaluate the number of CFU/ml for the key-periodontopathogens. Study concluded benefits of one-stage full-mouth disinfection in the treatment of patients suffering from severe adult periodontitis probably results from the full-mouth scaling and root planing within 24 h rather than the beneficial effect of chlorhexidine.

Charles CH, Mostler KM, Bartels LL, Mankodi SM (2004)[23] study was done to compare antiplaque and antigingivitis effects of a Chlorhexidine and an essential oil mouthrinse it was a 6 month clinical trial. In the study 108 subjects age 20- 57 were randomly allocated in 3 groups: essential oil mouthrinse (Listerine antiseptic), 0.12% CHX (peridex) or 5% hydroalcohol negative control. Oral soft tissue examination at baseline, GI index, PI index, Volpe- Manhold calculus index and Lohene extrinsic tooth stain index following scaling was done. Rinsing twice daily with the mouthwash adjunct to mechanical oral hygiene was told. Clinical variables were tested at 3 and 6 months. The study concluded that the essential mouthrinse and CHX had comparable antiplaque and antigingivtis effect.

Rajabalian S, Mohammadi M, Mozaffari B (2009)[24] conducted a study on Cytotoxicity evaluation of Persica mouthwash on cultured human and mouse cell lines in the presence and absence of fetal calf serum. In the study the toxic effects of four dilutions of Persica and CHX mouthwashes on KB, Saos-2, J744 A1, and gingival fibroblast cells were evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The effect of fetal calf serum (FCS) components on the cytotoxicity of these mouthwashes was also investigated. Results indicate that both Persica and CHX mouthwashes are toxic to macrophage, epithelial, fibroblast, and osteoblast cells in a concentration-dependent manner.

Li W, Wang RE, Finger M, Lang NP (2012)[25] conducted a study to evaluate the anti – gingivitis effect of a chlorhexidine (CHX) mouthwash with or without an Anti- discoloration System. In this study 26 healthy dental students were included assigned to 3 groups: group P (placebo), group T1 (0.12% CHX), group T2 (0.12% CHX with ADS). Participants were asked to rinse 10 ml sample twice daily. The clinical parameters, taken are: discoloration index (DI), plaque index (PI), gingival index (GI) were assessed on day 0, 7, 14, 21. After the completion of

the study he concluded that CHX with ADS appeared to be effective in preventing stains on the teeth. The ability of CHX mouthwash of preventing plaque accumulation and gingivitis was also greatly hampered by the addition of ADS. Infact, the CHX mouthwash with ADS showed no superior effect over water on maintenance of oral hygiene or prevention of gingivitis.

Singh V, Pathak AK, Sareen S, Mahesh P, Goel K (2015)[26] studied the comparative evaluation of topical application of turmeric gel and 0.2% chlorhexidine gluconate gel in prevention of gingivitis. A total of 40 subjects of both the sexes from age group 20-35 years. Simple random sampling was followed and the participants were assigned to two groups 'A' and 'B' of 20 participants each. Group A subjects were advised 0.2% chlorhexidine gluconate gel. Group B 30 subjects were advised experimental (turmeric) gel. Based on the observations of the study, it can be concluded that chlorhexidine gluconate as well as turmeric gel can be effectively used as an adjunct to mechanical plaque control in prevention of plaque and gingivitis. Chlorhexidine gluconate gel has been found to be more effective when antiplaque and anti-inflammatory properties were considered. The effect of turmeric observed may be because of its anti-inflammatory action. The antiplaque action of chlorhexidine gluconate is due to its substantivity. Substantivity of tumeric is required to be further studied.

Prasad KA et al (2015)[27] conducted a study on anti – plaque efficacy of herbal and 0.2% chlorhexidine gluconate mouthwash. 100 preclinical dental students were randomized into three groups (0.2% chlorhexidine, Saline and herbal mouthwash). All the groups were made to refrain from their regular mechanical oral hygiene measures and were asked to rinse with given respective mouthwashes for 4 days. The gingival and plaque scores are evaluated on 1 and 5 day, and differences were compared statistically. Concluded that within the limitations of this study chlorhexidine gluconate and herbal mouthwash (Hiora) showed similar anti plaque activity with latter showing no side effects.

Nadkerny PV, Ravishankar PL, Pramod V, Agarwal LA, Bhandari S (2015)[28] conducted a comparative evaluation of the efficacy of probiotic and chlorhexidine mouthrinses on clinical inflammatory parameters of gingivitis. The study was designed for a period of 4 week on 45 systemically healthy subjects between 20 and 30 years having chronic gingivitis. The study population was divided into three groups. Group A - 15 subjects were advised experimental

(probiotic) mouthwash. Group B - 15 subjects were advised positive control (chlorhexidine) mouthwash and Group C - 15 subjects into a negative control group (normal saline). Oral prophylaxis was done for all groups at baseline. After the proper oral hygiene instructions, all the three groups were instructed to rinse their mouth with 10 ml of their respective mouthrinse, undiluted for 1 min twice daily, 30 min after brushing. Clinical parameters such as plaque index PI, GI, and OHI-S were assessed at baseline, 2 weeks and 4 weeks, respectively. The study concluded probiotic mouthrinses tested was effectively used as an adjunct to mechanical plaque control in the prevention of plaque and gingivitis. Thus, the probiotic mouthrinse has a great therapeutic potential.

Deshmukh MA et al (2017)[29] conducted a comparative evaluation of the efficacy of probitotic, herbal and chlorhexidine mouthwash on gingival health. A group of 45 healthy subjects in the age group of 18-21 years received complete supragingival scaling at baseline and study variables OHI-S, PI and GI were recorded. Subjects were then randomly divided into three groups (15 in each group) and were randomly intervened with three different mouthwashes i.e., HiOra mouthwash, CHX mouthwash and Probiotic mouthwash. Variables were again recorded on the seventh and 14th day after use of mouthwashes and data obtained was subjected to statistical analysis. The study concluded that herbal and probiotic mouthwashes can prove to be effective alternatives to CHX with minimal side effects.

ALOE VERA:-

Recent dental literature has shown empirical evidence of use of Aloe vera in periodontics. Some of the studies are enumerated as follow:-

Bhat G, Kudva P, Dodwad V (2011)[30] conducted a study on Aloe Vera: Nature's soothing healer to periodontal disease. In this study a total 15 subjects were evaluated for clinical parameters like PI, GI, and PPD at baseline, following by scaling and root planning. Test site comprised of SRP followed by intra-pocket placement of Aloe Vera gel, which was compared with the control site in which only SRP was done. Clinical parameters were compared between the two sites at 1 and 3 months from baseline. Result showed encouraging findings in clinical parameters of the role of Aloe Vera gel as a drug for local delivery.

Pradeep AR, Agrawal E, Naik BS (2012)[31] study was conducted to assess the clinical and microbiologic effects of commercially available dentifrice containing Aloe Vera. In this study 90 patients diagnosed with chronic generalized gingivitis were selected and randomly divided into 3 groups: group 1- placebo toothpaste, group 2- toothpaste with Aloe Vera, group 3- toothpaste with polymer and triclosan. GI, PI was taken and microbiologic count was assessed at baseline, 6, 12 and 24 weeks. Result showed toothpaste containing Aloe Vera showed significant improvement in GI and PI scores, as well as microbiologic counts compared with placebo.

Sudarshan R, Annigeri RG, Sree GV (2012)[32] conducted a study of aloe vera in the treatment of oral submucous fibrosis. Twenty study subjects with OSMF were included in the study. Patients were divided into two groups. There were 10 patients in each group; group A subjects received 5 mg of aloe vera gel to be applied topically three times daily for 3 months and group B subjects received antioxidant capsules twice daily for 3 months. The results were analyzed with paired't' test and unpaired't' test. Study concluded that Aloe vera group showed a better treatment response compared to the antioxidants group. It reduces the burning sensation and improves mouth opening thereby enhanced the patients' compliance. It proves to be a relatively safe, can be applied topically, easily available, economical, noninvasive, and efficacious in the treatment for OSMF.

Ajmera N, Chatterjee A, Goyal V (2013)[33] conducted a study on aloe vera and its effect on gingivitis. In this study forty-five patients who were diagnosed with plaque-induced gingivitis were included in the study. They were divided into three groups with fifteen patients in each group. Group 1 was asked to rinse with 10 ml of aloe vera mouthwash twice daily for three months. Group 2 were treated with scaling only. Group 3 patients were asked to rinse with aloe vera mouthwash and scaling was done. The clinical changes were evaluated with Loe and Silness gingival index (1963) and Muhlemann and Son's Sulcus bleeding index (1971) at baseline, after one month and three months, respectively. The result suggested reduction in gingival inflammation in all the three groups, but it was more in the aloe vera mouthwash and scaling group. Hence, it was concluded that aloe vera had a significant anti-inflammatory property. Thus, it can be used as an adjunct to mechanical therapy for treating plaque-induced gingivitis.

Karim B et al (2014)[34] study was conducted to evaluate the effect of Aloe Vera on Periodontal Health. In the study 345 healthy subjects were randomly allocated in 3 groups: test group (n=115) - mouthwash contains Aloe Vera, Control group (n=115) - CHX group, distilled water-placebo (n=115). GI and PI index were assessed at day 0, 15, 30. Subjects were asked to rinse with the stated mouthwash twice daily during 30 day period. The result showed at Aloe Vera mouthrinse was equally effective in reducing periodontal indices as CHX. Significant reduction on plaque and gingivitis in Aloe Vera and CHX group. Aloe Vera mouthwash showed no side effects as seen with CHX.

Chhina S et al (2016)[35] conducted a randomized clinical study for comparative evaluation of aloe vera and 0.2% chlorhexidine gluconate mouthwash efficacy on de-novo plaque formation. This was a randomized, single blind, parallel, controlled clinical study with 90 healthy participants, with mean age of 27.19 ± 12.08 years. After thorough oral prophylaxis, participants were instructed to discontinue mechanical plaque control. Participants were divided randomly into three groups; pure Aloe vera mouthwash was dispensed to the test group; control group received 0.2% chlorhexidine gluconate mouthwash; in Placebo group, flavored distilled water was used as oral rinse twice daily. Effect on 4-day de novo plaque formation was assessed by comparing pre-rinsing Quigley Hein Modified Plaque Scores were analyzed statistically using analysis of variance and Student's t-test. Study concluded that herbal mouthwash containing Aloe vera mo has comparable antiplaque efficacy as the gold standard 0.2% chlorhexidine gluconate with fewer side effects and can be considered as an alternative.

Rezaei S et al (2016)[36] conducted a study to compare the efficacy of herbal mouthwash with chlorhexidine on gingival index of intubated patients in intensive care unit. The herbal mouthwas coantined *Salvadora persica* ethanol extract and aloe vera gel. Seventy-six intubated patients (18-64 years old with mean age 40.35 ± 13.2) in ICU were admitted to this study. The patients were randomly divided into two groups: (1) Herbal mouthwash and (2) chlorhexidine solution. Before the intervention, the GIs was measured by modified GI device into two groups. The mouth was rinsed by mouthwashes every 2-3 h for 4 days. 2 h after the last intervention, GIs were determined. The results of this study introduce a new botanical extract mouthwash with dominant healing effects on GI (1.5 ± 0.6) higher than that of synthetic mouthwash, chlorhexidine (2.31 ± 0.73).

Vangipuram S, Bhashyam M (2016)[37] conducted a comparative efficacy of aloe vera mouthwash and chlorhexidine on periodontal health. Thirty days randomized controlled trial was conducted among 390 dental students. The students were randomized into two intervention groups namely Aloe Vera (AV) chlorhexidine group (CHX) and one control (placebo) group. Plaque index and gingival index was recorded for each participant at baseline, 15 days and 30 days. The findings were than statistically analyzed, ANOVA and Post Hoc test were used. Study concluded that being an herbal product AloeVera has shown equal effectiveness as Chlorhexidine. Hence can be used as an alternative product for curing and preventing gingivitis.

CURCUMIN:-

Owing to its excellent antiinflammatory, antimicrobial and wound healing properties use of turmeric and its derivatives has gained a momentum in the recent research in periodontics. Some of the contemporary research with empirical evidence is being enumerated below:-

Mali MA, Behal R, Gilda SS (2012)[38] conducted a comparative evaluation of 0.1% turmeric mouthwash with 0.2% chlorhexidine gluconate in prevention of plaque and gingivitis: A clinical and microbilogical study. 60 subjects, 15 years and above, with mild to moderate gingivitis were recruited. Study population was divided into two groups. Group A-30 subjects were advised chlorhexidine gluconate mouthwash. Group B-30 subjects were advised experimental (turmeric) mouthwash. Both the groups were advised to use 10 ml of mouthwash with equal dilution of water for 1 min twice a day 30 min after brushing. Parameters were recorded for plaque and gingival index at day 0, 14, and 21 day. Subjective and objective criteria were assessed after 14 and 21 day. The N-benzoyl-l-arginine-p- nitroanilide (BAPNA) assay was used to analyze trypsin like activity of "red" complex microorganisms. Study concluded chlorhexidine gluconate as well as turmeric mouthwash can be effectively used as an adjunct to mechanical plaque control in prevention of plaque and gingivitis. Both the mouthwashes have comparable anti-plaque, anti-inflammatory and anti-microbial properties.

Muglikar S, Patil KC, Shivswami S, Hegde R (2013)[39] studied the efficacy of curcumin in the treatment of chronic gingivitis. Thirty patients aged 20-40 years with generalised chronic gingivitis were included in the study. They were randomly divided into 3 groups of 10 each. In group 1, patients underwent scaling and root planing followed by chlorhexidine mouthwash

(SRP/CHX Gr-1); in group 2, patients underwent scaling and root planing followed by curcumin mouthwash (SRP/CUR Gr-2); in group 3, and patients underwent only scaling and root planing (SRP Gr-3). Gingival and plaque indices were recorded at baseline (day 0) and 7, 14 and 21 days. Differences between the groups were statistically analysed. Study concluded curcumin is comparable to chlorhexidine as an anti-inflammatory mouthwash. Thus, it can be considered as an effective adjunct to mechanical periodontal therapy.

Subasree S, Murthykumar K, Naveed N (2014)[40] overviewed an article on Effects of Turmeric on Oral Health. He mentioned in a study made Waghmare et al. about 100 subjects were randomly selected. GI, PI were recorded at 0, 14, 21 days. It was found that CHX as well as turmeric mouthwash can be effectively used as in addition to mechanical plaque control methods in the prevention of plaque and gingivitis. Turmeric mouthwash prepared by dissolving 10 mg of curcumin extract in 100 ml of distilled water. The effect of turmeric observed may be because of its anti-inflammatory action. Reduction in total microbial count was observed in both the groups.

Kandwal A, Mamgain KR, Mamgain P (2015)[41] studied the comparative evaluation of turmeric gel with 2% chlorhexidine gluconate gel for the treatment of plaque induced gingivitis. 60 patients with plaque-induced gingivitis were divided into two groups, Group A was given turmeric gel and Group B was given chlorhexidine gel for 21 days in vaccupress trays. Plaque and gingival index were taken at baseline, 14 days and 21 days. Subjective and objective criteria were evaluated at 14 and 21 days. Study concluded that both groups reported a comparable reduction in plaque and gingival index. Turmeric gel reported better acceptance due to pleasant odor and no staining of teeth in comparison to chlorhexidine gel that reported a bitter taste and staining of teeth.

Sudhakar J et al (2015)[42] conducted a study on evaluation of anti- inflammatory effects of Curcumin gel as an adjunct to scaling and root planning. In this study 30 patients with chronic localized or generalized periodontitis aged between 25 and 60 years with pocket depth of 5-7 mm affecting at least two non-adjacent sites were included. In the experimental site scaling and root planning was performed, followed by placement of the curcumin gel and periodontal pack application. In the control site, subgingival scaling alone was performed followed by periodontal pack application. Parameters included were: (PI), (GI), (PPD) and (CAL). These parameters

were recorded on day 0, 30 and 45 days. Significant reduction in mean was observed in PI, GI, PPD and gain in clinical attachment level were demonstrated in both the groups from baseline to 45 days. Study concluded Curcumin can be effectively used along with scaling and root planning.

Pulikkotil SJ, Nath S (2015)[43] conducted a study on effects of curcumin on cervicular levels of IL-Iβ and CCL28 in experimental gingivitis. In this study 60 systemically healthy selected subjects were randomly assigned to one of three topical antigingivitis gels. Each gel was applied twice daily for 10 minutes as the sole method of oral hygiene for 29 days on the test quadrant only. Modified gingival index (MGI), plaque index (PI), bleeding on probing (BOP) and probing depth (PD) were assessed at baseline, 29 days and 60 days. Estimation of IL-1β and CCL28 levels in gingival crevicular fluid was done at baseline and at 29 days. It concluded the anti-inflammatory potential of topical curcumin was similar to CHX-MTZ but superior to CHX in affecting IL-1β and CCL28 levels.

NEEM:-

Some of the contemporary research on neem with empirical evidence is being enumerated below:-

Wolinsky LE, Mania S, Nachnani S, Ling S (1996)[44] studies the inhibiting effect of aqueous *Azadirachta indica* (neem) extract upon bacterial properties influencing in vitro plaque formation. Neem stick extracts were screened for minimal bacterial growth inhibition (MIC) against a panel of streptococci by means of a broth dilution assay. Initial bacterial attachment was quantified by the measurement of the adhesion of 3H-labeled Streptococcus sanguis to saliva-conditioned synthetic hydroxyapatite. Aggregating activity of the neem stick extracts upon a panel of streptococci was also examined. No inhibition of bacterial growth was observed among the streptococcal strains tested in the presence of < or = 320 micrograms/mL of the neem stick extract. The pre-treatment of S. sanguis with the neem stick extract or the gallotannin-enriched extract from Melaphis chinensis at 250 micrograms/mL resulted in a significant inhibition of the bacterial adhesion to saliva-conditioned hydroxyapatite. Pre-treatment of saliva-conditioned hydroxyapatite with the neem stick or gallotannin-rich extract prior to exposure to bacteria yielded significant reductions in bacterial adhesion. The Neem stick extract and the

gallotannin-enriched extract from Melaphis chinensis inhibited insoluble glucan synthesis. Incubation of oral streptococci with the neem stick extract resulted in a microscopically observable bacteria aggregation. These data suggest that neem stick extract can reduce the ability of some streptococci to colonize tooth surfaces.

Vanka A, Tandon S, Rao SR, Udupa N, Ramkumar P (2001)[45] conducted a study on the effect of indigenous neem mouthwash on streptococcus mutans and lactobacilli growth. In the present study, the antibacterial effect of Neem mouthwash against salivary levels of streptococcus mutans and lactobacillus has been tested over a period of 2 months. Also its effect in reversing incipient carious lesions was assessed. While streptococcus mutans was inhibited by Neem mouthwashes, with or without alcohol as well as chlorhexidine, lactobacillus growth was inhibited by chlorhexidine alone. The initial data appears to prove its effect in inhibiting S. mutans and reversing incipient carious lesions, longer term clinical trials are essential.

Chatterjee A, Saluja M, Singh N, Kandwal A (2011)[46] conducted a study to evaluate the antigingivitis and antiplaque effect of an *Azadirachta indica* (neem) mouthrinses on plaque induced gingivitis. In the study 45 subjects with plaque induced gingivitis were taken and divided into three groups. Group I (15 ml neem) mouthwash twice daily, Group II (15 ml of CHX) mouthwash twice daily; group III (15 ml saline) twice daily. BOP and Gingivitis were evaluated by Muhlemann and Sons sulcus bleeding index and loe and silness GI index. Result showed that A. indica- based mouthwash is equally effective in reducing periodontal indices as CHX . Significant reduction in GI, PI in both groups as compared to placebo.

Verma PU, Dixit J (2012)[47] conducted a study on the development of a HGF cell line for the evaluation of a novel mouthwash from *Azadirachta indica* with chlorhexidine. The present study attempts to assess the influence of Chlorhexidine (CHX) and Neem Extract (NE) on Cultured Human Gingival Fibroblasts (hGF). Fibroblasts were derived from healthy gingival biopsy specimens harvested aseptically. The effects of CHX and NE were evaluated on cultured hGF through morphological and biochemical assays. Morphological studies with hGF indicate altered morphology beyond 1% CHX. However, NE shows similar results at higher concentrations. Cytotoxicity and Antioxidant analysis of NE displays remarkable safety as compared with CHX with less than 32% cytotoxicity even at 100% conc. CHX beyond 1% concentration exhibits

toxic effect on hGF at 1 minute time exposure. However, NE does not adversely affect the fibroblasts even up to 50% concentration showing less toxic effect in comparison with CHX on these cells. The cytoprotective, oral friendly quality of NE emphasize the superiority of NE over CHX.

Kaur KR, Singh PM, Chopra R, Bhatia A (2014)[48] conducted a study in evaluating the Efficacy of Three Commercially Available Herbal Mouthwashes in Treatment of Chronic Gingivitis. In the study 40 patient's (18-30) years which chronic marginal gingivitis were taken. After scaling and polishing they were randomly divided into 4 groups of 10 patients each group A (control): rinsed with normal water, test group B: Neem mouthwash, test group c(all fresh mouthwash), group D : Hiora mouthwash twice daily for 21 days. Clinical parameters were OHI, GI, API were assessed at baseline 7 and 21 days. Result showed significant improvement in all clinical parameters.

Jalauddin M, et al (2017)[49] conducted a comparative evaluation of neem 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. 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.

Place of the study where it is conducted:-

A clinical longitudinal prospective study was carried out in the Department of Periodontics, Babu Banarsi Das College of Dental Sciences (BBDCODS), Lucknow, India. Ethical clearance was obtained from ethical committee of BBDCODS; Patients fulfilling the following inclusion and exclusion criteria were selected from the OPD of the periodontology department of BBDCODS.

Study subjects

Humans

Study sample and size

50 patients

- Group CHX - 10 (Control Group)
- Group A - 10
- Group B - 10 (Test Group)
- Group C - 10
- Group D - 10

Eligibility Criteria

Patients will be selected based upon the following inclusion and exclusion criteria.

- **Inclusion Criteria:-**
  - Patients in the age group of 18-35 years, irrespective of gender.
  - Patients with plaque Index (Silness and Loe, 1964) and Gingival Index(Loe and Silness, 1963) score > 1.5 are included.
  - Patient with good general health.

- **Exclusion Criteria:-**
  - Pregnant and lactating women.
  - Smokers, tobacco or pan chewers.
  - Patients with no antibiotic or anti- inflammatory drug therapy for the last 3 months.

- Patient with history of any periodontal therapy in last 6 months.
- Patients with removable prosthesis or orthodontic appliances.
- Patients with sensitivity to any mouthwash.
- Non cooperative patients.

MATERIALS:-

1. Mouth mirror and UNC – 15 Probe (HU-Friedy)
2. Explorer (no.23)
3. Mouthwash A (Aloe vera (base) 0.5 gm neem, and curcumin) Group A
4. Mouthwash B (Aloe vera (base) 1gm neem and curcumin) Group B
5. Mouthwash C (Aloe vera (base) 1.5gm neem and curcumin) Group C
6. Mouthwash D (Aloe vera (base) 2gm neem and curcumin) Group D
7. CHX (HEXIDINE 0.2%) Group CHX

STUDY DESIGN:-

A clinical longitudinal prospective study was carried out in the Department of Periodontics, Babu Banarsi Das College of Dental Sciences (BBDCODS), Lucknow.

Preparation of Formulations:-

Aloe vera, curcumin and neem mouthwash which are used in test groups, were prepared and standardized accordingly in collaboration with CIMAP (Central Institute for Medicinal and Aromatic Plants) Lucknow. Furthermore, the CHX mouthwash used in control group is commercially available under the trade mark HEXIDINE with concentration 0.2%.

(a) Base : Aloe Vera Juice

Freshly harvested aloe vera leaves were collected from CSIR-CIMAP Experimental farms at Lucknow. The leaves were thoroughly washed with distilled water to remove any contaminants. The upper, lower and sides were cut and the outer leaf portion was removed. The inner mucilage was collected and homogenized for 30-45 minutes. The homogenized liquid was filtered through a fine mesh to obtain clear juice. Food grade stabilizers and preservatives were added to prevent microbial and fungal growth.

(b) Herbal Extracts: Fresh neem leaves, bark and rhizomes of turmeric were collected from CIMAP experimental farm and dried under controlled conditions at 40-45°C. The dried herbs were extracted with hydro alcoholic mixture in fixed ratio in room temperature. The extracts were concentrated under low temperatures and vacuum to prevent degradation of thermo labile constituents. The concentrated extracts were stored under cold conditions prior to formulations.

(c) Formulations were prepared by mixing different quantities of the herbal extracts using aloe vera juice as base. The concentrated formulations were stored under cold conditions prior to trials by diluting with RO water in specific ratios. The formulations formed were divided into 4 groups according to the concentrations of herbal extracts with aloe vera juice base.

1. Group A (0.5gm neem and curcumin)
2. Group B (1gm neem and curcumin)
3. Group C (1.5gm neem and curcumin)
4. Group D (2gm neem and curcumin)

CLINICAL PARAMETERS:-

Clinical parameters recorded in the study are as follows:-

1) Plaque index (Silness J and Loe H in 1964). The six index teeth examined were 16, 12, 24, 36, 32, 44 surfaces examined were disto-facial, mesio-facial, and lingual surface.

Scoring Criteria:-

Score	Criteria
0	.No plaque
1	A film of plaque adhering to the free margin and adjacent area of tooth, the plaque may be seen by using probe on tooth surface.
2	Moderate accumulation of soft

	deposits within the gingival pocket, or the tooth and gingival margin which can be seen with naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingiva margin.

Calculation:

- Plaque index for a tooth: Scores added and divided by four.
- Plaque index for the individual: Indices for each of the teeth are added and then divided by the total number of teeth examined.

Interpretation of Plaque index:

Rating	**Scores**
Excellent	0
Good	0.1-0.9
Fair	1.0-1.9
Poor	2.0-3.0

2) Gingival index (Loe H and Silness J 1963) the six index teeth examined were 16, 12, 24, 36, 32, 44 surfaces examined were disto-facial, mesio-facial and lingual surface.

Scoring Criteria:-

Score	**Criteria**
0	Absence of inflammation/ normal

	gingiva.
1	Mild inflammation, slight change in color, slight edema, no bleeding of probing.
2	Moderate inflammation, moderate glazing, redness, edema and hypertrophy, bleeding on probe.
3	Severe inflammation, marked redness and hypertrophy ulceration. Tendency to spontaneous bleeding.

Calculation:-

- Gingival index for a tooth: Scores added and divided by four.
- Gingival index for the individual: Indices for each of the teeth are added and then divided by the total number of teeth examined.

Interpretation of Gingival index:-

- 0.1-1.0 – Mild gingivitis
- 1.1-2.0 – Moderate gingivitis
- 2.1-3.0 – Severe gingivitis

3) Pocket probing depth (PPD):- Pocket probing was done on four surfaces per tooth. Surfaces included were Mesio-buccal, Buccal, Disto-buccal, and Lingual.

PROCEDURE:

Each patient included in the study was examined on the basis of exclusion and inclusion criteria. The treatment procedure was fully explained to the patient and a duly signed written consent form was taken from each patient before initiation of the procedure. Clinical parameters PI, GI and PPD at baseline were recorded. All patients underwent scaling and root planing, polishing and oral hygiene instructions were given. Patients were instructed to rinse with their assigned mouthwash (10ml) twice daily for 30 seconds over a period of 28 days. They were recalled for re-evaluation on 7^{th}, 14^{th}, 21^{st} and 28^{th} day and all clinical parameters were recorded and plaque control measures were reinforced.

At the end of the study, the entire data thus collected was subjected to suitable statistical analysis and interpretation for final results.

LIST OF ILLUSTRATIONS

LIST OF TABLES

Statistical Analysis

Results are presented in mean±SE. The statistical analysis was performed using PRISM 5.1 software. Two way analysis of variance (ANOVA) was applied for deriving the significance within the group (Intra Group) using TUKEY'S test and deriving the significance among the groups (Inter Group) using BONFERRONI'S test. The p-value <0.05 was considered statistically significant.

CLINICAL PARAMETERS:-

Intergroup Comparison of Plaque Index between Group A and B at baseline 7, 14, 21 and 28 day (Table 1)

Intergroup plaque index score were recorded at these time intervals in Group A and B

At baseline the mean PI score for group A was 2.14±0.15 and for group B was 1.96±0.10 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group A was 2.00±0.14 and for group B was 1.87±0.09 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group A was 1.91±0.12 and for group B was 1.84±0.10 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group A was 1.86±0.13 for group B was 1.79±0.10 (p-value >0.05) which was statistically non-significant.

Day 28 the mean PI score for group A was 1.80±0.13 and for group B was 1.73±0.11 (p-value >0.05) which was statistically non-significant.

Table 1: Inter-group comparison of Group A and B at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
A	2.14±0.15	2.00±0.14	1.91±0.12	1.86±0.13	1.80±0.13
B	1.96±0.10	1.87±0.09	1.84±0.10	1.79±0.10	1.73±0.11
p-value	0.32	0.45	0.64	0.68	0.68

Intergroup Comparison of PI between Group A and C at baseline 7, 14, 21 and 28 day (Table 2)

Intergroup plaque index score were recorded at these time intervals in Group A and C

At baseline the mean PI score for group A was 2.14±0.15 and for group C was 2.31±0.14 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group A was 2.00±0.14 and for group C 1.95±0.14 was (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group A was 1.91±0.12 and for group C was 1.95±0.14 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group A was 1.86±0.13 for group C was 1.34±0.13 (p-value <0.05) which was statistically significant

Day 28 the mean PI score for group A was 1.80±0.13 and for group C was 1.00±0.09 (p-value <0.05) which was statistically significant

Table 2: Inter-group comparison between Group A and C at baseline, 7, 14, 21 and 28 days.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
A	2.14±0.15	2.00±0.14	1.91±0.12	1.86±0.13	1.80±0.13
C	2.31±0.14	1.95±0.14	1.95±0.14	1.34±0.13	1.00±0.09
P-value	0.41	0.78	0.07	0.008	0.0001

Intergroup Comparison of PI between Group A and D at baseline 7, 14, 21 and 28 day (Table 3)

Intergroup plaque index score were recorded at these time intervals in Group A and D

At baseline the mean PI score for group A was 2.14±0.15 and for group D was 1.93±0.11 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group A was 2.00±0.14 and for group D 1.85±0.11 was (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group A was 1.91±0.12 and for group D was 1.82±0.12 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group A was 1.86±0.13 for group D was 1.76±0.12 (p-value >0.05) which was statistically non-significant.

Day 28 the mean PI score for group A was 1.80±0.13 and for group D was 1.66±0.10 (p-value >0.05) which was statistically non-significant.

Table 3: Inter-group comparison of Group A and D at baseline 7, 14, 21 and 28 day

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
A	2.14±0.15	2.00±0.14	1.91±0.12	1.86±0.13	1.80±0.13
D	1.93±0.11	1.85±0.11	1.82±0.12	1.76±0.12	1.66±0.10
P-value	0.029	0.42	0.58	0.56	0.40

Intergroup Comparison of PI between Group A and CHX at baseline 7, 14, 21 and 28 day (Table 4)

Intergroup plaque index score were recorded at these time intervals in Group A and CHX

At baseline the mean PI score for group A was 2.14±0.15 and for group CHX was 2.58±0.12 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group A was 2.00±0.14 and for group CHX was 2.23±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group A was 1.91±0.12 and for group CHX was 1.76±0.14 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group A was 1.86±0.13 for group CHX was 1.32±0.13 (p-value <0.05) which was clinically significant.

Day 28 the mean PI score for group A was 1.80±0.13 and for group CHX was 1.03±0.12 (p-value <0.05) which was clinically significant.

Table 4: Inter-group comparison of Group A and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
A	2.14±0.15	2.00±0.14	1.91±0.12	1.86±0.13	1.80±0.13
CHX	2.58±0.12	2.23±0.11	1.76±0.14	1.32±0.13	1.03±0.12
P-value	0.03	0.212	0.430	0.008	0.000389

Intergroup Comparison of PI between Group B and C at baseline 7, 14, 21 and 28 day (Table 5)

Intergroup plaque index score were recorded at these time intervals in Group B and C

At baseline the mean PI score for group B was 1.96±0.10 and for group C was 2.31±0.14 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group B was 1.87±0.09 and for group C was 1.95±0.14 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group B was 1.56±0.10 and for group C was 1.56±0.14 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group B was 1.79±0.10 for group C was 1.34±0.13 (p-value <0.05) which was statistically significant.

Day 28 the mean PI score for group B was 1.73±0.11 and for group C was 1.00±0.09 (p-value <0.05) which was statistically significant.

Table 5: Inter-group comparison of Group B and C at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
B	1.96±0.10	1.87±0.09	1.84±0.10	1.79±0.10	1.73±0.11
C	2.31±0.14	1.95±0.14	1.56±0.14	1.34±0.13	1.00±0.09
P-value	0.049	0.66	0.13	0.01	0.00005

Intergroup Comparison of PI between Group B and D at baseline 7, 14, 21 and 28 day (Table 6)

Intergroup plaque index score were recorded at these time intervals in Group B and D

At baseline the mean PI score for group B was 1.96±0.10 and for group D was 1.93±0.11 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group B was 1.87±0.09 and for group D was 1.85±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group B was 1.56±0.10 and for group D was 1.82±0.12 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group B was 1.79±0.10 for group D was 1.76±0.12 (p-value >0.05) which was statistically non-significant.

Day 28 the mean PI score for group B was 1.73±0.11 and for group D was 1.66±0.10 (p-value >0.05) which was statistically non-significant.

Table 6: Inter-group comparison of Group B and D at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
B	1.96±0.10	1.87±0.09	1.84±0.10	1.79±0.10	1.73±0.11
D	1.93±0.11	1.85±0.11	1.82±0.12	1.76±0.12	1.66±0.10
P-value	0.88	0.90	0.89	0.81	0.63

Intergroup Comparison of PI between Group B and CHX at baseline 7, 14, 21 and 28 day (Table 7)

Intergroup plaque index score were recorded at these time intervals in Group B and CHX

At baseline the mean PI score for group B was 1.96±0.10 and for group CHX was 2.58±0.12 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group B was 1.87±0.09 and for group CHX was 2.23±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group B was 1.56±0.10 and for group CHX was 1.76±0.14 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group B was 1.79±0.10 for group CHX was 1.32±0.13 (p-value <0.05) which was statistically significant.

Day 28 the mean PI score for group B was 1.73±0.11 and for group CHX was 1.03±0.12 (p-value <0.05) which was statistically significant.

Table 7: Inter-group comparison of Group B and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
B	1.96±0.10	1.87±0.09	1.84±0.10	1.79±0.10	1.73±0.11
CHX	2.58±0.12	2.23±0.11	1.76±0.14	1.32±0.13	1.03±0.12
P-value	0.001	0.068	0.670	0.0011	0.0003

Intergroup Comparison of PI between Group C and D at baseline 7, 14, 21 and 28 day (Table 8)

Intergroup plaque index score were recorded at these time intervals in Group C and D

At baseline the mean PI score for group C was 2.31±0.14 and for group D was 1.93±0.11 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group C was 1.95±0.14 and for group D was 1.85±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group C was 1.56±0.14 and for group D was 1.56±0.14 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group C was 1.34±0.13 for group D was 1.76±0.12 (p-value >0.05) which was statistically non-significant.

Day 28 the mean PI score for group C was 1.00±0.09 and for group D was 1.66±0.10 (p-value <0.05) which was statistically significant.

Table 8: Inter-group comparison of Group C and D at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
C	2.31±0.14	1.95±0.14	1.56±0.14	1.34±0.13	1.00±0.09
D	1.93±0.11	1.85±0.11	1.56±0.14	1.76±0.12	1.66±0.10
P-value	0.05	0.612	0.188	0.067	0.0001

Intergroup Comparison of PI between Group C and CHX at baseline 7, 14, 21 and 28 day (Table 9)

Intergroup plaque index score were recorded at these time intervals in Group C and CHX

At baseline the mean PI score for group C was 2.31±0.14 and for group CHX was 2.58±0.12 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group C was 1.95±0.14 and for group CHX was 2.23±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group C was 1.56±0.14 and for group CHX was 1.76±0.14 (p-value >0.05) which was statistically non-significant.

Day 21 the mean PI score for group C was 1.34±0.13 for group CHX was 1.32±0.13 (p-value >0.05) which was statistically non-significant.

Day 28 the mean PI score for group C was 1.00±0.09 and for group CHX was1.03±0.12 (p-value >0.05) which was statistically non-significant.

Table 9: Inter-group comparison of Group C and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
C	2.31±0.14	1.95±0.14	1.56±0.14	1.34±0.13	1.00±0.09
CHX	2.58±0.12	2.23±0.11	1.76±0.14	1.32±0.13	1.03±0.12
P-value	0.16	0.12	0.31	0.94	0.85

Intergroup Comparison of PI between Group D and CHX at baseline 7, 14, 21 and 28 day (Table 10)

Intergroup plaque index score were recorded at these time intervals in Group D and CHX

At baseline the mean PI score for group D was 1.93±0.11 and for group CHX was 2.58±0.12 (p-value >0.05) which was statistically non-significant.

At day 7 the mean PI score for group D was 2.23±0.11 and for group CHX was 2.23±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PI score for group D was 1.76±0.14 and for group CHX was 1.76±0.14 (p-value >0.05) which was statistically non-significant.

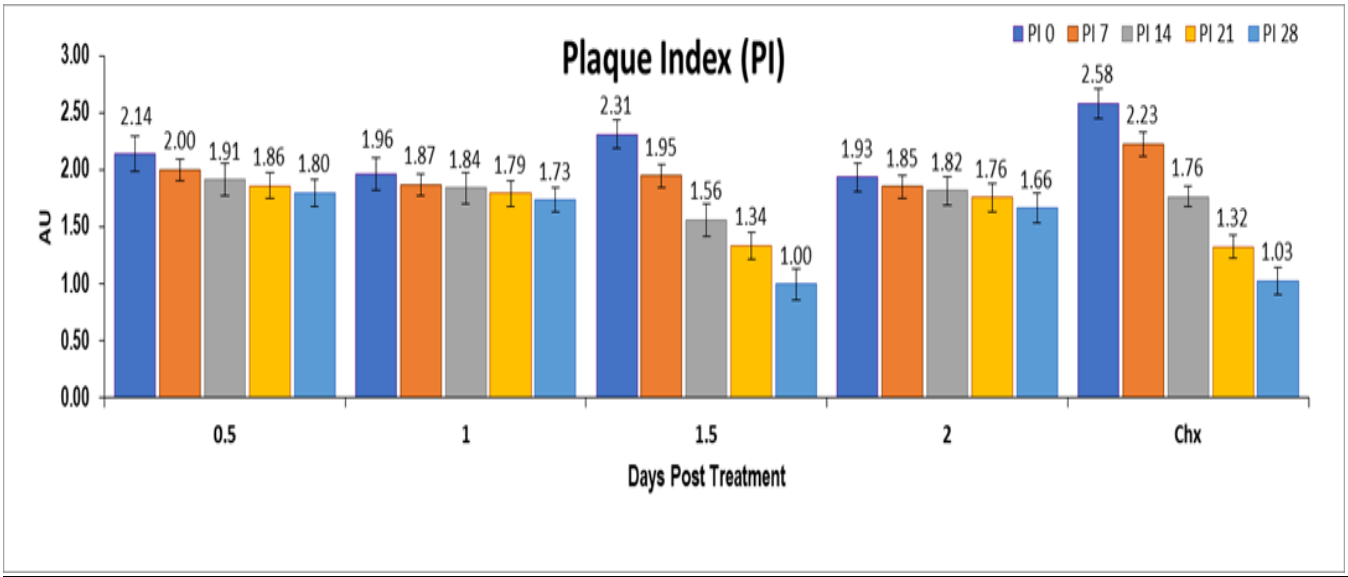
Day 21 the mean PI score for group D was 1.32±0.13 for group CHX was 1.32±0.13 (p-value >0.05) which was statistically non-significant.

Day 28 the mean PI score for group D was 1.03±0.12 and for group CHX was1.03±0.12 (p-value <0.05) which was statistically significant.

Table 10: Inter-group comparison of Group D and CHX at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
D	1.93±0.11	1.85±0.11	1.56±0.14	1.76±0.12	1.66±0.10
CHX	2.58±0.12	2.23±0.11	1.76±0.14	1.32±0.13	1.03±0.12
P-value	0.001	0.12	0.78	0.31	0.001

Graph 1:- Inter-group comparison of PI between Group A, B, C, D and CHX at Baseline, 7, 14, 21 and 28 day



Intergroup Comparison of Gingival Index between Group A and B at baseline 7, 14, 21 and 28 day (Table 11)

Intergroup gingival index score were recorded at these time intervals in Group A and B

At baseline, the mean GI reading for group A was 2.11±0.12, and group B was 2.06±0.16 (p-value >0.05) which was statistically non-significant.

At 7 day, the mean GI reading for group A was 2.02±0.12, B was 1.99±0.15 (p-value >0.05) which was statistically non-significant.

At 14 day, the mean GI reading for group A was 1.97±0.12, B was 1.94±0.15 (p-value >0.05) which was statistically non-significant.

At day 21, the mean GI reading for group A was 1.93±0.012, B was 1.88±0.14 (p-value >0.05) which was statistically non-significant).

At day 28, the mean GI reading for group A was 1.85±0.11, B was 1.79±0.013 (p-value >0.05) which was statistically non-significant.

Table 11: Inter-group comparison of Group A and B on baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
A	2.11±0.12	2.02±0.12	1.97±0.12	1.93±0.12	1.85±0.11
B	2.06±0.16	1.99±0.15	1.94±0.15	1.88±0.14	1.79±0.13
P-value	0.93	0.98	0.87	0.80	0.75

Intergroup Comparison of GI between Group A and C at baseline 7, 14, 21 and 28 day (Table 12)

Intergroup gingival index score were recorded at these time intervals in Group A and C

At baseline, the mean GI reading for Group A was 2.11±0.12, group C was 2.15±0.10 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI reading for group A was 2.02±12, group C was 1.78±1.10 (p-value >0.05) which was statistically non-significant.

At day 14 the mean GI reading for group A was 1.97±0.12, and C was 1.46±0.07 (p-value <0.05) which was statistically significant.

At day 21 the mean GI reading for group A was 1.93±0.12 and C was 1.44±0.07 (p-value <0.05) which was statistically significant.

At day 28 the mean GI reading for group was 1.85±0.11 and C was 1.00±0.09 (p-value <0.05) which was statistically significant.

Table 12: Inter-group comparison of Group A and C at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 Day**	**14 Day**	**21 Day**	**28 Day**
A	2.11±0.12	2.02±0.12	1.97±0.12	1.93±0.12	1.85±011
C	2.15±0.10	1.78±1.10	1.46±0.07	1.44±0.07	1.00±0.09
P-value	0.15	0.25	0.001	0.0004	0.001

Intergroup Comparison of GI between Group A and D at baseline 7, 14, 21 and 28 day (Table 13)

Intergroup gingival index score were recorded at these time intervals in Group A and D

At baseline, the mean GI reading for group A was 2.11±0.12, and group D was 1.96±0.14 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI reading for group A was 2.02±0.12, and group D was 1.87±0.13 (p-value >0.05) which was statistically non-significant.

At day 14 the mean GI reading for group A was 1.97±0.12, and group D was 1.81±0.13 (p-value >0.05) which was statistically non-significant.

At day 21 the mean GI reading for group A was 1.44±0.07, and group D was 1.75±0.12 (p-value >0.05) which was statistically non-significant.

At day 28 the mean GI reading for group A was 1.85±011 and group D was 1.67±0.09 (p-value >0.05) which was statistically non-significant.

Table 13: Inter-group comparison of Group A and D at baseline 7, 14, 21 and 28 day

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
A	2.11±0.12	2.02±0.12	1.97±0.12	1.93±0.12	1.85±011
D	1.96±0.14	1.87±0.13	1.81±0.13	1.75±0.12	1.67±0.09
P-value	0.44	0.45	0.36	0.26	0.001

Intergroup Comparison of GI between Group A and CHX at baseline 7, 14, 21 and 28 day (Table 14)

Intergroup gingival index score were recorded at these time intervals in Group A and CHX

At baseline, the mean GI reading for group A was 2.11±0.12, and group CHX was 2.22±0.13 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI reading for group A was 2.02±0.12 and group CHX was 1.76±0.07 (p-value >0.05) which was statistically non-significant.

At day 14 the mean GI reading for group A was 1.97±0.12 and group CHX was 1.44±0.10 (p-value <0.05) which was statistically significant.

At day 21 the mean GI reading for group A was 1.93±0.12 and group CHX was 1.05±0.08 (p-value <0.05) which was statistically significant.

At day 28 the mean GI reading for group A was 1.85±011 and group CHX was 0.96±0.10 (p-value >0.05) which was statistically significant.

Table 14: Inter-group comparison of Group A and CHX at baseline 7, 14, 21 and 28 day.

Group	Baseline	7 day	14 day	21 day	28 day
A	2.11±0.12	2.02±0.12	1.97±0.12	1.93±0.12	1.85±0.11
CHX	2.22±0.13	1.76±0.07	1.44±0.10	1.05±0.08	0.96±0.10
P-value	0.01	0.15	0.0001	0.0004	0.001

Intergroup Comparison of GI between Group B and C at baseline 7, 14, 21 and 28 day (Table 15)

Intergroup gingival index score were recorded at these time intervals in Group B and C

At baseline, the mean GI reading for group B was 2.06±0.16, and group C was 2.15±0.10 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI reading for group B was 1.99±0.15 and group C was 1.78±1.10 (p-value >0.05) which was statistically non-significant.

At 14 day the mean GI reading for group B was 1.94±0.15 and group C was 1.46±0.07 (p-value >0.05) which was statistically significant.

At day 21 the mean GI reading for group B was 1.88±0.14 and group C was 1.44±0.07 (p-value >0.05) which was statistically significant.

At day 28 the mean GI reading for group B was 1.79±0.13 and group C was 1.00±0.09 (p-value >0.05) which was statistically significant.

Table 15: Inter-group comparison of Group B and C at baseline 7, 14, 21 and 28 day.

Group	Baseline	7 day	14 day	21 day	28 day
B	2.06±0.16	1.99±0.15	1.94±0.15	1.88±0.14	1.79±0.13
C	2.15±0.10	1.78±1.10	1.46±0.07	1.44±0.07	1.00±0.09
P-value	0.64	0.25	0.008	0.00015	0.00008

Intergroup Comparison of GI between Group B and D at baseline 7, 14, 21 and 28 day (Table 16)

Intergroup gingival index score were recorded at these time intervals in Group B and D

At baseline, the mean GI reading for group B was 2.06±16, and group D was 1.96±0.14 (p- value >0.05) which was statistically non-significant.

At 7 day the mean GI reading for group B was 1.99±0.15 and D was 1.87±0.13 (p-value >0.05) which was statistically non-significant.

At 14 day the mean GI reading for group B was 1.94±0.15 and group D was 1.67±0.09 (p-value >0.05) which was statistically non-significant.

At day 21 the mean GI reading for group B was 1.88±0.14 and group D was 1.05±0.08 (p-value <0.05) which was statistically significant.

At day 28 the mean GI reading for group B was 1.79±0.13 and group D was 1.67±0.09 (p-value >0.05) which was statistically non-significant.

Table 16: Inter-group comparison of Group B and D at baseline 7, 14, 21 and 28 day.

Group	Baseline	7 day	14 day	21 day	28 day
B	2.06±0.16	1.99±0.15	1.94±0.15	1.88±0.14	1.79±0.13
D	1.96±0.14	1.87±0.13	1.81±0.13	1.75±0.12	1.67±0.09
P-value	0.62	0.55	0.51	0.49	0.46

Intergroup Comparison of GI between Group B and CHX at baseline 7, 14, 21 and 28 day (Table 17)

Intergroup gingival index score were recorded at these time intervals in Group B and CHX

At baseline, the mean GI reading for group B was 2.06±16, and group CHX was 2.22±0.13 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI reading for group B was 1.99±0.15 and group CHX was 1.76±0.07 (p-value >0.05) which was statistically non-significant.

At 14 day the mean GI reading for group B was 1.94±0.15 and group CHX was 1.44±0.10 (p-value <0.05) which was statistically non- significant.

At 21 day the mean GI reading for group B was 1.88±0.14 and group CHX was 1.05±0.08 (p-value <0.05) which was statistically significant.

At day 28 the mean GI reading for group B was 1.79±0.13 and group CHX was 0.96±0.10 (p-value <0.05) which was statistically significant.

Table 17: Inter-group comparison of Group B and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
B	2.06±0.16	1.99±0.15	1.94±0.15	1.88±0.14	1.79±0.13
CHX	2.22±0.13	1.76±0.07	1.44±0.10	1.05±0.08	0.96±0.10
P-value	0.44	0.19	0.011	0.00007	0.00007

Intergroup Comparison of GI between Group C and D at baseline 7, 14, 21 and 28 day (Table 18)

Intergroup gingival index score were recorded at these time intervals in Group C and D

At baseline, the mean GI reading for group C was 2.15±0.10, and group D was 1.96±0.14 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI for group C was 1.78±1.10 and group D was 1.87±0.13 (p-value >0.05) which was statistically non-significant.

At day 14 the mean GI for group C was 1.46±0.07 and group D was 1.81±0.13 (p-value >0.05) which was statistically non-significant.

At day 21 the mean GI for group C was 1.44±0.07 and group D was 1.75±0.12 (p-value <0.05) which was statistically significant.

At day 28 the mean GI for group C was 1.00±0.09 and group D was 1.67±0.09 (p-value <0.05) which was statistically significant.

Table 18: Inter-group comparison of Group C and D at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
C	2.15±0.10	1.78±1.10	1.46±0.07	1.44±0.07	1.00±0.09
D	1.96±0.14	1.87±0.13	1.81±0.13	1.75±0.12	1.67±0.09
P-value	0.27	0.58	0.027	0.0004	0.00005

Intergroup Comparison of GI between Group C and CHX at baseline 7, 14, 21 and 28 day (Table 19)

Intergroup gingival index score were recorded at these time intervals in Group C and CHX

At baseline, the mean GI reading for group C was 2.15±0.10, and group CHX was 2.22±0.13 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI for group C was 1.78±1.10 and group CHX was 1.76±0.07 (p-value >0.05) which was statistically non-significant.

At day 14 the mean GI for group C was 1.46±0.07 and group CHX was 1.44±0.10 (p-value >0.05) which was statistically non-significant.

At day 21 the mean GI for group C was 1.44±0.07 and group CHX was 1.05±0.08 (p-value >0.05) which was statistically non-significant.

At day 28 the mean GI for group C was 1.00±0.09 and group CHX was 0.96±0.10 (p-value >0.05) which was statistically non-significant.

Table 19: Inter-group comparison of Group C and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**
C	2.15±0.10	1.78±1.10	1.46±0.07	1.44±0.07	1.00±0.09
CHX	2.22±0.13	1.76±0.07	1.44±0.10	1.05±0.08	0.96±0.10
P-value	0.66	0.90	0.87	0.42	0.75

Intergroup Comparison of GI between Group D and CHX at baseline 7, 14, 21 and 28 day (Table 20)

Intergroup gingival index score were recorded at these time intervals in Group D and CHX

At baseline, the mean GI reading for group D was 1.96±0.14, and group CHX was 2.22±0.13 (p-value >0.05) which was statistically non-significant.

At 7 day the mean GI for group D was 1.87±0.13 and group CHX was 1.76±0.07 (p-value >0.05) which was statistically non-significant.

At day 14 the mean GI for group D was 1.81±0.13 and group CHX was 1.44±0.10 (p-value >0.05) which was statistically non-significant.

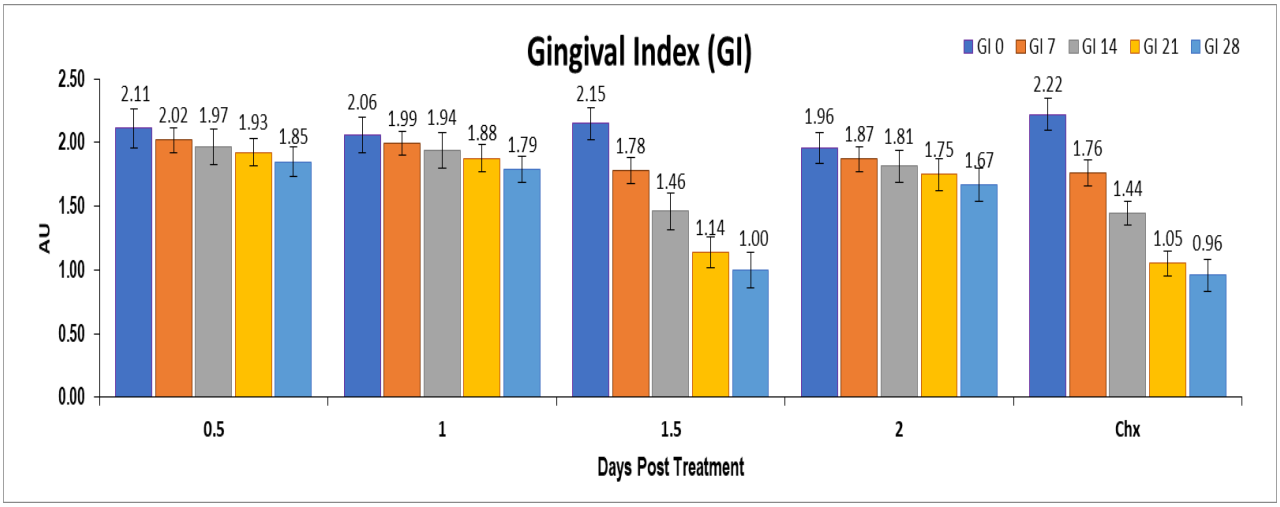
At day 21 the mean GI for group D was 1.75±0.12 and group CHX was 1.05±0.08 (p-value <0.05) which was statistically significant.

At day 28 the mean GI for group D was 1.67±0.09 and group CHX was 0.96±0.10 (p-value <0.05) which was statistically significant.

Table 20: Inter-group comparison of group D and CHX at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
D	1.96±0.14	1.87±0.13	1.81±0.13	1.75±0.12	1.67±0.09
CHX	2.22±0.13	1.76±0.07	1.44±0.10	1.05±0.08	0.96±0.10
P-value	0.18	0.498	0.036	0.00002	0.00004

Graph 2:- Inter-group comparison of GI between Group A, B, C, D and CHX at Baseline, 7, 14, 21 and 28 day



Intergroup Comparison of Pocking Probing Depth between Group A and B at baseline 7, 14, 21 and 28 day (Table 21)

Intergroup PPD score were recorded at these time intervals in Group A and B:-

At baseline the mean PPD score for group A was 5.99±0.18 and for group B was 5.71±0.32 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group A was 5.79±0.22 and for group B was 5.67±.31 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group A was 5.85±0.19 and for group B was 5.60±0.32 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group A was 5.90±0.21 and for group B was 5.56±0.37 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group A was 5.74±0.21 and for group B was 5.42±0.36 (p-value >0.05) which was statistically non-significant.

Table 21: Inter-group comparison of Group A and B at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
A	5.99±0.18	5.79±0.22	5.85±0.19	5.90±0.21	5.74±0.21
B	5.71±0.32	5.67±.31	5.60±0.32	5.56±0.37	5.42±0.36
P-value	0.45	0.75	0.51	0.42	0.44

Intergroup Comparison of PPD between Group A and C at baseline 7, 14, 21 and 28 day (Table 22)

Intergroup PPD score were recorded at these time intervals in Group A and C:-

At baseline the mean PPD score for group A was 5.99±0.18 and for group C was 5.90±0.31 (p-value

At 7 day the mean PPD score for group A was 5.79±0.22 and for group C was 5.83±0.30 (p-value

At day 14 the mean PPD score for group A was 5.85±0.19 and for group C was 5.80±0.31 (p-value

At day 21 the mean PPD score for group A was 5.90±0.21 and for group C was 5.80±0.35 (p-value

At day 28 the mean PPD score for group A was 5.74±0.21 and for group C was 5.64±0.36 (p-

Table 22: Inter-group comparison of Group A and C at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
A	5.99±0.18	5.79±0.22	5.85±0.19	5.90±0.21	5.74±0.21
C	5.90±0.31	5.83±0.30	5.80±0.31	5.80±0.35	5.64±0.36
P-value	0.80	0.91	0.89	0.79	0.80

Intergroup Comparison of PPD between Group A and D at baseline 7, 14, 21 and 28 day (Table 23)

Intergroup PPD score were recorded at these time intervals in Group A and D:-

At baseline the mean PPD score for group A was 5.99±0.18 and for group D 1.93±0.11 was (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group A was 5.79±0.22 and for group D was 1.85±0.11 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group A was 5.85±0.19 and for group D was 1.82±0.12 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group A was 5.90±0.21 and for group D was 1.76±0.12 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group A was 5.74±0.21 and for group D was 1.66±0.10 (p-value >0.05) which was statistically non-significant.

Table 23: Inter-group comparison of Group A and D at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
A	5.99±0.18	5.79±0.22	5.85±0.19	5.90±0.21	5.74±0.21
D	1.93±0.11	1.85±0.11	1.82±0.12	1.76±0.12	1.66±0.10
P-value	0.94	0.68	0.83	0.90	0.96

Intergroup Comparison of PPD between Group A and CHX at baseline 7, 14, 21 and 28 day (Table 24)

Intergroup PPD score were recorded at these time intervals in Group A and CHX:-

At baseline the mean PPD score for group A was 5.99±0.18 and for group CHX was 5.78±0.30 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group A was 5.79±0.22 and for group CHX was 5.72±0.29 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group A was 5.85±0.19 and for group CHX was 5.68±0.30 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group A was 5.90±0.21 and for group CHX was 5.65±0.35 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group A was 5.74±0.21 and for group CHX was 5.49±0.35 (p-value >0.05) which was statistically non-significant.

Table 24: Inter-group comparison of Group A and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
A	5.99±0.18	5.79±0.22	5.85±0.19	5.90±0.21	5.74±0.21
CHX	5.78±0.30	5.72±0.29	5.68±0.30	5.65±0.35	5.49±0.35
P-value	0.55	0.85	0.65	0.53	0.54

Intergroup Comparison of PPD between Group B and C at baseline 7, 14, 21 and 28 day (Table 25)

Intergroup PPD score were recorded at these time intervals in Group B and C:-

At baseline the mean PPD score for group B was 5.71±0.32 and for group C was 5.90±0.31 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group B was 5.67±.31 and for group C was 5.83±0.30 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group B was 5.60±0.32 and for group C was 5.80±0.31 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group B was 5.56±0.37 and for group C was 5.80±0.35 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group B was 5.42±0.36 and for group C was 5.64±0.36 (p-value >0.05) which was statistically non-significant.

Table 25: Inter-group comparison of Group B and C at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
B	5.71±0.32	5.67±.31	5.60±0.32	5.56±0.37	5.42±0.36
C	5.90±0.31	5.83±0.30	5.80±0.31	5.80±0.35	5.64±0.36
P-value	0.67	0.71	0.66	0.64	0.67

Intergroup Comparison of PPD between Group B and D at baseline 7, 14, 21 and 28 day (Table 26)

Intergroup PPD score were recorded at these time intervals in Group B and D:-

At baseline the mean PPD score for group B was 5.71±0.32 and for group D was 6.01±0.34 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group B was 5.67±.31 and for group D was 5.96±0.33 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group B was 5.60±0.32 and for group D was 5.93±0.35 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group B was 5.56±0.37 and for group D was 5.85±0.37 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group B was 5.42±0.36 and for group D was 5.77±0.40 (p-value >0.05) which was statistically non-significant.

Table 26: Inter-group comparison of Group B and D at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
B	5.71±0.32	5.67±.31	5.60±0.32	5.56±0.37	5.42±0.36
D	6.01±0.34	5.96±0.33	5.93±0.35	5.85±0.37	5.77±0.40
P-value	0.52	0.53	0.50	0.58	0.52

Intergroup Comparison of PPD between Group B and CHX at baseline 7, 14, 21 and 28 day (Table 27)

Intergroup PPD score were recorded at these time intervals in Group B and CHX:-

At baseline the mean PPD score for group B was 5.71±0.32 and for group CHX was 5.78±0.30 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group B was 5.67±.31 and for group CHX was 5.72±0.29 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group B was 5.60±0.32 and for group CHX was 5.68±0.30 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group B was 5.56±0.37 and for group CHX was 5.65±0.35 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group B was 5.42±0.36 and for group CHX was 5.49±0.35 (p-value >0.05) which was statistically non-significant.

Table 27: Inter-group comparison of Group B and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
B	5.71±0.32	5.67±.31	5.60±0.32	5.56±0.37	5.42±0.36
CHX	5.78±0.30	5.72±0.29	5.68±0.30	5.65±0.35	5.49±0.35
P-value	0.88	0.90	0.85	0.86	0.89

Intergroup Comparison of PPD between Group C and D at baseline 7, 14, 21 and 28 day (Table 28)

Intergroup PPD score were recorded at these time intervals in Group C and D:-

At baseline the mean PPD score for group C was 5.90±0.31 and for group D was 6.01±0.34 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group C was 5.83±0.30 and for group D was 5.96±0.33 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group C was 5.80±0.31 and for group D was 5.93±0.35 (p-value >0.05) which was statistically non-significant.

At day 21 the mean PPD score for group C was 5.80±0.35 and for group D was 5.85±0.37 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group C was 5.64±0.36 and for group D was 5.77±0.40 (p-value >0.05) which was statistically non-significant.

Table 28: Inter-group comparison of Group C and D at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
C	5.90±0.31	5.83±0.30	5.80±0.31	5.80±0.35	5.64±0.36
D	6.01±0.34	5.96±0.33	5.93±0.35	5.85±0.37	5.77±0.40
P-value	0.80	0.78	0.78	0.91	0.81

Intergroup Comparison of PPD between Group C and CHX at baseline 7, 14, 21 and 28 day (Table 29)

Intergroup PPD score were recorded at these time intervals in Group C and CHX:-

At baseline the mean PPD score for group C was 5.90±0.31 and for group CHX was 6.01±0.34 (p-value

At 7 day the mean PPD score for group C was 5.83±0.30 and for group CHX was 5.96±0.33 (p-value

At day 14 the mean PPD score for group C was 5.80±0.31 and for group CHX was 5.93±0.35 (p-value

At day 21 the mean PPD score for group C was 5.80±0.35 and for group CHX was 5.85±0.37 (p-value

At day 28 the mean PPD score for group C was 5.64±0.36 and for group CHX was 5.77±0.40 (p-value

Table 29: Inter-group comparison of Group C and CHX at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
C	5.90±0.31	5.83±0.30	5.80±0.31	5.80±0.35	5.64±0.36
CHX	5.78±0.30	5.72±0.29	5.68±0.30	5.65±0.35	5.49±0.35
P-value	0.78	0.79	0.79	0.76	0.76

Intergroup Comparison of PPD between Group D and CHX at baseline 7, 14, 21 and 28 day (Table 30)

Intergroup PPD score were recorded at these time intervals in Group C and CHX:-

At baseline the mean PPD score for group D was 6.01±0.34 and for group CHX was 6.01±0.34 (p-value >0.05) which was statistically non-significant.

At 7 day the mean PPD score for group D was 5.96±0.33 and for group CHX was 5.96±0.33 (p-value >0.05) which was statistically non-significant.

At day 14 the mean PPD score for group D was 5.93±0.35 and for group CHX was 5.93±0.35 (p-value >0.05) which was statistically non-significant.

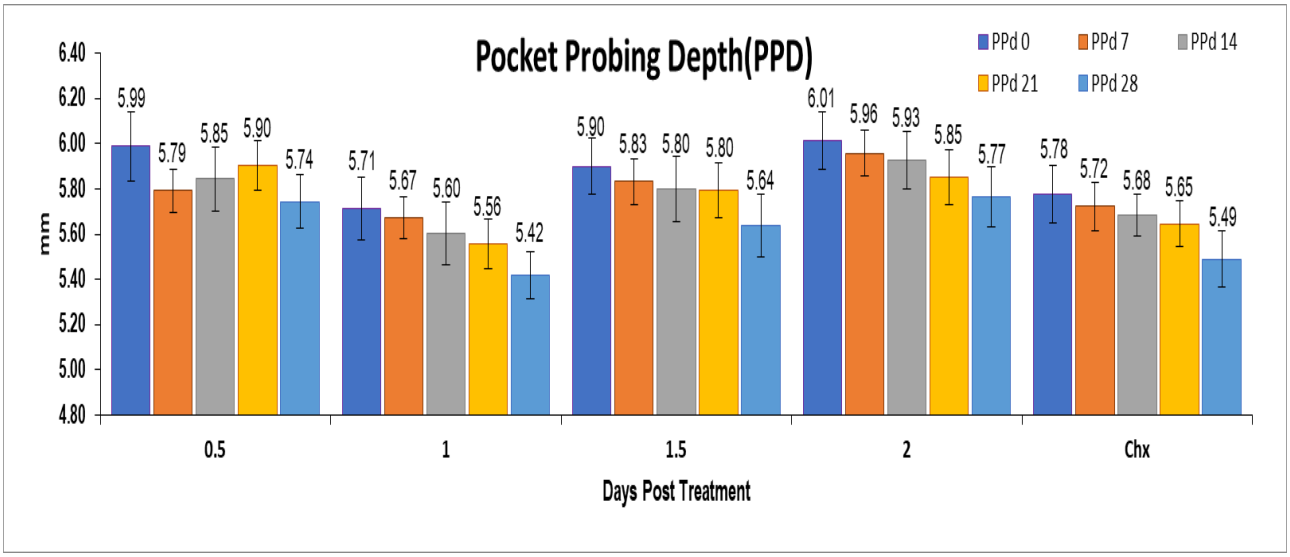
At day 21 the mean PPD score for group D was 5.85±0.37 and for group CHX was 5.85±0.37 (p-value >0.05) which was statistically non-significant.

At day 28 the mean PPD score for group D was 5.77±0.40 and for group CHX was 5.77±0.40 (p-value >0.05) which was statistically non-significant.

Table 30: Inter-group comparison of Group D and CHX at baseline 7, 14, 21 and 28 day.

Group	Baseline	Day 7	Day 14	Day 21	Day 28
D	6.01±0.34	5.96±0.33	5.93±0.35	5.85±0.37	5.77±0.40
CHX	5.78±0.30	5.72±0.29	5.68±0.30	5.65±0.35	5.49±0.35
P-value	0.61	0.60	0.60	0.69	0.60

Graph 3:- Inter-group comparison of PPD between Group A, B, C, D and CHX at Baseline, 7, 14, 21 and 28 day



INTRA GROUP:-

Comparison of Plaque Index between Group A, Group B, Group C, Group D, Group CHX at Baseline 7, 14, 21 and 28 day (Table 31).

In group A, the mean plaque index at baseline was 2.14±0.15 that reduced to 2.00±0.14 at 7 day, showing a reduction of 0.14±0.03. This change was found to statistically non-significant (p-value >0.05).

In group A, the mean plaque index at baseline was 2.14±0.15 that reduced to 1.91±0.12 at 14 day, showing a reduction of 0.23±0.03.This change was found to be statistically non-significant (p-value >0.05).

In group A, the mean plaque index at baseline was 2.14±0.15 that reduced to 1.86±0.13 at 21 day, showing a reduction of 0.28±0.002. This change was found to be statistically non-significant (p-value >0.05).

In group A, the mean plaque index at baseline was 2.14±0.15 that reduced to 1.80±0.13 at 28 day, showing a reduction of 0.34±0.02. This change was found to be statistically non-significant (p-value >0.05).

In group B, the mean plaque index at baseline was 1.96±0.10 that reduced to 1.87±0.09 at 7 day, showing a reduction of 0.09±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group B, the mean plaque index at baseline was 1.96±0.10 that reduced to 1.84±0.10 at 14 day, showing a reduction of 0.12±0. This change was found to be statistically non-significant (p-value >0.05).

In group B, the mean plaque index at baseline was 1.96±0.10 that reduced to 1.79±0.10 at 21 day, showing a reduction of 0.17±0. This change was found to be statistically non-significant (p-value >0.05).

In group B, the mean plaque index at baseline was 1.96±0.10 that reduced to 1.73±0.11 at 28 day, showing a reduction of 0.23±0.01. This change was found to be statistically non-significant (p-value>0.05).

In group C, the mean plaque index at baseline was 2.31±0.14 that reduced to 1.95±0.14 at 7 day, showing a reduction of 0.36±0. This change was found to be statistically non-significant (p-value >0.05).

In group C, the mean plaque index at baseline was 2.31±0.14 that reduced at 1.56±0.14 at 14 day, showing a reduction of 0.75±0. This change was found to be statistically significant (p-value <0.05).

In group C, the mean plaque index at baseline was 2.31±0.14 that reduced to 1.34±0.13 at 21 day, showing a reduction of 0.97±0.01. This change was found to be statistically significant (p-value <0.05)

In group C, the mean plaque index at baseline was 2.31±0.14 that reduced to 1.00±0.09 at 28 day, showing a reduction of 1.31±0.05. This change was found to be statistically significant (p-value <0.05).

In group D the mean plaque index at baseline was 1.93±0.11 that reduced to 1.85±0.11 at 7 day, showing a reduction of 0.08±0. This change was found to be statistically non-significant (p-value >0.05).

In group D, the mean plaque index at baseline was 1.93±0.11 that reduced to 1.82±0.12 at 14 day, showing a reduction of 0.11±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group D, the mean plaque index at baseline was 1.93±0.11 that reduced to 1.76±0.12 at 21 day, showing a reduction of 0.17±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group D, the mean plaque index at baseline was 1.93±0.11 that reduced to 1.66±0.10 at 21 day, showing a reduction of 0.27±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group D, the mean plaque index at baseline was 1.93±0.35 that reduced to 1.66±0.32 at 28 day, showing a reduction of 0.27±0.02. This change was found to be statistically non-significant (p-value >0.05

In group CHX the mean plaque index at baseline was 2.85±0.12 that reduced to 2.23±0.11 at 7 day, showing a reduction of 0.62±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group CHX the mean plaque index at baseline was 2.85±0.12 that reduced to 1.76±0.14 at 14 day, showing a reduction of 1.09±0.02. This change was found to be statistically significant (p-value <0.05).

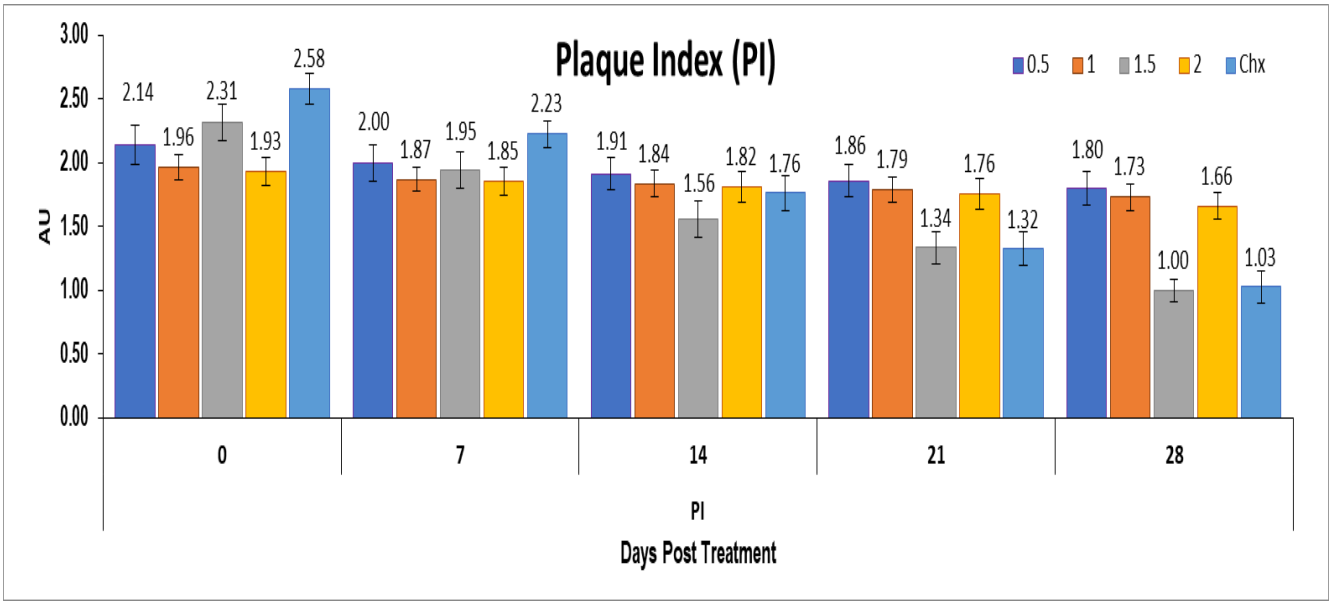
In group CHX the mean plaque index at baseline was 2.85±0.12 that reduced to 1.32±0.13 at 21 day, showing a reduction of 1.53±0.01. This change was found to be statistically significant (p-value <0.05).

In group CHX the mean plaque index at baseline was 2.85±0.12 that reduced to 1.03±0.12 at 28 day, showing a reduction of 1.82±0. This change was found to be statistically significant (p-value <0.05).

Table 31-: Intra-group comparison of PI for different groups from baseline to 7, 14, 21 and 28 day

Groups	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**	**p-value**	**Statistical Significance**
A	2.14±0.15	2.00±0.14	1.91±0.12	1.86±0.13	1.80±0.13	>0.05	NS
B	1.96±0.10	1.87±0.09	1.84±0.10	1.79±0.10	1.73±0.11	>0.05	NS
C	2.31±0.14	1.95±0.14	1.56±0.14	1.34±0.13	1.00±0.09	<0.05	S
D	1.93±0.11	1.85±0.11	1.82±0.12	1.76±0.12	1.66±0.10	>0.05	NS
CHX	2.85±0.12	2.23±0.11	1.76±0.14	1.32±0.13	1.03±0.12	<0.05	S

Graph 4:- Intra-group comparison of PI between the groups A, B, C, D and CHX at baseline 7, 14, 21 and 28 day.



Comparison of Gingival Index between Group A, Group B, Group C, Group D, Group CHX at Baseline 7, 14, 21 and 28 day (Table 32).

Intra-Group:-

In group A, the mean gingival index at baseline was 2.11±0.12 that reduced to 2.02±0.12 at 7 day, showing a reduction of 0.09±0.02. This change was found to statistically non-significant (p-value >0.05).

In group A, the mean GI at baseline was 2.11±0.12 that reduced to 1.97±0.12 at 14 day, showing a reduction of 0.14±0.02. This change was found to statistically non-significant (p-value >0.05).

In group A, the mean GI at baseline was 2.11±0.12 that reduced to 1.93±0.12 at 21 day, showing a reduction of 0.14±0.02. This change was found to be statistically non-significant (p-value >0.05).

In group A the mean GI at baseline was 2.11±0.12 that reduced to 1.85±0.11 at 28 day showing a reduction of 0.26±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group B the mean GI at baseline was 2.06±0.16 that reduced to 1.99±0.15 at 7 day showing a reduction of 0.07±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group B the mean GI at baseline was 2.06±0.16 that reduced to 1.94±0.15 at 14 day showing a reduction of 0.12±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group B the mean GI at baseline was 2.06±0.16 that reduced to 1.88±0.14 at 21 day showing a reduction of 0.018±0.02. This change was found to be statistically non-significant (p-value >0.05).

In group B the mean GI at baseline was 2.06±0.16 that reduced to 1.79±0.13 day 28 showing a reduction of 0.27±0.03. This change was found to be statistically non-significant (p-value >0.05)

In group C the mean GI at baseline was 2.15±0.10 that reduced to 1.78±0.10 at day 7 showing a reduction of 0.37±.01. This change was found to be statistically significant (p-value <0.05).

In group C the mean GI at baseline was 2.15±0.10 that reduced to 1.46±0.07 at 14 day, showing a reduction of 0.69±0.03. This was found to be statistically significant (p-value <0.05).

In group C the mean GI at baseline was 2.15±0.10 that reduced to 1.14±0.07 at 21 day showing a reduction of 1.01±0.03. This change was found to be statistically significant (p-value <0.05).

In group C the mean GI at baseline was 2.15±0.10 that reduced to 1.00±0.09 at 28 day showing a reduction of 1.15±0.01. This change was found to be statistically significant (p-value <0.05).

In group D the mean GI at baseline was 1.96±0.14 that reduced to 1.87±0.13 at 7 day showing a reduction of 0.09±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group D the mean GI at baseline was 1.96±0.14 that reduced to 1.81±0.13 at 14 day showing a reduction of 0.15±0.01. This change was found to be statistically non-significant (p-value >0.05).

In group D the mean GI at baseline was 1.96±0.14 that reduced to 1.75±0.12 at 21 day showing a reduction of 0.21±0.02. This change was found to be statistically non-significant (p-value >0.05).

In group D the mean GI at baseline was 1.96±0.14 that reduced to 1.67±0.09 at 28 day showing a reduction of 0.29±0.05. This change was found to be statistically non-significant (p-value >0.05).

In group CHX the mean GI at baseline was 2.22±0.13 which was reduced to 1.76±0.07 at 7 day showing a reduction of 0.46±0.06. This change was found to be statistically significant (p-value <0.05).

In group CHX the mean GI at baseline was 2.22±0.13 which was reduced to 1.44±0.10 at 14 day showing a reduction of 0.78±0.03. This change was found to be statistically significant (p-value <0.05).

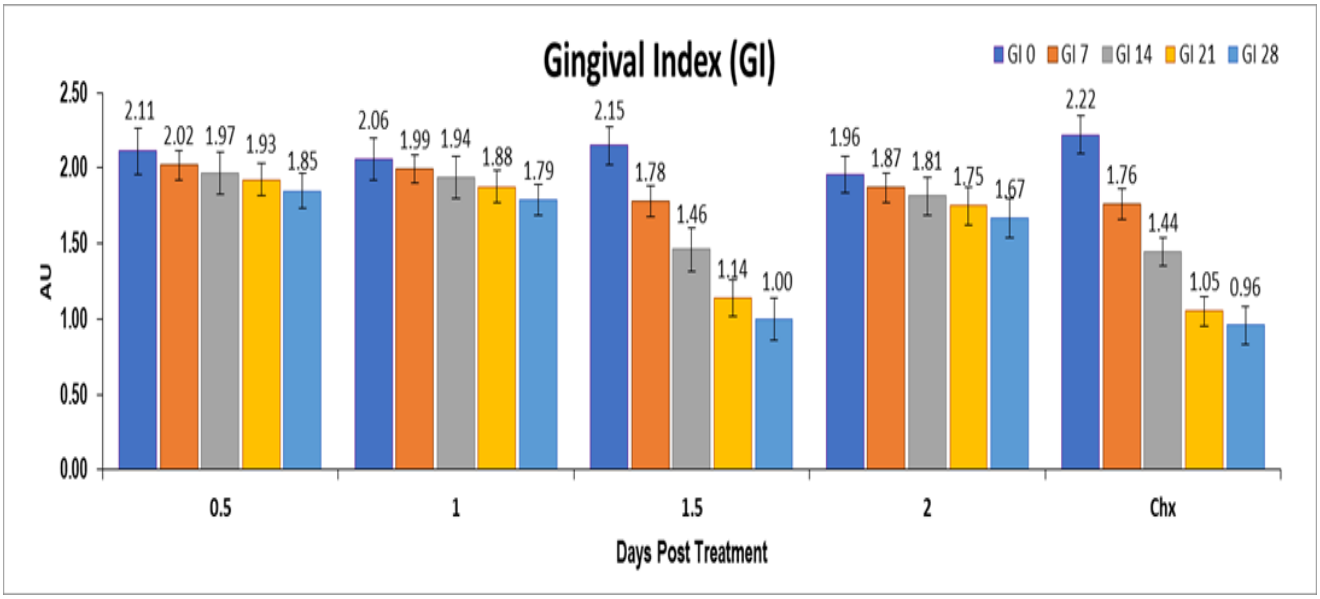
In group CHX the mean GI at baseline was 2.22±0.13 which was reduced to 1.05±0.08 at 21 day showing a reduction of 1.17±0.05. This change was found to be statistically significant (p-value <0.05).

In group CHX the mean GI at baseline was 2.22±0.13 which was reduced to 0.96±0.10 at day 28 showing a reduction of 1.26±0.03. This change was found to statistically significant (p-value <0.05).

Table 32-: Intra-group comparison of GI between the groups at baseline 7, 14, 21 and 28 day.

Group	**Baseline**	**7 day**	**14 day**	**21 day**	**28 day**	**p-value**	**Statistical significance**
A	2.11±0.12	2.02±0.12	1.97±0.12	1.93±0.12	1.85±0.11	>0.05	NS
B	2.06±0.16	1.99±0.15	1.94±0.15	1.88±0.14	1.79±0.13	>0.05	NS
C	2.15±0.10	1.78±0.10	1.46±0.07	1.14±0.07	1.00±0.09	<0.05	S
D	1.96±0.14	1.87±0.13	1.81±0.13	1.75±0.12	1.67±0.09	>0.05	NS
CHX	2.22±0.13	1.76±0.07	1.44±0.10	1.05±0.08	0.96±0.10	<0.05	NS

Graph 5:- :- Intra-group comparison of GI between the groups A, B, C, D and CHX at baseline 7, 14, 21 and 28 day**.**



Comparison of Pocket Probing Depth between Group A, Group B, Group C, Group D, Group CHX at Baseline 7, 14, 21 and 28 day (Table 33).

Intra-Group:-

In group A the mean PPD at baseline was 5.99±0.18 which reduced to 5.79±0.22 at 7 day showing a reduction of 0.2±0.04. This change was found to be statistically non-significant (p-value >0.05).

In group A the mean PPD at baseline was 5.99±0.18 which reduced to 5.85±0.19 at 14 day showing a reduction of 0.14±0.01. This change was found to be statistically non-significant (p-value <0.05).

In group A the mean PPD at baseline was 5.99±0.18 which reduced to 5.90±0.21 at day 21 showing a reduction of 0.09±0.03. This change was found to be statistically non-significant (p-value <0.05).

In group A the mean PPD at baseline was 5.99±0.18 which reduced to 5.74±0.21 at 28 day showing a reduction of 0.25±0.03. This change was found to statistically non-significant (p-value <0.05).

In group B the mean PPD at baseline was 5.71±0.32 which reduced to 5.67±0.31 at 7 day showing a reduction of 0.04±0.01. This change was found to be statistically non-significant (p-value <0.05).

In group B the mean PPD at baseline was 5.71±0.32 which reduced to 5.60±0.32 at 14 day showing a reduction of 0.11±0.01. This change was found to be statistically non-significant (p-value <0.05).

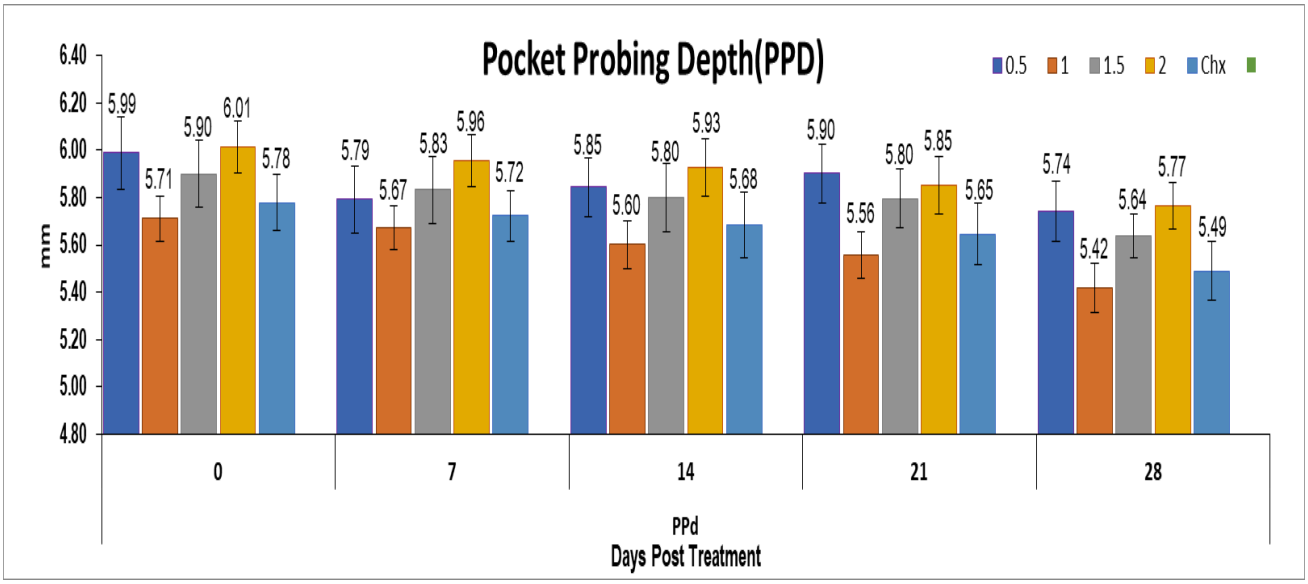
In group B the mean PPD at baseline was 5.71±0.32 which reduced to 5.56±0.37 at 21 day showing a reduction of 0.15±0.05. This change was found to be statistically non-significant (p-value <0.05).

In group B the mean PPD at baseline was 5.71±0.32 which reduced to 5.42±0.36 at day 28 showing a reduction of 0.29±0.03. This change was found to be statistically non-significant (p-value <0.05).

Table 33-: Intra-group comparison of PPD between the groups at baseline 7, 14, 21 and 28 day.

Group	**0 day**	**7 day**	**14 day**	**21 day**	**28 day**	**p-value**	**Clinical significance**
A	5.99±0.18	5.79±0.22	5.85±0.19	5.90±0.21	5.74±0.21	>0.05	NS
B	5.71±0.32	5.67±0.31	5.60±0.32	5.56±0.37	5.42±0.36	>0.05	NS
C	5.90±0.31	5.83±0.30	5.80±0.31	5.80±0.35	5.64±0.36	>0.05	NS
D	6.01±0.34	5.96±0.33	5.93±0.35	5.85±0.37	5.77±0.40	>0.05	NS
CHX	5.78±0.30	5.72±0.29	5.68±0.30	5.65±0.35	5.49±0.35	>0.05	NS

Graph 6:- Intra-group comparison of PPD between the groups A, B, C, D and CHX at baseline 7, 14, 21 and 28 day.



DISCUSSION

This clinical longitudinal prospective study was designed to evaluate the clinical effects of various herbal mouthwashes containing Aloe vera, Neem, Curcumin and comparing it with Chlorhexidine mouthwash on periodontal diseases.

Dental plaque formed on the gingival margin and adjacent tooth surface causes inflammation of gingiva. The bacteria in the plaque release toxins which cause swelling, redness and bleeding of gingiva. Periodontitis is a more severe and destructive gingival disease which may progress irreversibly in breaking down periodontal structures. Bacteria in the dental plaque are of the main factors causing periodontal inflammation; therefore careful plaque control is very important. As it is impossible to eliminate oral bacteria causing dental plaque, it is important to achieve plaque control by limiting growth of harmful bacteria. However, mechanical plaque removal is inadequately performed by most members of the population.[50,48] The need for additional help in controlling bacterial plaque provides the rationale for patients to use antimicrobial mouthwash in addition to their mechanical oral hygiene regimens. In the recent years, use of mouthwash has been on the increase as it is relatively easy to use for maintaining oral hygiene.[51]

Chlorhexidine is a broad spectrum bisbiguanide antiseptic; it is a strong base and is practically insoluble in water.[52] CHX use for chemical plaque control. This family of rinses is mainly indicated for use as adjuncts to mechanical cleaning, in specific clinical situations where mechanical oral hygiene is difficult, such as post-surgery, in individuals with inter-maxillary fixation, in fixed appliance orthodontic therapy and in individuals with intellectual and physical disabilities.[53] CHX mouthwash is mainly available in concentrations of 0.1%, 0.12% or 0.2%. The effect of CHX on the microbial biofilm is dose-dependent.[54] The optimum dose of CHX in a mouthwash is considered to be 20 mg twice daily equivalent to 10 mL of 0.2% CHX mouthwash (20 mg) or 15 mL of 0.12% CHX mouthwash (18 mg).[55] A rinse time of 30 seconds appears to be effective and acceptable although 60-second rinse times are also advocated. But its long-term usage may result in various adverse effects most common being the formation of brown staining on the teeth and oral tissues, particularly the tongue.[53] Other less common local adverse effects have also been reported including supragingival calculus accumulation, oral mucosal lesions and altered taste sensation. Parotid gland swelling has been reported following CHX mouthwash use. There have been rare reports of type 1 hypersensitivity reactions to CHX used in the mouth or on

the lips.[56] These local adverse effects limit the use of CHX to short or moderate term use in specific clinical circumstances, the adverse effects are transient and resolve once CHX mouthwash use has ceased. The occurrence of side effects tends to be reduced with lower CHX concentrations. Hence, search for an effective and safe alternative to CHX mouthwash has led to introduction of various herbal products in dentistry which are without any major side effects, can be used for longer duration are cheap and locally available.[57] Natural herbs when used in mouthwashes have shown significant advantages over the chemical ones.[58.59]

Considering the limitations in present assessment, an attempt was made to evaluate three common medicinal plants from Indian flora representatives for assessment of their use in periodontics. These herbs were Aloe vera, Neem and Curucmin. The purpose for taking them as representatives is their vast utility as medicinal plants in traditional Indian medicine.

Aloe vera which is used as a base in the mouthwash is a potential anti-bacterial agent which is said to be very effective in fighting the bacteria and preventing gingival and periodontal disease.[60] Aloe vera has demonstrated antibacterial action against a range of bacteria particularly against Streptococcus mutans, which account for its anti-plaque action. Some of the constituents of Aloe vera like Vitamin C, hyaluronic acid and dermatan sulfate are involved in collagen synthesis, and hence provide relief in swelling and bleeding gums. It exhibits strong antiseptic action in gingival pockets where normal cleaning is difficult.[61, 62] CHX, sodium hypochlorite, amine fluoride and cetylpyridinium chloride are widely used as mouthwashes and irrigating agents that can inhibit the growth of potentially pathogenic oral bacteria.[63] Although these antimicrobial agents are widely used, side effects such as immediate hypersensitivity reactions, toxicity, tooth staining and other side effects have been reported. Moreover, it has been reported that CHX and sodium hypochlorite possess cytotoxicity toward human periodontal ligament cells, inhibit protein synthesis, and affect mitochondrial activity, thus having detrimental effects on oral tissues.[64] CHX also has some negative side effects such as oral mucosal erosion, discoloration of teeth, and bitter taste.

Turmeric or *Curcuma longa* used as a chief constituent in the mouthwash is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. Current research has focused on turmeric's antioxidant, hepatoprotective, anti-inflammatory, anti-carcinogenic and antimicrobial properties, in addition to its use in gastric ulcer (also can cause ulcer at high doses), cardiovascular disease and gastrointestinal disorders, antioxidant and wound healing. The major constituent, curcumin (diferuloylmethane) is the most important fraction of *Curcuma longa*.[16] Owing to its excellent anti-inflammatory, antimicrobial and wound healing properties use of turmeric and its derivatives has gained a momentum in the recent research in periodontics.

Neem (*Azadirachta indica)* used as a chief constituent 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.[65,66] Other than this, the leaf extract of neem has also shown superior antiviral and antihyperglycemic activity *in vitro* and *in vivo* on animals.[67] Neem leaves have been used in the treatment of gingivitis and periodontitis. The possible mechanism of anti-inflammatory action of neem is by inhibiting prostaglandin E and 5 HT and thus reducing the inflammation. The antibacterial action can be explained by "Azadiachtin" that is known to destroy bacterial cell wall and thus inevitably inhibit the growth of bacteria, also the breakdown of cell wall disturb osmotic pressure and leads to cell death.[68]

The study was designed to evaluate Group A, B, C, D (Test Groups) to compare with Group CHX (Control Group) on clinical parameters GI, PI and PPD. Result reveled that there was improvement in all the groups but there was significant improvement seen in group C and it was comparable to CHX.

CLINICAL – PARAMETERS:-

Inter-group comparison of PI :-

Upon Inter-group comparison of Group A statistically significant result was found with Group C at 21 and 28 day interval and with group CHX at 21 and 28 day interval.

Upon Inter-group comparison was Group B statistically result was found with Group C at 21 and 28 day interval and with group CHX at 21 and 28 day interval.

Inter-group comparison of PI in group C statistically significant result was found with Group D at 28 day interval.

Inter-group comparison of Group D statistically significant result was found with Group CHX at 28 day interval.

Inter-group comparison of GI:-

Upon Inter-group comparison of GI of Group A statistically significant result was found with Group C at 14, 21 and 28 day interval and Group CHX at 14, 21 and 28 day interval.

Similarly upon Inter-group comparison of Group B statistically significant was found with Group C at 14, 21 and 28 day interval and Group CHX at 21 and 28 day.

Inter-group comparison of Group and C and D significant result was found at 21 and 28 day.

Inter-group comparison of Group D significant result was found with Group CHX at 21 and 28 day.

Inter-group comparison of PPD:-

In Inter-group comparison of the mean PPD in all the groups the results were statistically non-significant.

Intra-group comparison of PI

Intra-group comparison of PI of Group C from baseline was found to be statistically significant at 7, 14, 21 and 28 day interval.

Similarly in Group CHX statistically significant result was found from baseline to 7, 14, 21 and 28 day.

Intra-group comparison of GI:-

Intra-group comparison of GI of Group C from baseline was found to be statistically significant at 7, 14, 21 and 28 day interval.

Similarly in Group CHX statistically significant result was found from baseline to 7, 14, 21 and 28 day.

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This is in accordance with study conducted by Lee SS, Zhang W, Li Y in (2004)[69] in which it was seen that magnesium lactate present in Aloe vera inhibits Histidine Decarboxylase, thereby preventing the formation of histamine from histidine in mast cells. The decrease in gingival index can also be attributed to presence of sterols as anti-inflammatory agents and lupeol as antiseptic analgesics.

Villalobos et al. (2001)[70] observed a significant reduction in plaque and gingivitis after a 30-day use of mouthrinses containing aloe vera associated with tooth brushing.

Also, de Oliveira et al. (2008)[71] found that both dentifrice containing aloe vera and dentifrice containing fluoride resulted in significant reduction of plaque and gingivitis, but no statistical significant difference was observed between them.

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bradykinin thereby reduce prostaglandin synthesis and inhibits oxidation of arachidonic acid, which might decrease inflammation and relieves pain.

Mali AM (2012)[38] compared 0.1% turmeric mouthwash with 0.2% CHX mouthwash in prevention of plaque and gingivitis and concluded turmeric to be as efficacious as at both controlling plaque and inflammation, the main culprits in causing gingivitis.

Waghmare PF (2011)[72] in this clinical and microbial study comparative evaluation of turmeric and CHX mouthwash was done on plaque and gingivitis both turmeric and CHX were equally effective at decreasing bacterial counts. Also, this study reported adverse events and tolerability found that turmeric resulted in teeth staining less often, and had a more pleasant taste compared to CHX.

Mendieta et al (1994), [73] Anderson et al (1997)[74] in their study using CHX and curcumin mouthwash yielded a statistically significant change in comparison to scaling and root planing on PI and GI and stated that the significant reduction of PI , GI in curucmin mouthwash could be due to its anti-inflammatory, anti-oxidant properties which resolve inflammation, while CHX acts as an anti-bacterial agent only.

Jalauddin M et al (2017)[49] concluded that 2% neem mouthwash can be used as an alternative to CHX mouthwash based on the reduced score of GI and PI.

Botelho et al. (2008)[75] reported that Aloe vera based mouthrinse is highly efficacious and that it may be used as an alternative therapy in the treatment of periodontal disease.

Patel and Ventakrishna (1988)[76] reported a significant reduction in PPD and gain in clinical attachment level by using neem.

Based on the efficacy of the data it is evident that the dose of 1.5gm was amongst the best in the dose dependent study ranging from 0.5 to 2 gm. Further, observation from the study concluded that the ratio of the herbal components is optimum at 1.5gm of herbal mouthwash to 0.2gm of CHX. CHX being pure compound acts at a much lower dose as compared to the herbal counterpart; however it is also associated with inherent toxicities at higher doses. On the other hand the herbal formulations used in this study though has higher dose but is equally efficacious. The formulation being from crude extracts have multiple phytoconstituents which act as synergy

with different mechanism either directly on the pathogen or indirectly through the stimulation of innate immune mechanism especially through increased mucosal response. In this study *Azadirachta indica* (neem), and Curcumin have been traditionally used and proven for anti-microbial and immunostimulatory response.

DISCUSSION

This clinical longitudinal prospective study was designed to evaluate the clinical effects of various herbal mouthwashes containing Aloe vera, Neem, Curcumin and comparing it with Chlorhexidine mouthwash on periodontal diseases.

Dental plaque formed on the gingival margin and adjacent tooth surface causes inflammation of gingiva. The bacteria in the plaque release toxins which cause swelling, redness and bleeding of gingiva. Periodontitis is a more severe and destructive gingival disease which may progress irreversibly in breaking down periodontal structures. Bacteria in the dental plaque are of the main factors causing periodontal inflammation; therefore careful plaque control is very important. As it is impossible to eliminate oral bacteria causing dental plaque, it is important to achieve plaque control by limiting growth of harmful bacteria. However, mechanical plaque removal is inadequately performed by most members of the population.[50,48] The need for additional help in controlling bacterial plaque provides the rationale for patients to use antimicrobial mouthwash in addition to their mechanical oral hygiene regimens. In the recent years, use of mouthwash has been on the increase as it is relatively easy to use for maintaining oral hygiene.[51]

Chlorhexidine is a broad spectrum bisbiguanide antiseptic; it is a strong base and is practically insoluble in water.[52] CHX use for chemical plaque control. This family of rinses is mainly indicated for use as adjuncts to mechanical cleaning, in specific clinical situations where mechanical oral hygiene is difficult, such as post-surgery, in individuals with inter-maxillary fixation, in fixed appliance orthodontic therapy and in individuals with intellectual and physical disabilities.[53] CHX mouthwash is mainly available in concentrations of 0.1%, 0.12% or 0.2%. The effect of CHX on the microbial biofilm is dose-dependent.[54] The optimum dose of CHX in a mouthwash is considered to be 20 mg twice daily equivalent to 10 mL of 0.2% CHX mouthwash (20 mg) or 15 mL of 0.12% CHX mouthwash (18 mg).[55] A rinse time of 30 seconds appears to be effective and acceptable although 60-second rinse times are also advocated. But its long-term usage may result in various adverse effects most common being the formation of brown staining on the teeth and oral tissues, particularly the tongue.[53] Other less common local adverse effects have also been reported including supragingival calculus accumulation, oral mucosal lesions and altered taste sensation. Parotid gland swelling has been reported following CHX mouthwash use. There have been rare reports of type 1 hypersensitivity reactions to CHX used in the mouth or on

the lips.[56] These local adverse effects limit the use of CHX to short or moderate term use in specific clinical circumstances, the adverse effects are transient and resolve once CHX mouthwash use has ceased. The occurrence of side effects tends to be reduced with lower CHX concentrations. Hence, search for an effective and safe alternative to CHX mouthwash has led to introduction of various herbal products in dentistry which are without any major side effects, can be used for longer duration are cheap and locally available.[57] Natural herbs when used in mouthwashes have shown significant advantages over the chemical ones.[58.59]

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Upon Inter-group comparison of Group A statistically significant result was found with Group C at 21 and 28 day interval and with group CHX at 21 and 28 day interval.

Upon Inter-group comparison was Group B statistically result was found with Group C at 21 and 28 day interval and with group CHX at 21 and 28 day interval.

Inter-group comparison of PI in group C statistically significant result was found with Group D at 28 day interval.

Inter-group comparison of Group D statistically significant result was found with Group CHX at 28 day interval.

Inter-group comparison of GI:-

Upon Inter-group comparison of GI of Group A statistically significant result was found with Group C at 14, 21 and 28 day interval and Group CHX at 14, 21 and 28 day interval.

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Inter-group comparison of Group and C and D significant result was found at 21 and 28 day.

Inter-group comparison of Group D significant result was found with Group CHX at 21 and 28 day.

Inter-group comparison of PPD:-

In Inter-group comparison of the mean PPD in all the groups the results were statistically non-significant.

Intra-group comparison of PI

Intra-group comparison of PI of Group C from baseline was found to be statistically significant at 7, 14, 21 and 28 day interval.

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with different mechanism either directly on the pathogen or indirectly through the stimulation of innate immune mechanism especially through increased mucosal response. In this study *Azadirachta indica* (neem), and Curcumin have been traditionally used and proven for anti-microbial and immunostimulatory response.

CONCLUSION

Within the limits of this clinical 28 days longitudinal control trial, it may be concluded that Group A, B, C and D containing Aloe vera, neem and curcumin had antiplaque and antigingivitis property.

Inter-group comparison of PI and GI:-

Group A showed insignificant mean reduction with Group B and D from baseline to 28 day interval, but showed clinically significant reduction with Group C and CHX at 21 and 28 day interval. For GI the reduction was significant even at 14^{th} day.

- Group B showed insignificant mean reduction with Group D from baseline to 28 day, but showed clinically significant reduction with Group C and CHX at 21 and 28 day. For GI the reduction was significant with Group C even at 14^{th} day
- Group C showed insignificant mean reduction with Group CHX but showed statistically significant reduction with Group D at 21 and 28 day.
- Group D showed mean reduction with Group CHX which was statistically significant at 21 and 28 day.

Inter-group comparison of PPD:-

- Inter-group mean reduction was seen in all the groups from baseline to 28 day but reduction was statistically non-significant.

Intra-group comparison of PI and GI:-

- Intra-group comparison of PI and GI for all the Groups showed significant reduction but Group C and CHX showed statistically significant reduction from baseline to 7, 14, 21 and 28 day interval.

Intra-group comparison of PPD:-

- In Intra-group comparison of the mean PPD in all the groups the results were statistically non-significant.

CONCLUSION

The findings of the present study suggest that all the herbal formulations in different concentration were found to be effective in controlling the plaque and maintaining the healthy gingival status therefore, the herbal mouthwash containing aloe vera, neem and curcumin can serve as a better alternative to chlorhexidine.

The present study was a short term study with small sample size; further clinical and microbiological studies with larger sample size are required to clarify and broaden our understanding of the role of these herbal mouthwash in periodontal disease.

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SUMMARY

The present study was conducted to evaluate the clinical effects of various herbal mouthwashes containing Aloe vera, Neem and Curcumin and comparing it with CHX mouthwash on periodontal diseases.

Aloe vera, curcumin and neem mouthwash which are used in (test groups), are prepared and standardized accordingly in collaboration with CIMAP Lucknow. Furthermore, the CHX mouthwash used in (control group) is commercially available under the trade mark HEXIDINE with concentration 0.2%. The formulations formed were divided into 4 groups according to the concentrations of herbal extracts with Aloe vera juice base Group A (0.5gm neem and curcumin), Group B (1gm neem and curcumin), Group C (1.5gm neem and curcumin) and Group D (2gm neem and curcumin).

A total of 50 subjects were taken from the OPD of Department of Periodontics aged between 18-35 years for the study. The study protocol was explained to all the patients. The patients, who fulfilled the criteria, were enrolled in the study. The subjects were divided into 5 groups Group CHX, A, B, C and Group D 10 subjects in each group. Clinical parameters PI, GI and PPD at baseline were recorded. All patients underwent scaling and root planing, polishing and oral hygiene instructions were given. Patients were instructed to rinse with their assigned mouthwash (10ml) twice daily for 30 seconds over a period of 28 days. They were recalled for re-evaluation on 7^{th}, 14^{th}, 21^{st} and 28^{th} day and all clinical parameters were recorded and plaque control measures were reinforced.

After collection of the data from baseline to 28 day, analysis was done and following results were obtained:

In Inter-group comparison of the mean PI and GI between Group A and C, Group A and CHX, Group B and C, Group B and CHX, Group C and D and Group D and CHX difference was statistically significant at 21 and 28 day interval, which showed that Group C and CHX showed reduced plaque and gingival index.

In Inter-group comparison of the mean PPD in all the groups the results were statistically non-significant.

In Intra-group comparison of the mean PI and GI in Group C and Group CHX showed a reduction in PI and GI from baseline to 28 day the difference was statistically significant. However, no reduction in PPD was seen within the groups.

Based on the efficacy of the data it is evident that the Group C having dose of 1.5gm was amongst the best in the dose dependent study ranging from 0.5 to 2 gm and showed antiplaque antigingivitis property. Further, observation from the study concluded that the ratio of the herbal components is optimum at 1.5gm of herbal mouthwash to 0.2% of CHX. CHX being pure compound acts at a much lower dose as compared to the herbal counterpart; however it is also associated with inherent toxicities at higher doses. On the other hand the herbal formulations used in this study though has higher dose but is equally efficacious. The formulation being from crude extracts have multiple phytoconstituents which act as synergy with different mechanism either directly on the pathogen or indirectly through the stimulation of innate immune mechanism especially through increased mucosal response. In this study *Azadirachta indica* (neem), and Curcumin have been traditionally used and proven for anti-microbial and immunostimulatory response.

The present study was a short term study with small sample size; further clinical and microbiological studies with larger sample size are required to clarify and broaden our understanding of the role of these herbal mouthwash in periodontal disease. Therefore, the findings of the present study suggest that the herbal mouthwash containing aloe vera, neem and curcumin can serve as a better alternative to 0.2% CHX.

LIST OF GRAPHS

ABSTRACT

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