COMPARATIVE EVALUATION OF THE EFFICACY OF *Azadirachta indica* INCORPORATED COLLAGEN FIBERS WITH CURCUMIN INCORPORATED COLLAGEN FIBERS -A RANDOMISED CLINICAL STUDY

Dissertation

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In

PERIODONTOLOGY

By

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Certificate

This is to certify that the dissertation entitled "**COMPARATIVE EVALUATION OF THE EFFICACY OF *Azadirachta indica* INCORPORATED COLLAGEN FIBERS WITH CURCUMIN INCORPORATED COLLAGEN FIBERS -A RANDOMISED CLINICAL STUDY"** is a bonafide work done *by* ***Dr Ekta dwivedi*** post graduate student, Department of Periodontology, under our guidance and supervision in partial fulfillment of the Master of Dental Surgery course during the academic session 2018-2021.

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"The most important function of education at any level is to develop the personality of the individual and the significance of his life to himself and to others."

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Dr Ekta Dwivedi

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CONTENTS

LIST OF TABLES

LIST OF FUGURES

LIST OF ILUSTRATIONS (PLATES)

LIST OF APPENDICES

LIST OF ABBREVIATIONS

PI	Plaque index
GI	Gingival index
PPD	Probing pocket depth
GCF	Gingival crevicular fluid
BOP	Bleeding on probing
AZA	*Azadirachtaindica*
CHX	Chlorhexidine
TC	Tetracycline
LDD	Local drug delivery
SRP	Scaling and root planing
MMP	Matrix Metallo Proteinases
PCR	Polymerase Chain Reaction
RAL	Relative attachment level
Spp	Species

The study was conducted in the Department of Periodontology, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh.The present study aimed to evaluate the time release pattern and compare the efficacy of *Azadirachta indica* (Neem) and *Curcuma longa* (curcumin) incorporated in collagen fiber in subjects suffering from moderate chronic periodontitis. Hence, the study was divided into *in-vitro* and *in-vivo* arm. In, *in-vitro* arm -the time release pattern of *Azadirachta indica* incorporated fibres and curcumin incorporated fibres were evaluated in School of Pharmacy, BBDU. *In-vivo* arm of study was aimed at evaluating and comparing Azadirachta indica (Neem) fibre with Curcumin fibre (Turmeric) as a local drug delivery (LDD) agent after 7 days, 14 days and 21 days of healing. The study was a Parallel Design Clinical Study with 2-groups,

Experimental Group-A: Scaling and Root Debridement + Neem fibres

Experimental Group-B: Scaling and Root Debridement + Curcumin fibres

A total of 40 patients [20 in each group] (age range: 25-65 years) based on inclusion and exclusion criteria were enrolled in this study and randomly distributed using lottery method. All 40 patients with at least 5-6 mm pocket after initial examination, were subjected to phase-I therapy which consisted of full mouth scaling and root debridement using hand and ultrasonic instruments. Following this, clinical parameters viz. Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD) at baseline were recorded. After recording clinical parameters at baseline, required proportion of the wet fibres were taken and were gently pushed into the pocket site using gingival cord packing instrument. The gingiva was subsequently adapted to close the entrance of the site and hand pressure was applied for 2-minutes to encourage haemostasis. After the placement of fibres in both the groups, the threaded sites were given a periodontal dressing for 1 week. All patients were recalled on 7^{th}, $14^{th}$and 21^{st} day post-therapy. *In- vitro* result analysis showed release of Neem constituents from collagen thread at 72 hours was 38.33% which there after remained constant when checked at 5^{th} and 7^{th} day. Approximately 76% curcumin was released from collagen thread within 5 days. Hence, therapeutic range of neem and curcumin will be administered before degradation of collagen starts. *In-vivo* a significant reduction in all the three clinical parameters at day 21. Inter- group comparison did not show significant difference. Hence, from this study it can be concluded that Neem incorporated collagen fibers and curcumin incorporated collagen fibers can be used as an adjunct to SRP, for the added benefits in the control of periodontal disease.

INTRODUCTION

Periodontal disease encompasses a number of pathological conditions, such as chronic periodontitis, systemic disease-associated periodontitis, aggressive periodontitis and necrotizing periodontitis, affecting the tooth supporting structures. It is well established that periodontal disease is caused by local bacterial infection with a pathogenic microflora within the periodontal pocket. The microflora involved in periodontitis is complex and composed mainly of gram negative anaerobic bacteria. The bacteria form a highly structured and complex biofilm in the periodontal pocket, later the biofilm reaches sub-gingivally and it becomes hard to remove during regular oral hygiene practices. Most commonly periodontal diseases are treated by mechanical procedures such as thorough scaling and root planing in conjunction with patient's proper plaque control. Although mechanical therapy may provide long term stability for many patients, but in several conditions due to the complex anatomy of the root and the site of lesion may hinder the treatment and prevent the sufficient removal of the bacterial load. The control of supra-gingival plaque is very essential to prevent re-colonization. Patient who could not achieve acceptable plaque control during or after the treatment mostly suffer from recurrent periodontitis, so oral hygiene is very important for successful outcome after the treatment.The periodontal pocket provides an ideal environment for the growth of anaerobic bacteria, thus for the effective treatment the antibacterial agents are used along with mechanical debridement in the treatment of periodontitis. Approaches to systemic antibiotics in periodontal therapy includes mainly single drug therapies with either Tetracyclines, Minocycline, Penicillins, Metronidazole or Clindamycin[1]. Indeed, side effects such as hypersensitivity, gastrointestinal intolerance and the development of bacterial resistance have been described[2]. Some studies also reported poor results due to the fact that the active product could not achieve an adequate concentration at the site of action or due to the inability of the active product to be retained locally for a sufficient period of time. These drawbacks would be markedly reduced if antimicrobial agents applied locally, although side effects effects such as gastrointestinal disturbances and development of antibiotic resistance cannot be totally ruled out[3].

Local delivery of antimicrobial agents into periodontal pocket has been extensively developed and investigated since late 1970's . It includes oral rinses, sub gingival irrigation and controlled release delivery systems. The local tissue concentration of a

drug can be enhanced by incorporating the active agent into controlled release delivery systems to be placed directly in the periodontal pocket. Sustained local drug delivery systems may also be recommended for sites which are considered difficult to instrument because of depth or anatomical complexity. They have been evaluated in several forms such as gels, strips, fibers, chips, ointments etc and using different antimicrobial agents[4]. Many chemical agents have been tesed as adjunct to mechanical methods which can reduce plaque associated gingivitis. Chlorhexidine, triclosan, povidine –iodone and various phenolic compounds have been used successfully as antiplaque agents However, side effects such as allergy, discolouration of teeth and unpleasant taste can occur when these chemicals are used for an extended period of time[5].

Over the last decade herbal medications turned out to be a popular form of therapy throughout the world when used in prophylaxis and treatment of various diseases. Many side-effects associated with modern medicines have been averted by using herbal medicines, and thus they are safer to use. Presently a large number of natural herbs are also known to halt the progression of periodontal disease. The herbs frequently used for treatment of periodontitis are Neem, turmeric, aloe-vera, clove and cinnamon.

Neem has been used in India and South Asia for thousands of years as the preferred tool for maintaining healthy gums and teeth. Brushing with Neem twigs and chewing Neem leaves and seeds after a meal has been the traditional dental care practice. With available modern preparations many people are now using commercial products that contain the same basic Neem components. The antibacterial activity of Neem has been evaluated and known from ancient times[6]. Neem has been considered to have various therapeutic activities such as astringent, antiseptic, insecticidal, antiulcer and for cleaning the teeth in pyorrhoea and other dental diseases. Other than this, leaf extract of the Neem showed superior antiviral activity in vitro[7] and anti hyperglycemic in vivo [8].

Leaves of the Neem have been used in the treatment of gingivitis and periodontitis. Neem has also showed better efficacy in the treatment of oral infections and plaque growth inhibition in treating periodontal disorder. Neem has also shown good in vitro, broad range antibacterial activity[9]. It removes toxins from the body, purifies the blood

and neutralizes damaging free radicals. So, locally it may also have an effect in enhancing healing. Neem extract contains Azadiractin, glycosides, sterols, luminols, flavonoids, Nimbin and Nimbidin[9].

Curcuma longa (Turmeric) is a widely used indian rhizomatous medicinal plant from the family Zingiberaceae. Curcumin Demethoxycurcumin (DMC, and Bisdemethoxycurcumin (BDMC) are the constituents of the turmeric and are collectively known as curcuminoids. Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) or Diferuloylmethane is well known for its different biological activities such as anti-inflammatory and anti-viral[11] , anti-oxidant, anti-cancer, anti-bacterial, anti-asthmatic, anti-arthritis, anti-diabetic, anti-venom, anti-obesity, wound healing, in depression and anxiety and other activities[12].*Curcuma longa* has been shown to exhibit anti-inflammatory biological activity against periodontitis [13].

As per the herbal products test of our literature search, no study has been found comparing two herbal products efficacy in subjects of moderate chronic periodontitis hence we have taken up this study with the aim of assessing the efficacy of the two herbs i.e neem and curcumin in collagen fibers as local drug delivery and comparing the outcome.

AIM AND OBJECTIVES

1. AIM

The aim of the study is to evaluate the time release pattern and compare the efficacy of *Azadirachta indica* (Neem) and *Curcuma longa* (curcumin) incorporated in collagen fiber in subjects suffering from moderate chronic periodontitis.

2. OBJETIVES

1. To evaluate the time release pattern of *Azadirachta indica* (Neem) and *Curcuma longa* (curcumin**)** incorporated fibers as local drug delivery system in *In vitro*

2. To evaluate the efficacy of *Azadirachta indica* (Neem) and *Curcuma longa* (curcumin**)** incorporated fibers as local drug delivery system in subjects suffering from moderate Chronic periodontitis.

3. To compare the efficacy of *Azadirachta indica* (Neem) incorporated fibers and *Curcuma longa* (curcumin**)** incorporated fibers.

REVIEW OF LITERATURE

MEDICINAL PROPERTIES OF NEEM:-

Khalid SA et al (1986)[14] examined in vitro anti-malarial activity against plasmodium falciparum on twenty one compounds isolated from nine medicinal plants used in traditional medicine which includes alkaloids lignans, triterpines, coumarins , limonoids and flavonoids. Most were relatively inactive ; limonoid , gedunin had an ICsO value roughly equivalent to quinine. In this protocol, the flavonoid quercetin purified from Diosma pilosa was found to have the same activity as a commercially obtained preparation. Simple radiometric assays for antimalarial activity can thus be used to rapidly screen purified plant material or secondary plant metabolites.

Van der Nat JM et al.(1987)[15] examined the immunomodulatory activity of *A. indica* bark extract showing strong anti- complementary effects which were dose and time-dependent and most pronounced in the classical complement pathway assay. A dose – dependent decrease in the chemiluminescence of polymorphonuclear leukocyte was observed and a dose-dependent increase in the migration inhibition factor by lymphocytes.

Ray A et al.(1996)[16] studied the effects of A. indica on the tests of humoral and cell mediated immune response after 3 weeks of oral Azadirachta indica (leaf extract) treatment in ovalbumin (antigen) immunized mice. The result suggested that tests for humoral immune responses, A. indica treated mice had higher IgM and IgG levels and higher anti- ovalbumin antibody titers when compared to the vehicle treated group. In tests for cell mediated immune responses, there is enhancement of macrophages migration inhibition and food pad thickness after *Azadirachta indica* treatment. These results suggest that possible immunopotentiating effects of *Azadirachta indica.*

Biswas K et al (2005)[17] Reviewed that neem is the most useful traditional plant with medicinal properties.Nimbdin, bitter crude extract has anti-inlammatory, antipyretic, antifungal ,antibacterial effects.Neem leaves contain cyclic trisulphide and cyclic tetrasulphide having antifungal properties.Further, medicinal uses of neem can be utilized as immune stimulants, a ntiulcer, antimalarial, anticarcinogenic, antioxidant, antiviral effects.

Mosaddek ASM et al (2008)[18] Conducted a study to compare the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone . The anti-inflammatory effect of aqueous extract of neem leaf (400 mg/kg body weight) was compared with that of dexamethasone (0.75 mg, intra peri-toneally) by administering one hour before the formalin injection and once daily for 7 days in rats. The percentage of inhibition of paw edema in case ofneem after 3, 6 hours, on day 3, 7 after formalin injection were 28, 40, 45, 58% respectively and that in case of dexamethasone after 3, 6 hours, on day 3, 7 after formalin injection were 43, 58, 61, 65% respectively. The reduction was statistically significant in each case ($p<0.001$). The study suggested that anti-inflammatory effect of neem extract is less than that produced by dexamethasone.

Paul R et al(2011)[19]reviewed the anticancer biology of neem leaves. All parts of this tree, particularly the leaves, bark, seed- oil and their purified products are widely used for treatment of cancer. The anticancer properties of the plant have been studied largely in terms of its preventive, protetive, tumor suppressive, immunomodulatory, and apoptotic effects against various types of cancer and their molecular mechanisms.

Hossain MA et al(2013)[20] investigated *in viro* antioxidant activity and characterize chemical constituents in different crude extracts of the leaves of *A. indica* by using modern GC-MS. The evaluation of antioxidant capacity of different crude extracts was in order of chloroform> butanol> ethyl acetate axtract> hexane exract> methanol extract.

Khetarpal S et al.(2014)[21] Compared the anti-bacterial and anti-inflammatory properties of neem, curcumin and aloe vera in conjunction with chlorhexidine as an intracanal medicament. Thirty three patients(33) within the age group of 25-40 years with two single rooted anterior teeth, presenting with periapical radiolucency requiring endodontic therapy were selected. These 33 patients were divided into two groups, in which one was the test and another was control in one single patient. The control samples were taken from all the 33 patients i.e (33 samples) in which chlorhexidine is used as intracanal medicament, whereas the test groups were divided into another three respective groups with 11 samples in each group on the basis of three herbal intracanal medicaments used in this study. The antimicrobial and anti-inflammatory property was assessed in our study using the microbial colony count

method and the Visual analogue pain scale method respectively. Test Group A(Neem) showed the highest antibacterial activity with maximum reduction in microbial colony count scores. and the test Group B(Curcumin) showed the highest anti-inflammatory activity with maximum reduction in the VAS pain score. On comparing test groups with control group and antimicrobial property was analyzed. The results were statistically non-significant when Group A(Neem) was compared with Control group(Chlorhexidine) and statistically significant when Group B(Curcumin) and Group C(Aloe vera) was compared with Chlorhexidine.

Verma UP et al(2018)[22] assessed the influence of CHX, NVC and NE on cultured human gingival fibroblasts (hGFs) using MTT assay and FACS analysis. MTT assay with hGFs indicated altered morphology with maximum cell death at 10% CHX, while NVC and NE showed similar results at a concentration of 75% and above. On FACS analysis, beyond 1%, CHX adversely affected the cell cycle phase distribution whereas NE exerted a detrimental effect only at 100%. Finally, it was concluded that membrane integrity is unaffected up to 50% exposure to NE, a crucial feature, makes us choose it as the best among the three mouth rinses tested.

Nguyen Duc Hanh et al(2020)[23] developed and evaluated neem gel formulation using gum karaya as gelling agent . he aim of this study was to develop and evaluate the neem gel formulation using gum karaya as a gel forming agent. The gelling ability of gum karaya and its mixtures with the other gel-forming agents (sodium alginate, carboxymethyl starch and hydroxypropyl methylcellulose) were demonstrated by rheology properties. Gum karaya was demonstrated to be a gel-forming agent with the pseudoplastic flow. Of three gelling agents, sodium alginate was selected as the best combined excipient with gum karaya due to the better elasticity improvement of the gum karaya gel in comparison with hydroxypropyl methylcellulose (HPMC) and carboxymethyl starch (CMS). The cause-effect relations between the three independent variables (the ratios of gum karaya, calcium chloride and sodium alginate) and the two dependent variables (spreadability and viscosity of neem gel) were investigated. The optimal neem gel formulation comprised gum karaya, calcium chloride and sodium alginate with the concentrations of 1.6%, 0.0435% and 1%, respectively. The optimal neem gel was evaluated for its various properties such as pH, homogeneity, spreadability, active compound quantitation by high performance

liquid chromatography (HPLC) and dynamic viscoelasticity behaviours. This study has reported for the first time the application of gum karaya in neem gel preparation. The results of the research may provide the useful data for further studies not only on the gum karaya as the gelling agent but also on the neem gel preparation for the treatment of skin diseases.

EFFICACY OF NEEM IN PERIODONTAL DISEASE

Pai MR et al (2004)[24] Conducted a study to evaluate the effectiveness of neem leaf extract against plaque formation in males over 6 weeks and salivery bacterial count was checked for streptococcus mutans and lactobacilli species. A mucoadhesive dental gel containing 25 mg of leaf exract was formulated and efficacy was checked for 6 weeks with commercially available chlorhexidine gluconate gel. The result suggested that neem extract gel reduced plaque index and bacterial count than control group.

Jain S et al (2012)[25] evaluated the therapeutic efficacy of neem chip in periodontal pockets with SRP as control. Probing pocket depth, clinical attachment level, Plaque index were evaluated at baseline, 6 weeks and 3 months and compared. In the test group result showed significant reduction in the probing depth and gingival index from baseline to 3 months compared to control group.

Antony VV et al. (2013)[26] Evaluated the efficacy of Azadirachta indica (Neem) extract gel as a local drug delivery in the treatment of patients with chronic periodontitis and evaluated the clinical as well as the microbiological benefits when used as an adjunct to scaling and root planning. The results showed improvements in the clinical parameters both at the control and the experimental sites. However, the experimental sites showed better results compared to the control site. The microbiological analysis proved a relative reduction of the periodontal pathogens at both the experimental as well as the control site with the control site showing a significantly higher reduction.

Abhishek KN et al (2015)[27] studied the effect of neem toothpaste on plaque and gingivitis. 30 students divided into 2 groups were included in the study . A washout phase of $2^{1/2}$ days were carried out for both the groups. Following propylaxis subjects

were randomly allocated into 2 groups . Statisticaly significant difference was observed with the use of neem toothpaste in terms of plaque index and gingival index.

Dhingra K et al(2016)[28] studied the systematic review was to evaluate the effectiveness of *Azadirachta indica* based herbal mouthrinse in improving plaque control and gingival health. These studies reprted that neem mouthrinse was as effective as CHX mouthrinse when used as adjunct to tooth brushing in reducing plaque and gingival inflammation in gingivitis patients.

Vennila K et al.(2016)[29] evaluated the efficacy of 10% whole Azadirachta indica (Neem) chip as an adjunct to scaling and root planning in chronic periodontitis. Twenty otherwise healthy patients with the bilateral periodontal probing depth of 5-6mm were included in the study. After scaling and root planning (SRP), 10% non absorbable neem chip was placed in the pocket in one side of the arch. Other side was done with SRP only. clinical parameter showed statistically improved on the neem chip sites and presence of P. gingivalis strains were significantly reduced on the neem chip sites.

Nimbulkar G et al.(2020)[30] studied the microbiological and clinical evaluation of neem gel and chlorhexidine gel on dental plaque and gingivitis in 20 to 30 years old adults and concluded that neem gel showed significant decrease in dental plaque, and microbial counts, which was comparable to chlorhexidine gel providing a good herbal alternative. No side effects were reported for use of neem gel over considered period of time.

STUDIES ON LOCAL DRUG DELIVERY SYSTEM

Shah JC et al.(2001)[31] reviewed that the cubic phases have been shown to deliver small molecule drugs and large proteins by oral and parentral routes in addition to local delivery in vaginal and periodontal cavity. Bio-degradibility, phase behavior , ability to drug deliver of varying sizes and polarity and the ability to enhance the chemical and/ or physical stability of incorporated drugs and proteins make the cubic phase gel an excellent candidate for use as a drug delivery matrix . However , shorter release duration and the extremity high viscosity may limit its use to specific applications such as periodontal , mucosal, vaginal and short acting oral and parentral drug delivery.

Renvert S et al (2008)[32] conducted a study to assess the clinical and microbiologic outcome of local administration of minocycline microsphere and chlorhexidine gel, in patients of peri-implantitis. Patients with probing depth >4 mm with bleeding and /or exudation on probing .Patients were randomly allocated to give either of two. Result concluded use of minocycline significantly reduced probing depths.

Sharma A (2012)[33] evaluated the efficacy of 1% ALN gel compared to a placebo gelas a local drug delivery system in adjunct to SRP for the treatment of 66 intrabony defects in patients with chronic periodontitis. The ALN gel was prepared by adding ALN to a polyacrylic acid-distilled water mixture. Clinical parameters were recorded at baseline, 2months, and 6 months. Radiographic parameters recorded at baseline and 6 months.Results suggested that he local delivery of 1% ALN into the periodontal pocket significantly reduced PD,CAL gain and improved bone fill compared to a placebo gel as an adjunct to SRP.

Dodwad et al.(2012)[34] reviewed that the adjunctive use of local drug delivery in conjunction with SRP may inhance the results in sites that don't respond to conventional therapy. Local drug delivery systems with controlled release properties have the potential to be used as a therapeutic component in the management of periodontal diseases . The clinician will need to make decisions based on the desired outcomes of the therapy.

Balappanavar AY (**2013)**[35] compared the effectiveness of 0.5% tea,2% neem, and 0.2% chlorhexidine mouthwashes on oral health. 30 healthy subjects wereselected and randomly assigned into 3 groups i.e group A -0.2% chlorhexidine gluconate, group B -2% neem, and group C 0.5 % tea, 10 subjects per group. Mean plaque and gingival scores were reduced over the 3 week trial period for experimental and control groups. Antiplaque effectiveness was highest in group C. Neem and tea showed comparative effectiveness on gingiva better than chlorhexidine.the salivary pH rise was sustained and significant in Group B and C compared to Group A.

Christopher J et al (2015)[36] conducted a meta-analysis of 72 articles on the effectiveness of SRP with or without te systemic antimicrobials, a systemic host modulator(subantimicrobial- dose doxycycline), locally delivered antimicrobials (chlorhexidine chips, doxycycline hyclate gel, and minocycline microspheres), and a

variety of non surgical lasers (photodynamic therapy with a diode laser, neodymium: yttrium-aluminium- garnet lasers, and erbium lasers).The panel judged 4 adjunctive therapies as beneficial with a moderate level of certainy: systemic subantimicrobial – dose doxycycline, systemic antimicrobials, chlorhexidine chips and photodynamic therapy with a diode laser.

Gupta A et al (2018)[37] examined the efficacy of local drug delivery system of ZLN gel as an adjunctto SRP for the treatment of human periodontal intrabony defects clinically and radiografically. In moderate to severely affected 40 chronic periodontitis patients , 40 intrabony defects(three walled and combined defects without involving furcation) were randomly divided into two groups and treated either with 0.05% ZLN gel or placebo gel (control group) after SRP. Clinical parameters were assessed at baseline and at 3 or 6 months using occlusal acrylic stent. Radiographic parameters were assessed at baseline and 6 months, utilizing ONIS 2.5 PROFESSIONAL and SYNGO software compatible with DentaScan to measure the volumetric bone changes in intrabony defects. Result showed ZLN gel applied sub gingivally in intrabony defects resulted in significant improvements both clinically and radiographically in defect depth and buccolingual width with volumetric defect gain.

MEDICINAL PROPERTIES OF CURCUMIN

Sebastia N et al (2012)[38] revieved the medicinal properties and health benefits of curcumin. Chemical investigations have concluded that medicinal properties of this preparation are due to its major polyphenolic compound (5-10% of dry weight), the curcumin (diferuloylmethane). This vivacious yellow compound has been also used as a spice, approved as food additive to flavor various types of curries and mustards. Biomedical investigations of curcumin have evidenced a wide range of molecular and cellular activities, most related to redox reactions and signal transduction. Many of the activities are related to its ability to suppress acute and chronic inflammation. Moreover, curcumin inhibits cancer development and progression. It has activity as a blocking agent, inhibiting the initiation of cancer and also as a suppressing agent, inhibiting malignant cell proliferation, thus, the progression of carcinogenesis (a clinical trial in Phase I about curcumin for the prevention of colon cancer has been completed). Another interesting aspect of curcumin's activity is the ability to exert

both radioprotective effects in normal cells and radiosensitizing effects in cancer cells. Curcumin is remarkably well tolerated, but its bioavailability is poor (a clinical trial concerning pharmacokinetics of curcumin in healthy volunteers had been completed in2007). Hence, its bioavailability has been tried to improve by dissolving curcumin in ambivalent solvents. However, all these promising results should be taken in caution because of some undesirable effects ofcurcumin have been detected.

Haneefa.M et al(2014)[39] reported an investigation of the bioactive "Curcumin" present in the crude plant extracts of 4 selected turmeric plants i.e. BSR-01, BSR-02, CL-101, CL-219. This investigation was carried out to determine and compare the quantitative amounts of curcumin that are present in 4 different varieties of turmeric. The extraction of the herb curcumin from turmeric was attempted by using a "Soxhlet" solvent extraction technique with 95% ethanol as a solvent, then the quantification of curcumin in turmeric was normally based on spectrophotometric measurement. The presence of curcumin was confirmed by UV-Visible and elemental analytical techniques. Morphology studies (SEM) and XRD crystal studies were also investigated.

Platia S et al (2016)[40] assessed the efficacy of 0.12 % chlorhexidine and Tumeric as Subgingival Irrigants in Patients of Chronic Periodoniitis. A total of 60 patients suffering from chronic periodontitis were enrolled in the study. Before irrigation, complete scaling and root planing was done. Total of 60 patients were randomly and equally divided into 3 test groups. Test group 1 comprised of Patients irrigated with 0.12% Chlorhexidine digluconate. Test group 2 comprised of Patients irrigated with freshly prepared 10%Turmeric solution. Test group 3 comprised of Patients irrigated with distilled water (control). All clinical parameters- Plaque index (Turesky-Gilmore-Glickman Modification of the Quigley Hein Plaque Index), Gingival index (Loe and Silness gingival index) and Periodontal pocket depth were assessed on day 0 after complete oral prophylaxis and again on day 7, 21 and 42.Out of the three subgingival irrigating solutions, good results were seen with Chlorhexidine, then with Turmeric and then for distilled water as an irrigating solution. Concluded that chlorhexidine has shown to be a potent therapeutic agent which has the properties of improving the periodontal status.

Xiao CJ et al (2018)[41] evauated protective effect and related mechanisms of curcumin in rat experimental periodontitis. The gingival fibroblasts were incubated with different concentrations of curcumin in the absence or presence of lipopolysaccharide (LPS). Concentrations of interleukin-1β(IL-1β), tumor necrosis factor-α (TNF-α), osteoprotegerin (OPG) and soluble receptor activator of nuclear factor kappa-B ligand (RANKL) culture supernatants of rat gingival fibroblasts were determined by enzyme linked immunosorbent assay. The nuclear fraction of rat gingival fibroblasts was extracted and nuclear factor kappa-B (NF-κB) activation was assessed by western blotting to elucidate related mechanisms. Curcumin was given every two days by oral gavage. The gingival inflammation and alveolar bone loss between the first and second molars were observed by hematoxylin and eosin staining. Collagen fibers were observed by picro-sirius red staining. Alveolar bone loss was assessed by micro-CT analysis.Result shown Curcumin attenuated the production of IL-1β and TNF-α in rat gingival fibroblasts stimulated by LPS, and

inhibited the LPS-induced decrease in OPG/sRANKL ratio and NF-κB activation. Curcumin significantly reduced gingival inflammation and modulated collagen fiber and alveolar bone loss in vivo

Dave DH et al (2018)[42] evaluated the efficacy of oral curcumin gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis. In this clinical study, forty participants with mild chronic periodontitis were included. Included participants underwent Phase I therapy, after which they were allocated into two groups (20 each), out of which only one group received curcumin gel for topical application. Plaque index (PI), bleeding on probing measured by sulcus bleeding index, probing pocket depth and clinical attachment level (CAL) were recorded at baseline and at the follow-up after 2 months. Both test and control groups showed statistically significant reduction in PI, sulcular bleeding index, pocket probing depth and CAL. Curcumin gel group showed statistically significant difference compared to the control group with respect to PI (<0.001), sulcular bleeding index (<0.001) and pocket probing depth (0.006). Concluded that Curcumin as an adjunct to SRP showed higher reduction in plaque accumulation, sulcular bleeding and and pocket probing depth as compared to SRP alone.

Vandana D et al(2019)[43] aimed to prepare a topical gel containing curcumin (CUR) for the treatment of microbial infections on skin. CUR was complexed with the β-cyclodextrin (β-CD) using kneading method in 1:1, 1:2, and 1:3 molar ratios and characterized. The inclusion complex with high aqueous solubility was loaded in the topical gel containing (2% CUR) which was prepared using carbopol, sodium CMC, and guar gum and evaluated for viscosity, spreadability, extrudability, pH, drug content, and *in vitro* diffusion studies. The *in vitro* anti-inflammatory activity of the gel was performed by albumin protein denaturation technique, the statistical analysis was done using ANOVA followed Dunnett's t-test. The antimicrobial activity of CUR was evaluated using standard strains of *Candida albicans* and *Escherichia coli* by agar well diffusion method. Result showed that the complexation of CUR had an increased solubility up to 103.09 times for 1:3 molar ratio with *in vitro* dissolution 90.64% for 60 min. The optimized formulation F9 had viscosity of 6500.3 cps and 97.5% *in vitro* drug diffusion for 8 h which follows zero-order release kinetics. *In vitro* anti-inflammatory activity studied showed that the CUR gel has a good potency for renaturation and was as effective as standard diclofenac with 76.9% inhibition (p=0.0507). CUR showed approx. 3 mm diameter of zone of inhibition against *C. albicans* and *E. coli*.

Rathi VD et al (2019)[44] evaluated the efficacy of Azadirachta indica (neem) and curcuma longa (turmeric)in extraction socket. 45 extraction site were divided in 3group of 15 each where apart from group left as control, remaining groups received neem and turmeric extracts respectively .The healing of extraction socket was assessed and compared with the control group. Delayed wound healing was found in the control group as compared to the group receiving herbal extracts.

Aniesrani DS et al (2016)[45] evaluated in vitro release pattern of curcumin loaded egg albumin nanoparticles prepared using acetone as desolvation agent and to study the effect of egg albumin concentration from nanoparticles on curcumin release from nanoparticles. An initial burst release of curcumin which is adsorbed on the surface of nanoparticles and further sustained release of entrapped curcumin upto 72 h was observed in all particles.

MATERIALS AND METHOD

STUDY SETTING

The study was conducted in the Department of Periodontology, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh. The study was commenced after the clearance from the Institutional Ethical committee and approval from BBD University (BBDU), Lucknow, Uttar Pradesh.

STUDY DESIGN

The study design included both *in-vitro* & *in-vivo* arms.

IN-VITRO ARM

The time release pattern of *Azadirachta indica* incorporated fibres and curcumin incorporated fibres were evaluated and compared in vitro in School of Pharmacy, BBDU. Commercially available vial, each containing 25mg of sterile type - I fibrillar collagen were procured from EUCARE PHARMA Pvt Ltd., Chennai.

Armamentarium

Following are the main required materials to conduct in-vitro evaluation.

1. Flasks and Micropipettes
2. Soxhlet Apparatus
3. KC Diffusion Cell
4. Spectrophotometer
5. Plants Products (neem leaves and turmeric rhizome)

Plant Taxonomy

Scientific names of the plants included in present study	*Azadirachta indica*	*Curcuma longa*
Common name	Neem	Turmeric (Haldi)
Domain	Eukaryota	Eukaryota
Kingdom	Plantae (Plants)	Plantae (Plants)
Sub-kingdom	Viridiplantae (Green Plants)	Viridiplantae (Green Plants)
Infra-kingdom	Streptophyta (Land Plants)	Streptophyta (Land Plants)
Super-division	Embryophyta	Embryophyta
Division	Tacheophyta (Vascular Plants)	Tacheophyta (Vascular Plants)
Sub-division	Spermatophytina (Seed Plants)	Spermatophytina (Seed Plants)
Class	Magnoliopsida	Magnoliopsida
Super-order	Rosanae	Lilianae (Monocots)
Order	Sapindales	Zingiberales
Family	Meliaceae (Mahogany)	Zingiberaceae (Ginger family)
Genus	Azadirachta	Curcuma (Hidden)
Species	Indica	Longa
Part used in present study	Leaves	Rhizome

Chemical Composition

NEEM (*Azadirachta indica)*

Azadirachtin (AZA, 1), the main active component of this plant, is a tetranortriterpenoid. Salanin 1,4- epoxiazadiradione, meliantrol, melianone, gedunin, nimboline, nimbin, Nimbidin, deacetilasalanin azadiractol azadirone, vilosinin, meliacarpine are other active components.

TURMERIC (*Curcuma longa*)

The main active components of the turmeric are the non-volatile curcuminoids (curcumin, desmethoxycurcumin, bisdemethoxycurcumin) and the volatile oil.

Collection and Validation of Plant Material(PLATE I,II,III)

Neem leaves and rhizomes of curcuma longa were collected from the medicinal garden of the School of Pharmacy, BBDU, and washed thoroughly with running tap water, followed by double distilled water to remove any foreign material or adherent soil and dirt etc. It was then dried under shade in cool dry place. The dried Neem leaves (500mg) and rhizomes (500mg) were powdered in grinder and was passed through sieve no.44 (moderately fine powder as per Indian Pharmacopoeia) to obtain powder of uniform size. It was then stored in airtight light resistant containers for further usage in study. A small pack of activated carbon was also placed in container to prevent moisture contamination. Neem leaves collected was collaborated and validated by CSIR- NATIONAL INSTITUTEOF SCIENCE COMMUNICATION AND INFORMATION RESOURCES DELHI. (ANNEXURE-3) (Ref.No.- NISCAIR/RHMD/ Consult/2020/3720-21-1). Collected rhizome of curcumin was collaborated and validated by CSIR- NATIONAL INSTITUTEOF SCIENCE COMMUNICATION AND INFORMATION RESOURCES DELHI. (ANNEXURE-4) (Ref. No.- NISCAIR/RHMD/ Consult/2020/3720-21-2).

PLATE I: NEEM LEAVES

PLATE II: UNIFORM SIZED POWDER OF NEEM

PLATE III: UNIFORM SIZED POWDER OF TURMERIC

Preparation of the Extract

Dried neem leaves powder and dried curcuma longa rhizomes powder were transferred to the two separate Soxhlet apparatus to prepare respective extracts.

Soxhlet Apparatus (PLATE IV,V,VI)

It was originally designed for the extraction of liquid from solid material, typically, used when the desired compound has a limited solubility in a solvent. A Soxhlet extractor has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non- volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction, the solvent is removed, by a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded. The extracts were then filtered through filter paper and was kept in an airtight amber coloured container.

Drug Diffusion Study (PLATE VII)

In vitro drug diffusion study was performed using KC Diffusion cell; with capacity of 50mL. KC Diffusion Cell: (Fig.2 Plate 4) In vitro studies help in investigating the mechanism of skin or mucosa permeation of the drug before it can be developed into a transdermal therapeutic system. Egg membrane was selected as semi permeable which was isolated by dipping hen egg in concentrated hydrochloric acid solution. This HCL

solution dissolved outer CaCo3 covering. Removal of the inner semi permeable membrane was done using needle and spatula. Phosphate buffer of pH- 7.4 was used as receptor medium simulating blood. This study was performed at room temperature i.e., 25 degree C and temperature was regulated by controlling the water flow inside KC diffusion cell. Two KC Diffusion cell system were used for two different extracts. Extracts was filled in donor chamber above the semi permeable membrane (egg membrane). Samples were collected at regular interval of1 hour or 7 hours. To get the overall release of components from the extract through semi permeable membrane; samples were collected at 72 hours, 5th day, 7th day. A fixed volume of sample (1ml) was collected at a time from the receiver chamber and the same volume of freshly prepared phosphate buffer was added into the same chamber to maintain the volume as earlier to assess the release of neem and curcumin constituents from the extract into tissues (through egg membrane).

1. INCORPORATION OF NEEM INTO STERILE COLLAGEN FIBRES: A single vial of type 1 sterile collagen fibres weighed 25 mg, were soaked into 5 ml of pure Neem extract. Total weight of the soaked fibres was taken. After 2 hrs, unbound Neem was drained out and then was weighed again, thus determining the amount of Neem extract absorbed by sterile collagen fibres.

2. INCORPORATION OF CURCUMIN INTO STERILE COLLAGEN FIBRES: A single vial of type 1 sterile collagen fibres weighed 25 mg, were soaked into 5 ml of pure curcumin extract. Total weight of the soaked fibres was taken. After 2 hrs, unbound curcumin was drained out and then was weighed again, thus determining the amount of curcumin extract absorbed by sterile collagen fibres.

PLATE IV: PRINCIPLE OF SOXHLET APPARATUS

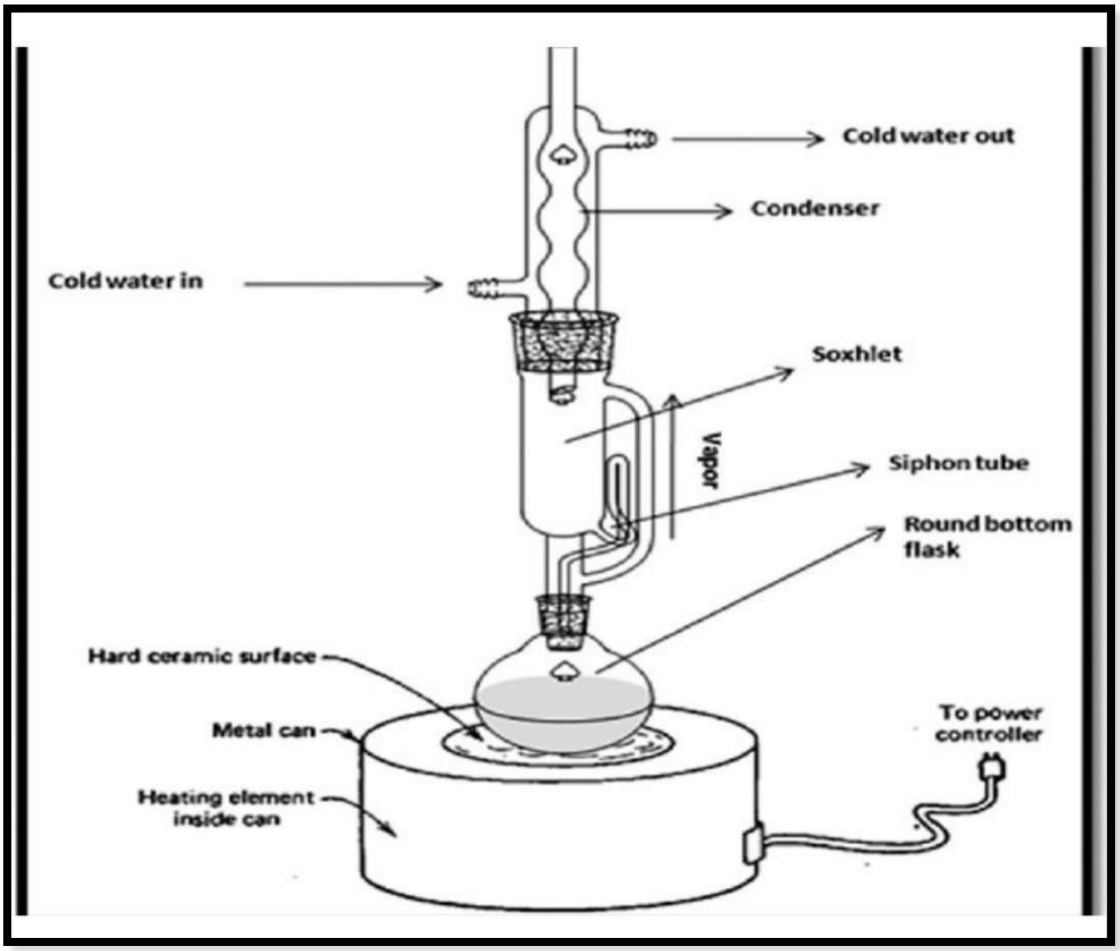


PLATE V: EXTRACTION PROCESS BY OF NEEM LEAVES BY SOXHLET APPARATUS

PLATE VI: EXTRACTION PROCESS OF CURCUMIN RHIZOMES BY SOXHLET APPARATUS

PLATE VII: K C DIFFUSION CELL

***IN-VIVO* ARM**

This arm of study was aimed at evaluating and comparing *Azadirachta indica* (Neem) fibre with Curcumin fibre (Turmeric) as a local drug delivery (LDD) agent after 7 days, 14 days and 21 days of healing. The study was a **Parallel Design Clinical Study** with 2-groups,

- **Experimental Group-1: Scaling and Root Debridement + Neem fibre**
- **Experimental Group-2: Scaling and Root Debridement + Curcumin fibres**

STUDY POPULATION (*in-vivo* arm)

The study population included the subjects selected from the patients visiting the Department of Periodontology, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh. A total of 40 patients [20 in each group] (age range: 25-65 years) based on inclusion and exclusion criteria were enrolled in this study and randomly distributed using lottery method.

SUBJECT SELECTION

INCLUSION CRITERIA

1. Systemically healthy patients
2. Patients with moderate chronic periodontitis with at least one pocket of 5-6 mm. [All the 6 sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) per tooth were examined for pocket depth and clinical attachment level. The site with deepest findings was included in the study]
3. Age 25 – 55 years
4. Adequate patient compliance

EXCLUSION CRITERIA

1. Presence of any systemic illness or drug allergies or any kind of tobacco usage
2. Pregnant and Lactating females
3. Patients who had or is taking any kind of systemic or local antimicrobial therapy, anti-inflammatory drugs for any purpose in the past 3 months including the use of mouth washes.

4. Site Specific: No caries, plaque retentive areas (restoration overhangs etc.), traumatic occlusion and ill-positioning of the tooth included.

Armamentarium (PLATE VIII)

Diagnostic instruments

Probe(UNC -15)

Mouth mirror

Tweezer

Explorer

Materials And Instruments Required For Fiber Insertion

Cumine scaler (Hu- Friedy USA)

Gracey curettes (Hu- Friedy USA)

Gingival cord packer

Neem incorporated collagen fibers

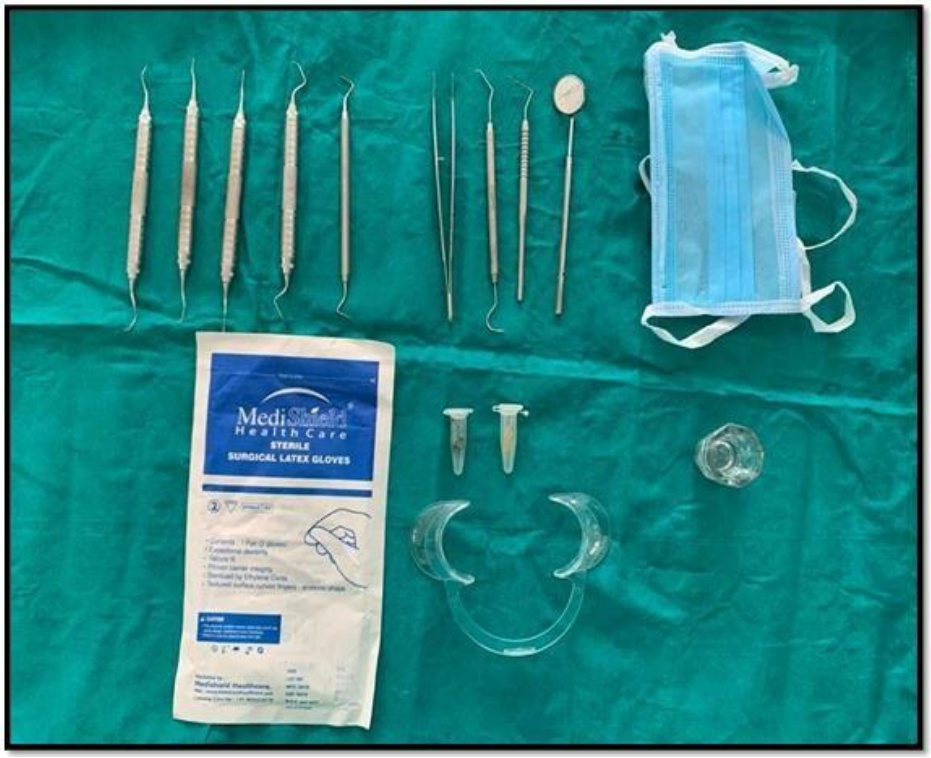
Curcumin incorporated fibers

Cheek retractor

Dapen Dish

Coe- pak

PLATE VIII: ARMAMENTARIUM



Sample Size

Sample size calculation:

$$\mathbf{n = 2(Z_\alpha + Z_\beta)^2 . \sigma^2 \div d^2}$$

where n (Total number of patients) came as 40 after Calculations (20 in Each Group)

$Z\alpha$ = 1.96 (95% Confidence Level)

Z_β = 1.28 (90% Power)

σ = 0.75 (Anticipated Standard Deviation)

d = 1.58 (Test Value of Difference between Means)

INFORMED CONSENT

Prior to initiating the study, all the patients included were informed of the purpose and design of this clinical study and were required to sign a written informed consent form failing of which led to their exclusion. A detailed medical and dental history was

taken from each patient and a detailed clinical examination including initial radiographs was performed.

SCALING AND ROOT DEBRIDEMENT

All 40 patients with at least one 5-6 mm pocket after initial examination, were subjected to phase-I therapy which consisted of full mouth scaling and root debridement using hand and ultrasonic instruments. Following this, clinical parameters at baseline were recorded.

CLINICAL PARAMETERS at Baseline, 7 days 14 days and at 21 Days

Under-mentioned clinical parameters were assessed at baseline, 7^{th} day, 14^{th} day and at 21^{st} day of healing:

- Plaque index (PI) [Silness & Low 1964][46]
- Gingival index (GI) [Low & Silness 1963][47]
- Probing pocket depth (PPD) -measured only at baseline and day 21

PLAQUE INDEX (PI) ***(Silness and Loe 1964)***

A mouth mirror and a dental explorer were used, after air drying of the teeth and gingiva lightly to assess plaque. The surfaces examined were the four gingival areas of the tooth i.e., the disto-facial, facial, mesio-facial, and lingual surfaces. Only plaque of the cervical 1/3 of the tooth was evaluated.

Scoring criteria

1. No plaque in the gingival area
2. A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may only be recognized by running a probe across the tooth surface.
3. Moderate accumulation of soft deposits within the gingival margin, which can be seen by the naked eye
4. Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

Individual tooth PI and full mouth plaque index score was recorded at baseline, 7th day ,14 day and 21st Day of healing.

Plaque index (full mouth) = Total score (full mouth) / No. of surfaces examined

GINGIVAL INDEX (GI) ***(Loe and Silness 1963)***

A mouth mirror and a UNC-15 graduated periodontal probe, Hu-friedy were used after drying the gingiva lightly. The tissues surrounding each tooth were divided into four gingival scoring units i.e., disto-facial papilla, facial margin, mesiofacial papilla, and entire lingual gingival margin.

Scoring criteria

1. Normal gingiva
2. Mild inflammation, slight change in colour, slight oedema, no bleeding on probing (BOP).
3. Moderate inflammation, redness, oedema, glazing, BOP
4. Severe inflammation, marked redness and oedema, ulceration, spontaneous bleeding

Individual tooth GI and full mouth gingival index score was recorded at baseline, 7^{th} day, 14^{th} day and 21^{st} Day of healing.

Gingival index (full mouth) = Total score (full mouth) / No. of surfaces examined

PROBING POCKET DEPTH MEASUREMENTS

PPD were determined at baseline and at 21^{st} day by using UNC-15 graduated periodontal probe *(Hu-friedy)* and were recorded to the nearest millimetres. No probing was done at 7^{th} and 14^{th} day as the healing is not completed.

LDD PROCEDURE (PLATE IX-XIV)

After recording clinical parameters at baseline, required proportion of the wet fibres were taken and was gently pushed into the pocket site using gingival cord packing instrument. The gingiva was subsequently adapted to close the entrance of the site and hand pressure was applied for 2-minutes to encourage haemostasis. After the placement of fibres in both the groups, the threaded sites were given a periodontal dressing *(Coe-pak^{TM} GC America Inc. Illinois, USA)* for 1 week. Patients were asked to refrain from oral hygiene aids in the concerned area for 1 week during which periodontal dressing was present in the mouth. All patients were recalled on 7th, 14th, 21st day post-therapy. Periodontal dressings were removed on 7th day and at-home oral hygiene measures were resumed and re-inforced. No sub-gingival instrumentation or probing was done till 21st day.

PLATE IX: BASELINE EVALUATION OF NEEM FIBERS

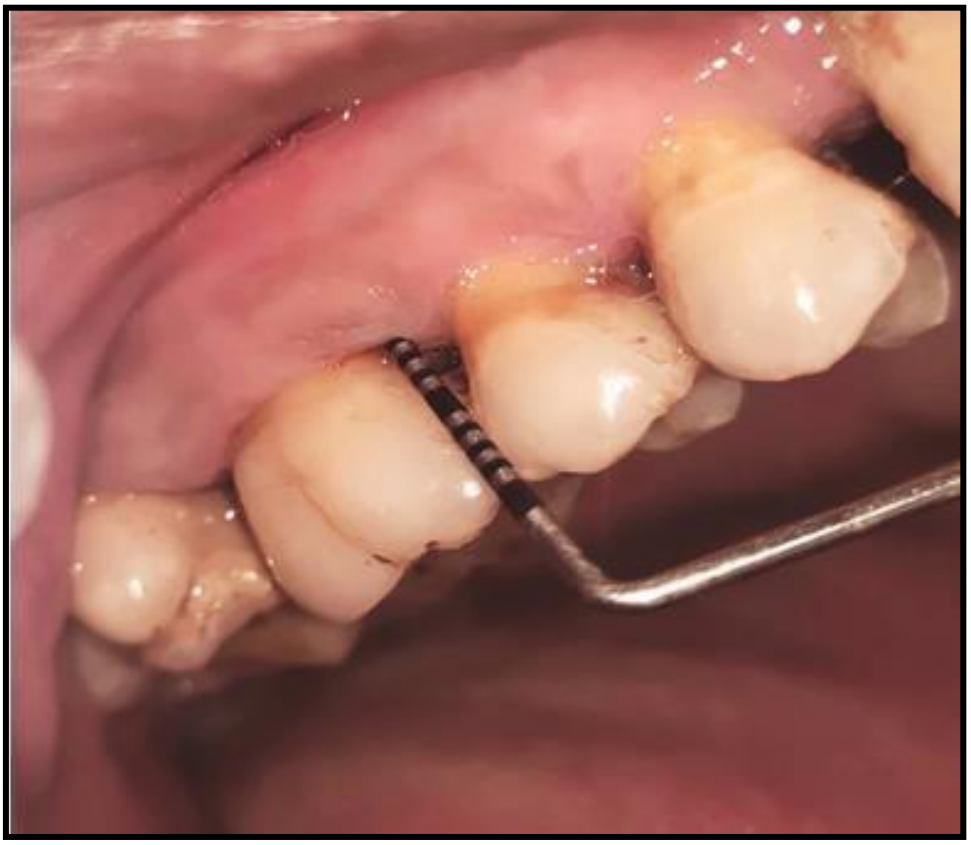


PLATE X: PLACEMENT OF NEEM FIBERS

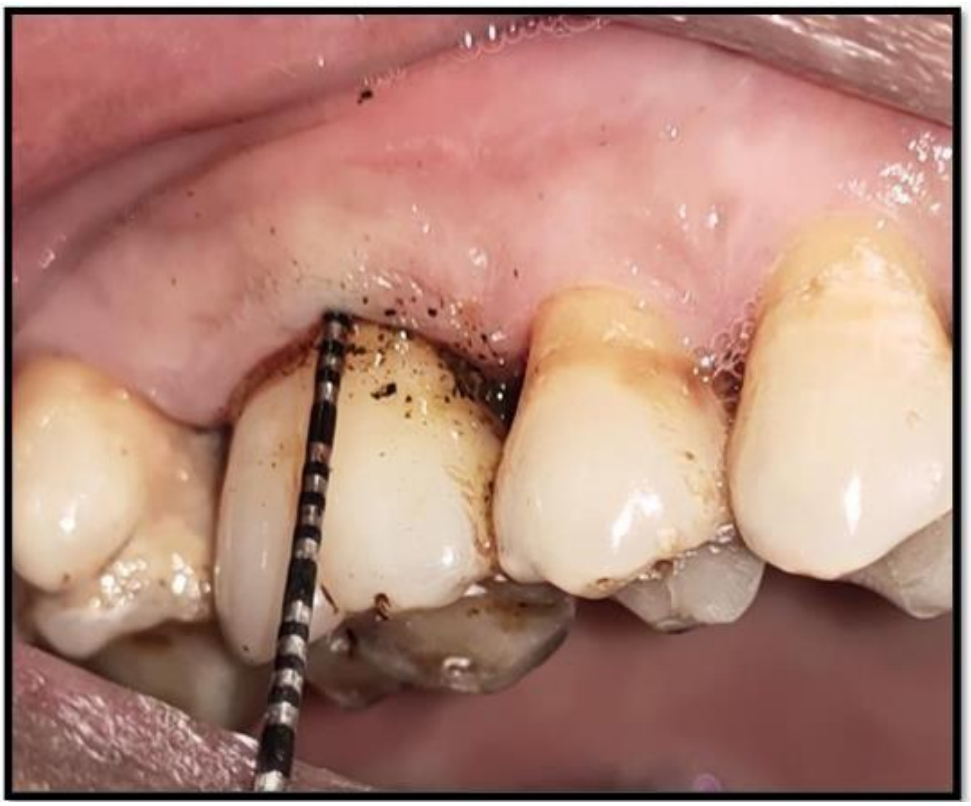


PLATE XI: EVALUATION ON DAY 21 FOR NEEM FIBERS

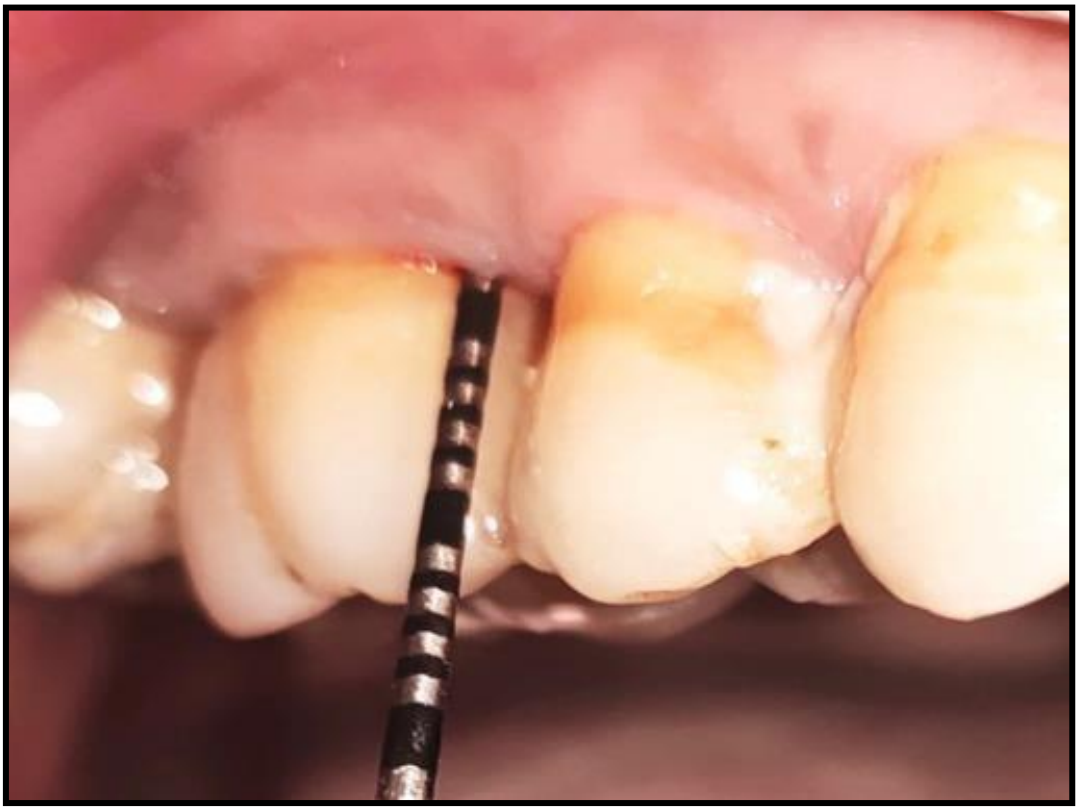


PLATE XII: BASELINE EVALUATION OF CURCUMIN FIBERS

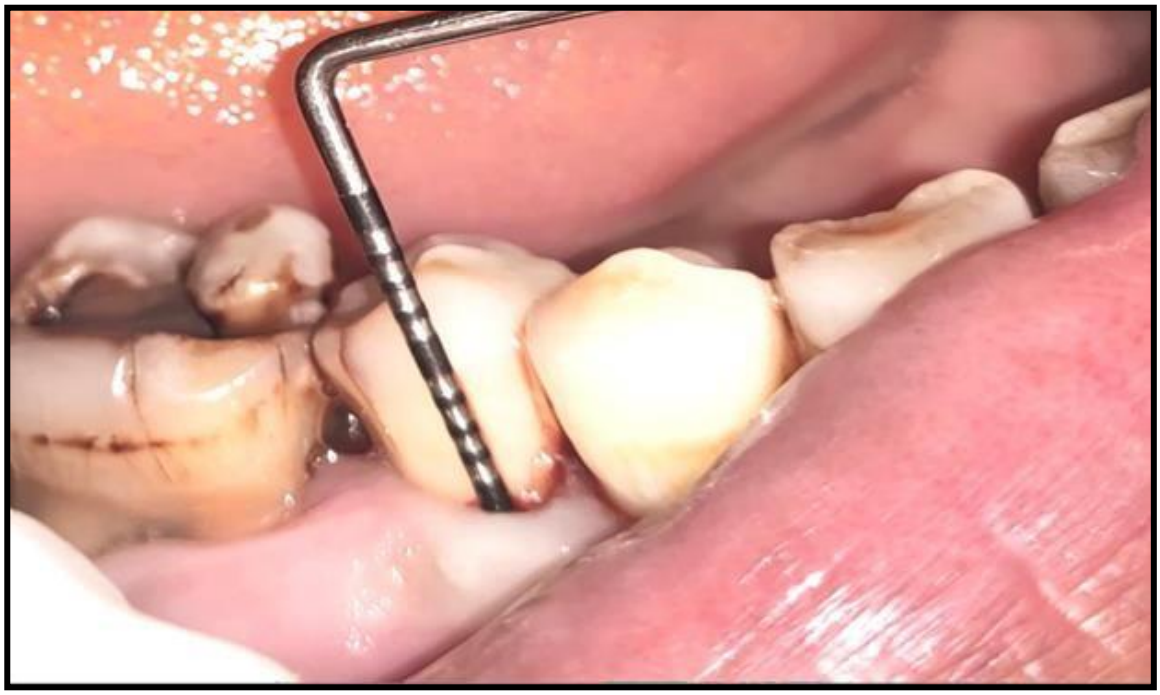


PLATE XIII: PLACEMENT CURCUMIN FIBERS

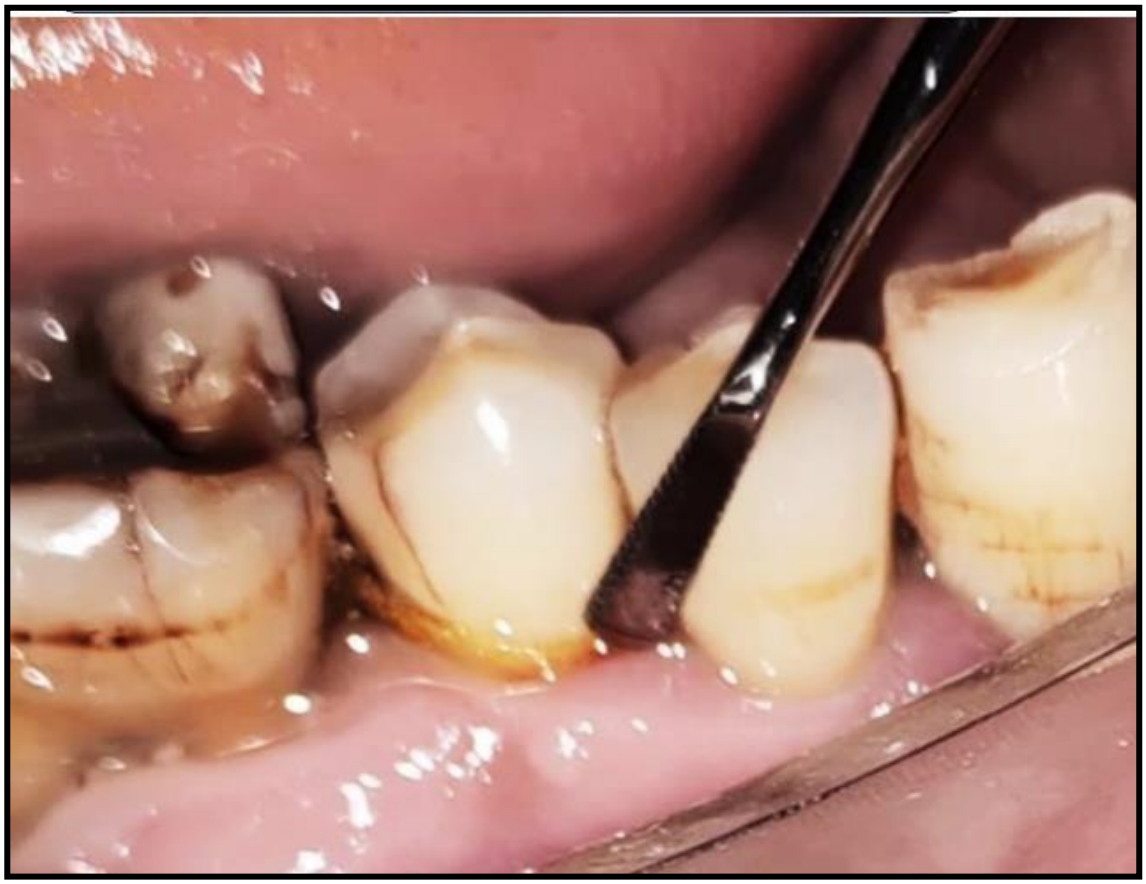
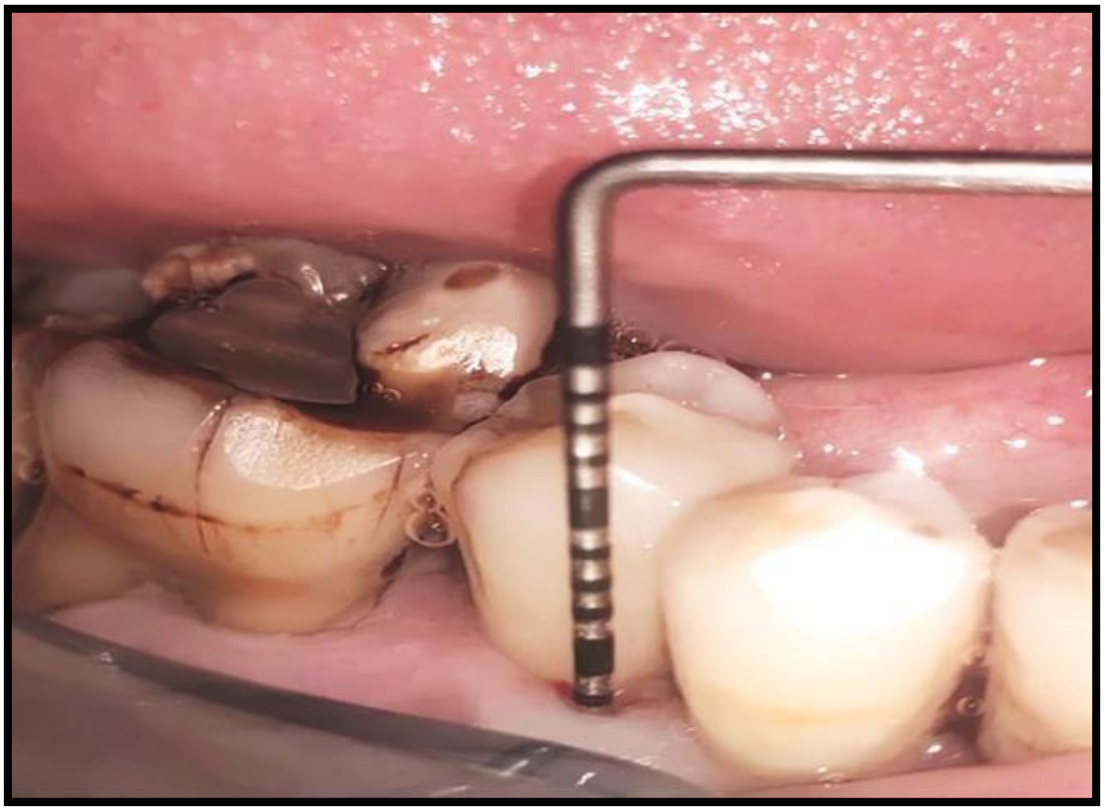


PLATE XIV: EVALUATION ON DAY 21 FOR CURCUMIN FIBERS



RESULTS AND OBSERVATIONS

The present study is a randomized clinical study deals with comparative clinical evaluation of the efficacy of *Azadirachta indica* (neem) incorporated collagen fibers with *Curcuma longa* (curcumin) incorporated collagen fibers as a local drug delivery agent. Total 40 teeth were selected and randomized equally into two groups and treated with scaling and root planning (SRP) along with *Azadirachta indica* (*Group A, n=20*) and SRP along with *Curcuma longa* (*Group B, n=20*).

The outcome measures of the study were clinical periodontal parameters viz. plaque index (PI), gingival index (GI) and probing pocket depth (PPD)). The outcome measures were assessed at pre treatment (day 0) and post treatment (day 7, 14 and 21) for PI and GI. The PPD was measured in millimetre (mm) at pre-treatment day 0 and day 21.

The objective of the study was (i) to evaluate the efficacy of *Azadirachta indica* incorporated fibers as local drug delivery system in subjects suffering from moderate chronic periodontitis, (ii) to evaluate the efficacy of *Curcuma longa* incorporated fibers as local drug delivery system in subjects suffering from moderate chronic periodontitis, and (iii) to compare the efficacy of *Azadirachta indica* incorporated fibers and *Curcuma longa* incorporated fibers in subjects suffering from moderate chronic periodontitis.

Outcome measure

I. Plaque index (PI)

The pre (day 0) and post (day 7, 14 and 21) PI score of two groups (Group A and Group B) is summarised in Table 1 and also depicted in Fig. 1. In both groups, the mean PI score also show marked decrease with time after the treatment and the decrease was evident higher in Group A as compared to Group B.

For each group, comparing the difference in mean PI score between the periods (i.e. intra group) (Table 2 and Fig. 2), Tukey test showed significant ($P < 0.001$) change/decrease in PI score of both groups at both day 14 and 21 as compared to pre-treatment (day 0). Moreover, in Group A, it also showed significant ($P < 0.001$) change/decrease at day 7 as compared to day 0. Further, in both groups, it also decreased significantly ($P < 0.01$ or $P < 0.001$) at both day 14 and 21 as compared to day 7. Furthermore, in Group A, it also decreased significantly ($P < 0.001$) at 21 as compared to day 14 but in Group B, it did not change/decrease significantly ($P > 0.05$) i.e. found to be statistically the same.

Similarly, for each period, comparing the difference in mean PI score between the groups (i.e. inter group) (Table 3 and Fig. 3), Tukey test showed similar ($P > 0.05$) PI score between the two groups at all periods i.e. did not differed significantly or found to be statistically the same.

However, at final evaluation, the net mean decrease (i.e. mean change from day 0 to day 21) in PI score of Group A (47.3%) was found 20.9% higher as compared to Group B (26.4%).

Table 1: Pre and post PI score of two groups over the periods

Time period	Group A (n=20)	Group B (n=20)
day 0	1.85 ± 0.59	1.80 ± 0.64
day 7	1.49 ± 0.54	1.68 ± 0.63
day 14	1.24 ± 0.46	1.41 ± 0.56
day 21	0.98 ± 0.45	1.33 ± 0.57

The pre and post PI score of two groups were summarised in Mean ± SD.

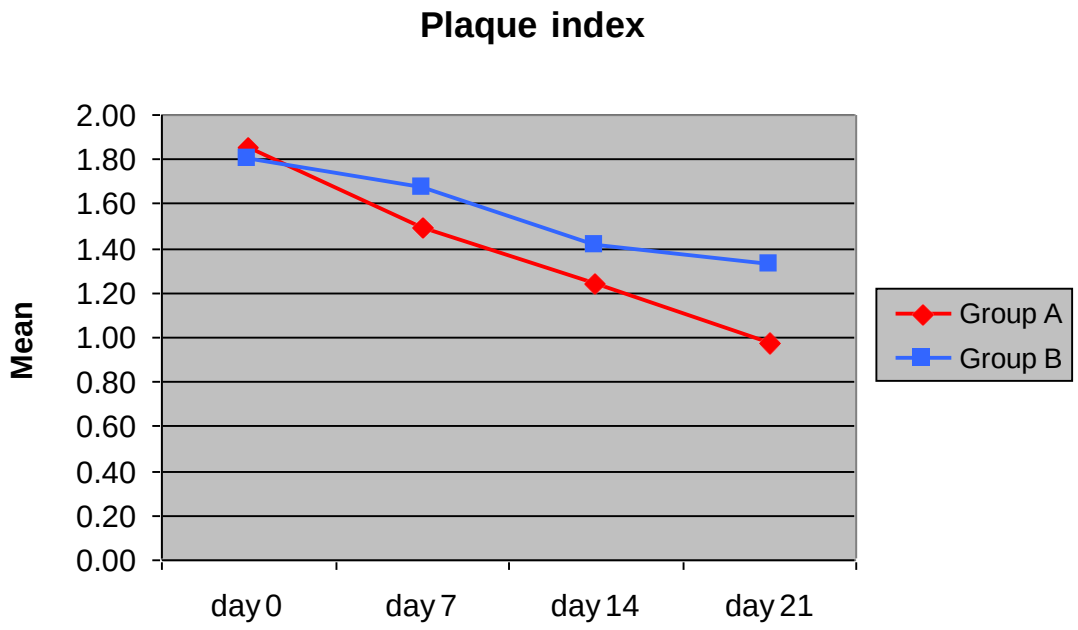
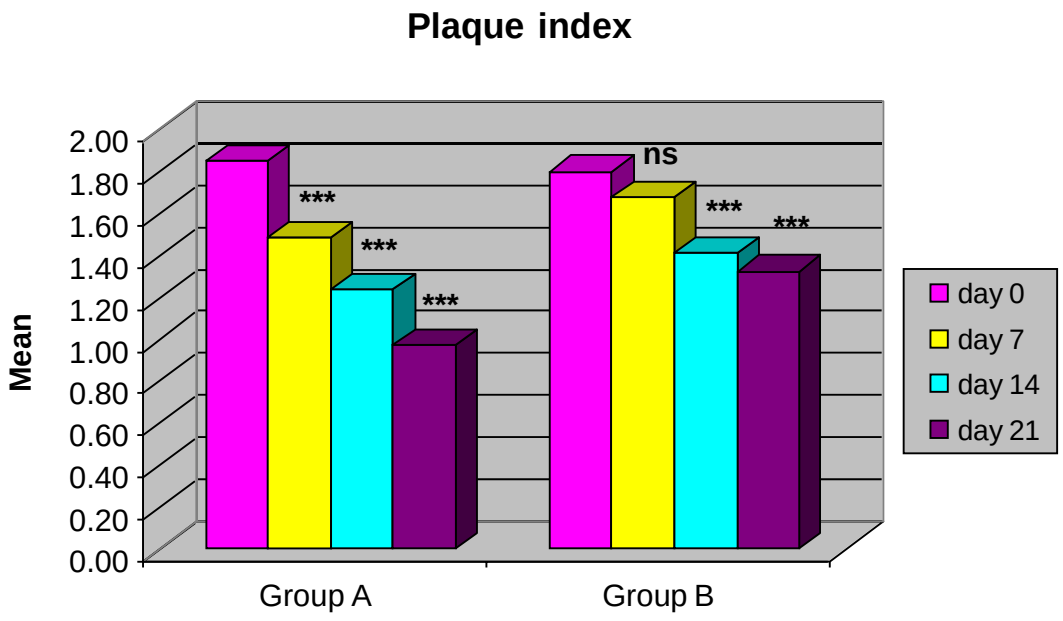


Fig. 1. Line graphs showing pre and post mean PI score of two groups over the periods.

Table 2: For each group, comparison (*P* value) of difference in mean PI score between the periods by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
day 0 vs. day 7	0.36	< 0.001	0.13	0.352
day 0 vs. day 14	0.61	< 0.001	0.39	< 0.001
day 0 vs. day 21	0.88	< 0.001	0.48	< 0.001
day 7 vs. day 14	0.25	0.001	0.26	< 0.001
day 7 vs. day 21	0.51	< 0.001	0.35	< 0.001
day 14 vs. day 21	0.26	< 0.001	0.09	0.779

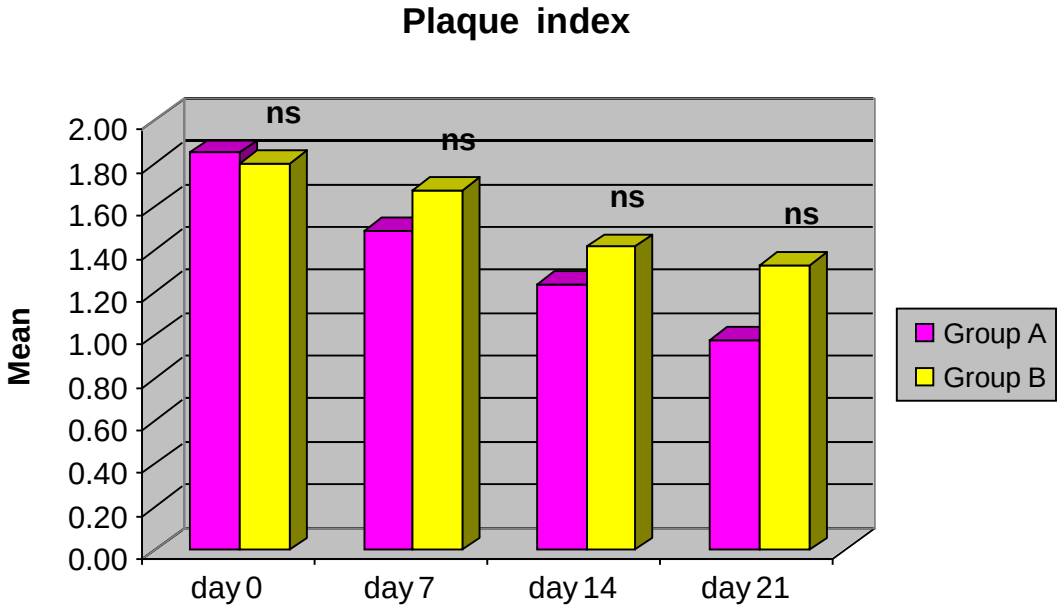


$^{ns}P > 0.05$ or $^{***}P < 0.001$- as compared to day 0

Fig. 2. For each group, bar graphs showing comparison of difference in mean PI score between the periods.

Table 3: For each period, comparison (*P* value) of difference in mean PI score between the groups by Tukey test

Time period	Group A vs. Group B	
	Mean difference	***P* value**
day 0	0.05	1.000
day 7	0.19	0.961
day 14	0.18	0.973
day 21	0.35	0.502



[ns]*P* > 0.05- as compared to Group A

Fig. 3. For each period, bar graphs showing comparison of difference in mean PI score between the groups.

II. Gingival index (GI)

The pre (day 0) and post (day 7, 14 and 21) GI score of two groups (Group A and Group B) is summarised in Table 4 and also shown in Fig. 4. After treatment, the mean GI score in both groups also show linear decrease with time and the decrease was evident higher in Group B as compared to Group A.

For each group, comparing the difference in mean GI score between the periods (i.e. intra group) (Table 5 and Fig. 5), Tukey test showed significant ($P < 0.01$ or $P < 0.001$) change/decrease in GI score of both groups at all post periods (day 7, 14 and 21) as compared to pre treatment (day 0). Further, in Group B, it also decreased significantly ($P < 0.001$) at both day 14 and 21 as compared to day 7. In contrast, in Group A, it decreased significantly ($P < 0.01$) at day 21 as compared to day 7. However, in both groups, it did not change/decrease significantly ($P > 0.05$) at day 21 as compared to day 14 i.e. found to be statistically the same.

Similarly, for each period, comparing the difference in mean GI score between the groups (i.e. inter group) (Table 6 and Fig. 6), Tukey test showed similar ($P > 0.05$) GI score between the two groups at all periods i.e. did not differed significantly or found to be statistically the same.

However, at final evaluation, the net mean decrease (i.e. mean change from day 0 to day 21) in GI score of Group B (39.7%) was found 10.9% higher as compared to Group A (28.8%).

Table 4: Pre and post GI score of two groups over the periods

Time period	Group A (n=20)	Group B (n=20)
day 0	1.56 ± 0.44	1.58 ± 0.51
day 7	1.34 ± 0.38	1.29 ± 0.36
day 14	1.23 ± 0.39	1.04 ± 0.37
day 21	1.11 ± 0.36	0.95 ± 0.37

The pre and post GI score of two groups were summarised in Mean ± SD.

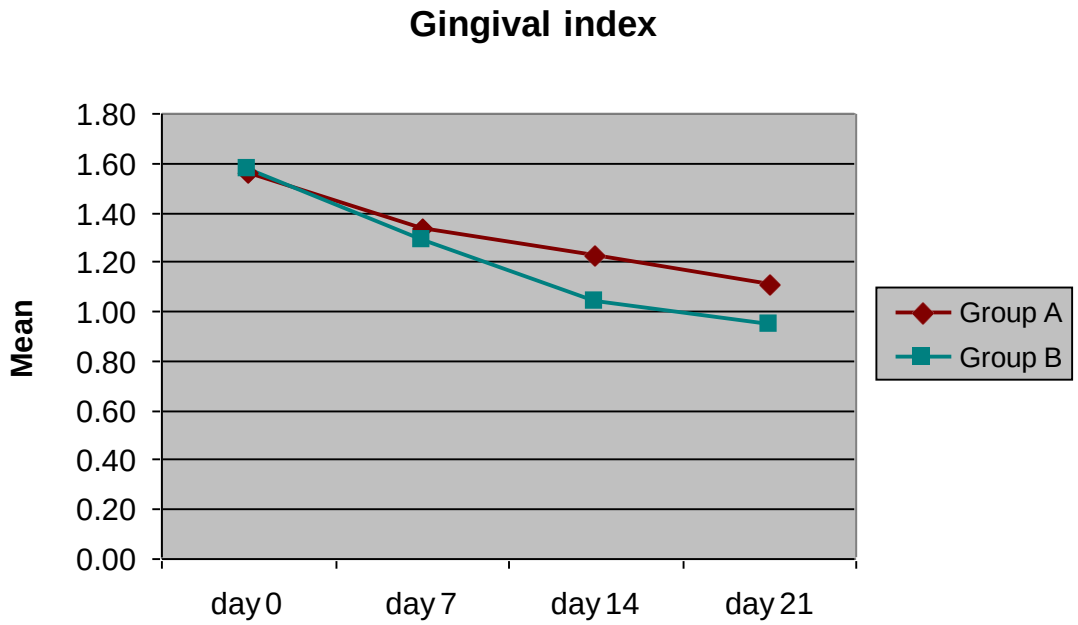
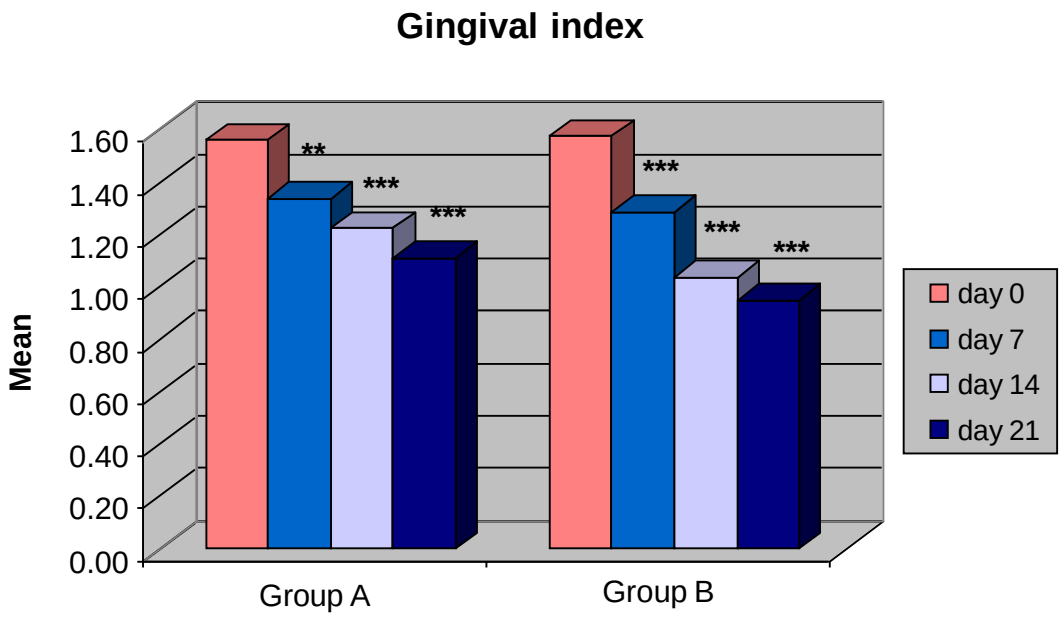


Fig. 4. Line graphs showing pre and post mean GI score of two groups over the periods.

Table 5: For each group, comparison (*P* value) of difference in mean GI score between the periods by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
day 0 vs. day 7	0.23	0.001	0.29	< 0.001
day 0 vs. day 14	0.34	< 0.001	0.54	< 0.001
day 0 vs. day 21	0.45	< 0.001	0.63	< 0.001
day 7 vs. day 14	0.11	0.418	0.25	< 0.001
day 7 vs. day 21	0.23	0.001	0.34	< 0.001
day 14 vs. day 21	0.11	0.418	0.09	0.727

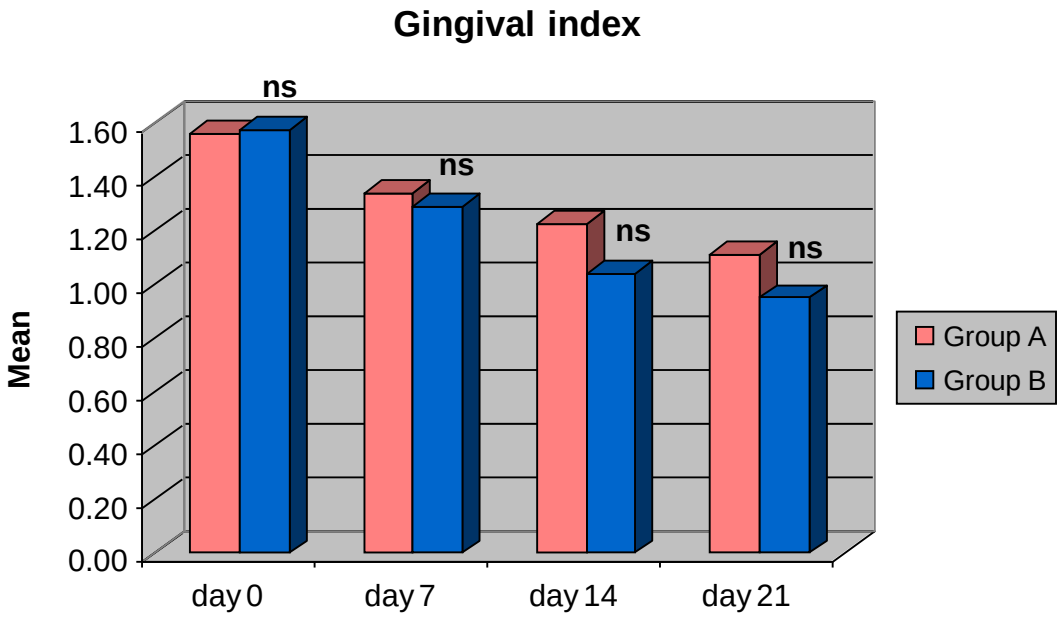


$^{**}P < 0.01$ or $^{***}P < 0.001$- as compared to day 0

Fig. 5. For each group, bar graphs showing comparison of difference in mean GI score between the periods.

Table 6: For each period, comparison (*P* value) of difference in mean GI score between the groups by Tukey test

Time period	Group A vs. Group B	
	Mean difference	***P* value**
day 0	0.01	1.000
day 7	0.05	1.000
day 14	0.19	0.815
day 21	0.16	0.902



[ns]*P* > 0.05- as compared to Group A

Fig. 6. For each period, bar graphs showing comparison of difference in mean GI score between the groups.

III. Probing pocket depth (PPD)

The pre (day 0) and post (day 21) PPD score (mm) of two groups (Group A and Group B) is summarised in Table 7 and also depicted in Fig. 7. After treatment, the mean PPD score in both groups decrease linearly with time and the decrease was evident higher in Group A as compared to Group B.

For each group, comparing the difference in mean PPD score between the periods (i.e. intra group) (Table 8 and Fig. 8), Tukey test showed significant ($P < 0.001$) change/decrease in PPD score of both groups at day 21 as compared to pre treatment (day 0). Further, in both groups, it also decreased significantly ($P< 0.001$) at day 21.

Similarly, at day 21, comparing the difference in mean PPD score between the groups (i.e. inter group) (Table 9 and Fig. 9), At day 21, the mean PPD score was of Group B was found significantly ($P < 0.05$) different and higher as compared to Group A.

However, at final evaluation, the net mean decrease (i.e. mean change from day 0 to day 21) in PPD score of Group A (48.2%) was found 17.8% higher as compared to Group B (30.4%).

Table 7: Pre and post PPD score (mm) of two groups over the period.

Time period	Group A (n=20)	Group B (n=20)
day 0	5.70 ± 0.92	5.60 ± 0.88
day 21	2.95 ± 0.69	3.90 ± 0.79

The pre and post PPD score of two groups were summarised in Mean ± SD.

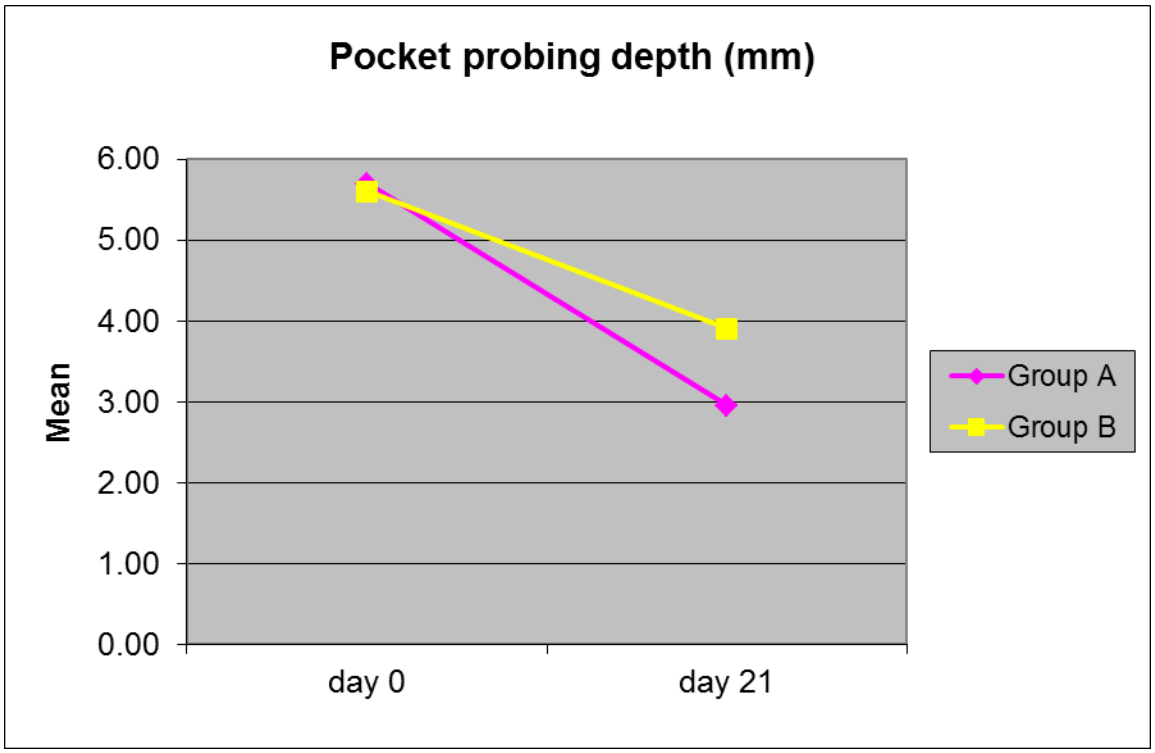
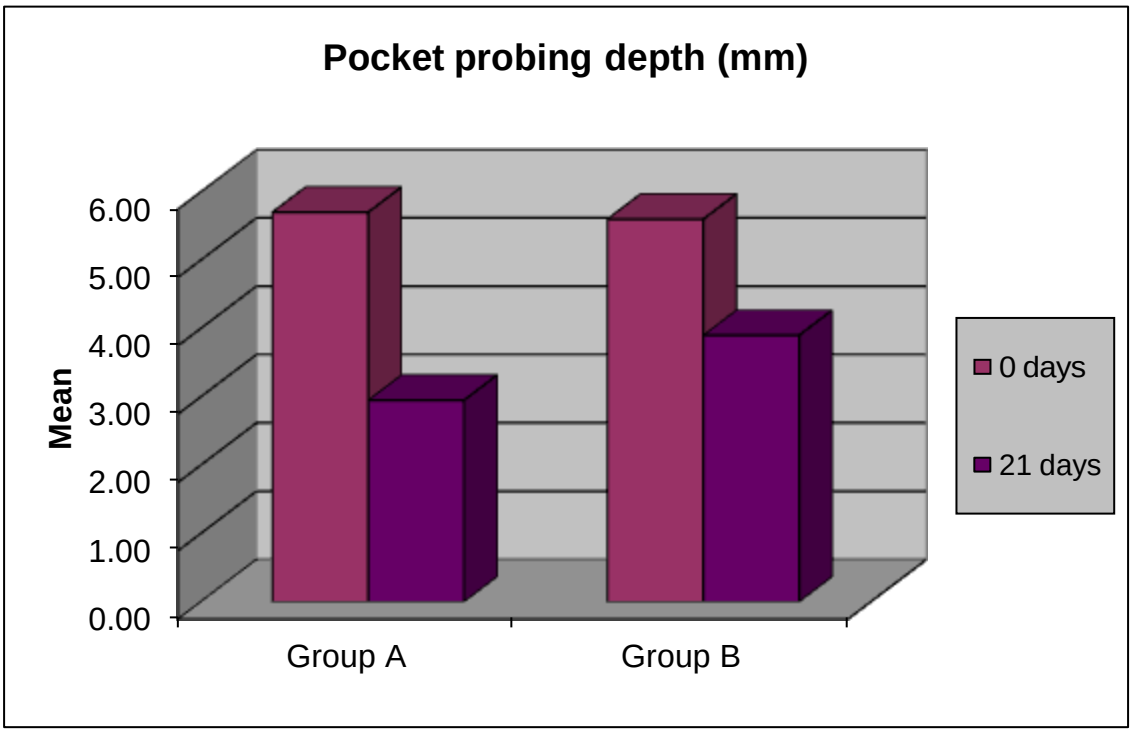


Fig. 7. Line graphs showing pre and post mean PPD score of two groups over the period.

Table 8: For each group, comparison (*P* value) of difference in mean PPD score (mm) between the period by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
day 0 vs. day 21	2.75	< 0.001	1.70	< 0.001

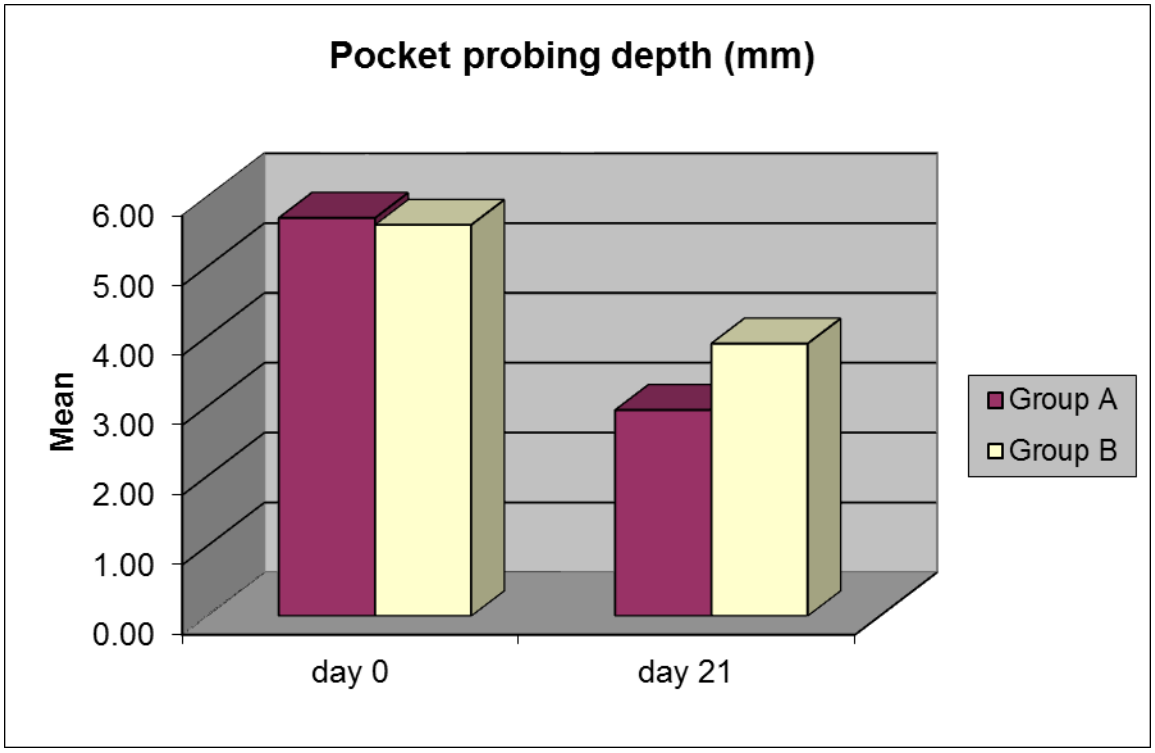


$^{***}P < 0.001$- as compared to day 0

Fig. 8. For each group, bar graphs showing comparison of difference in mean PPD score between the periods.

Table 9: For each period, comparison (*P* value) of difference in mean PPD score (mm) between the groups by Tukey test

Time period	Group A vs. Group B	
	Mean difference	***P* value**
day 0	0.10	1.000
day 21	0.95	0.021



$^{ns}P > 0.05$ or $^{*}P < 0.05$- as compared to Group A

Fig. 9. For each period, bar graphs showing comparison of difference in mean PPD score between the groups.

DISCUSSION

Periodontitis is a pathologic manifestation of host-microbial interaction occurring at biofilm-gingival interface and is an inflammatory condition. The nature of the periodontal disease depends on the interaction among the bacterial agent, the environment, and the host's defense mechanisms to the bacterial assault mainly composed of gram-negative anaerobic bacteria[48]. The periodontal treatment aims to eradicate gingival inflammation, bleeding, periodontal pocket depth and arrest destruction of soft tissue and bone by removal of the bacterial deposits from the tooth surface and to shift the pathogenic microbiota to one compatible with periodontal health. Therapeutic approach includes mechanical scaling and root planing (SRP) [49]. The effectiveness of this method is limited due to the lack of accessibility in deep periodontal pocket[50]. Putative pathogens associated with periodontal diseases are susceptible to a variety of antiseptics and antibiotics[51,52]. Elimination or adequate suppression of putative periodontopathic microorganisms in the subgingival microbiota is essential for periodontal healing. For the effective treatment, the antibiotic must reach the depth of the pocket and produce gingival fluid concentrations higher than the minimum inhibitory concentrations (MIC) of the suspected pathogens [53]. Systemic administration of antimicrobial has been useful in treating periodontal pockets, but repeated and long-term use of systemic antibiotics possess potential danger including resistant strains and superimposed infections. Local administration, therefore, provide a useful answer to these problems. The principal requirement for effectiveness of this form of therapy is that the agent reaches the base of the pocket and is maintained there by means like reservoir for an adequate time for the antimicrobial effect to occur [54]. It was in the year 1979 when Dr. Max Goodson et al[55]. first proposed the concept of controlled delivery in the treatment of periodontitis. Allopathic medications are successfully used as local drug delivery agents. Recently usage of herbal products (neem, turmeric, aloe-vera, lemon grass, green tea, tea tree oil, oak, coriander, babul, pomegranate etc.) has increased as LDD agent owing to their relatively safe nature [56].

As per our literature search, no study has been found comparing two herbal products efficacy as LDD agents in the form of collagen fibers in subjects of moderate chronic periodontitis. In the present report we utilized two natural extracts for the purpose of LDD viz. neem and turmeric impregnated in collagen fibers. The aim was to evaluate the time release pattern and compare the efficacy of *Azadirachta indica* (Neem) against *Curcuma longa* (curcumin) in subjects suffering from moderate chronic periodontitis. Hence, the study was divided in in-vitro and in-vivo arm.

Objective of the *in vitro* study is -To evaluate the time release pattern of *Azadirachta indica* (Neem) and *Curcuma longa* (curcumin**)** incorporated fibers as local drug delivery system. Drug diffusion study was performed to check the time release pattern, using KC diffusion cell. The release of neem extract from collagen thread through egg membrane upto 7 hours at 1 hour interval and later at 72 hour was assessed. At 1st hour 3.26% of extract was released which rapidly increased to 6.61% at 2 hours. It further increased to 17.62% at 5 hour. The neem was seen to release at a slower rate with increasing time i.e 18.27% and 18.84% at 6th and 7th hour respectively. Release was further evaluated at 72 hours which came to be 23.85% followed by a constant rate of release at 5th and 7th day. The curcumin released from curcumin extract, was rapid in the first 12 h. Approximately 90% curcumin was released from curcumin exract within 120 h, while 76% curcumin was released from curcumin fiber within 120 h. Rate of release of the drug from the collagen vehicle, as in any resorbable control drug delivery device, is dependent on the rate of degradation of the vehicle. The degradation of collagen in a physiologic environment is mainly governed by a variety of factors and more specifically by a group of degradation enzymes known as matrix metalloproteinases (MMPs). Several of these enzymes are tissue derived, while some of them are bacterial in origin. P. *gingivalis* has the highest collagenolytic activity, and these enzymes could potentially act on the collagen vehicle in the present study and produce a variable degradation rate. In view of the data thus generated, it is pertinent to state that the degradation of collagen when placed in human body will take substantial time to begin and by that time therapeutic level of drugs were already achieved as seen by release pattern of Neem and curcumin in vitro.

In-vivo arm of this clinical study compared the efficacy of neem impregnated collagen fibers and turmeric (curcumin) extract impregnated collagen fibers as LDD agents in the treatment of moderate chronic periodontitis cases in terms of probing Pocket Depth (PPD), Plaque Index (PI) and Gingival Index (GI). A total of 40 teeth were selected and randomized equally into two groups and treated with scaling and root planing (SRP) + *Azadirachta indica* fibers (Group A, n=20) and SRP + *Curcuma longa* (Group B, n=20). The clinical parameters were assessed at pre-treatment (day 0) and post-treatment (day 7, 14 and 21). The PPD was measured in millimetres (mm).

Regarding, post-operative complications, no adverse tissue responses, infections, or unusual patient experiences were noticed. These were just clinical observations, and no parameters were used to assess the conditions.

Regarding PI, in the intra-group comparison between follow-up timings (7^{th},14^{th},21^{st} Day), statistically significant ($p<0.001$) differences/reduction were found in both the groups (A & B) when compared to baseline (day 0) data. In group-A, statistically significant ($p<0.001$) reductions were found at 7^{th} day compared to day 0. Further there was statistically significant reduction ($p<0.001$) between 7^{th} and 14^{th}, 21^{st} day. In group-A, the reduction was statistically significant ($p<0.001$) between 14^{th} and 21^{st} day, while in group-B it was statistically insignificant ($p>0.05$). When comparing between the groups, the differences between PI reduction at each follow-up visit (7^{th},14^{th},21^{st} Day) were statistically insignificant ($p>0.05$). Net mean PI reduction of group-A was 20.9% higher for whole 21-day follow-up period.

Regarding GI, in the intra-group comparison between follow-up timings (7^{th},14^{th},21^{st} Day), statistically significant ($p<0.001$) differences/reduction were found in both the groups (A & B) when compared to baseline (day 0) data. In group-B, statistically significant ($p<0.001$) reductions were found at 14^{th}, 21^{st} day compared to 7^{th} day. While in group-A, the reduction was statistically significant ($p<0.001$) at 21^{st} day when compared with 7^{th} day. However, in both groups, it did not change/reduce significantly ($P<0.05$) at day 21 as compared to day 14 i.e., found to be statistically insignificant. When comparing between the groups, the differences between GI reduction at each follow-up visit (7^{th},14^{th},21^{st} Day) were statistically insignificant ($p>0.05$). Net mean GI reduction of group-B was 10.9% higher for whole 21-day follow-up period.

PPD, when comparing intra-group between follow-up timings, statistically significant ($p<0.001$) differences/reduction were found in both the groups (A & B) when data were compared at day 21 to baseline (day 0). Net mean PPD reduction of group-A was 17.8% higher for whole 21-day follow-up period. This intergroup difference was significant ($P<0.05$).

These results were in accordance with previous research data which is quiet sparse. Vennila K (2016) [57] in a randomized split mouth study investigated the efficacy of 10% whole *Azadirachta indica* (neem) chip as an adjunct to SRP and found that clinical parameters (including PPD) were statistically improved on the neem chip sites and presence of P. gingivalis strains were significantly reduced on the neem chip sites ($P=<0.05$). Nagasri M (2013) [58] found that the local application of curcumin in conjunction with SRP have showed

improvement in periodontal parameters and has a beneficial effect in patients with chronic periodontitis (P=0.001).

As a whole Neem group (group-A) produced higher net improvement in PI and PPD which can be explained by antimicrobial effects of neem. It is suggested that bioactive materials found in neem leads to the presence of 'Gallo-tannins' during the early stages of plaque formation that could effectively reduce the number of bacteria available for binding to the tooth surface by increasing their physical removal from the oral cavity through aggregate formation. Additionally, the effective inhibition of glucosyltransferase activity and the reduced bacterial adhesion to saliva coated hydroxyl appetite suggest some potential anti-plaque activity [59].

Curcumin group presented with an overall higher GI reduction. This can be attributed to anti-inflammatory and host-modulatory properties of curcumin (turmeric). The active constituents of turmeric include the three curcuminoids: Curcumin (di-feruloyl-methane), de-methoxy-curcumin, and bis-demethoxy-curcumin, as well as volatile oils (turmerone, atlantone, and zingiberone), sugars, proteins, and resins. Curcumin exhibits anti-inflammatory, antioxidant, anticarcinogenic, antiviral, and antimicrobial activities. Curcumin modulates the inflammatory response by down-regulating the activity of cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase enzymes and inhibits the production of the inflammatory cytokines [60]. Izui S et al (2015) [61] investigated the antibacterial effect of curcumin on periodontopathic bacteria, particularly *P. gingivalis* and suggested that Curcumin also possesses antibacterial activity against periodontopathic bacteriae and may be a potent agent for preventing periodontal diseases. However, curcumin has very poor bioavailability that is a major barrier in its clinical efficacy. Many studies showing very low, even undetectable concentrations in the blood and extraintestinal tissues. Reasons postulated are due to its poor absorption, rapid metabolism, chemical instability and systemic elimination.

If we compare the results of neem and curcumin extracts as LDD agents to standard allopathic LDD agents, they were found to be equally effective. A meta-analysis on LDD agents (doxycycline, minocycline, tetracycline, and chlorhexidine) found that there is statistically significant improvement in PPD, GI & PI scores. Compared with each other chlorhexidine was most effective followed by tetracycline, minocycline and doxycycline. Weighted mean PPD reduction with adjunctive therapy was 3.06 ± 1.06 and mean reduction scores for BOP was 0.92±0.3 and the plaque score was 1.16 ± 0.57 which is in accordance to our study (Table-1,4,7).

The low toxicity and low cost of these herbs should encourage further investigation leading to a better understanding and their application to oral health.

The design of the study was parallel armed which reduces the power of the study and is a short coming of this report which can be overcome by using a split-mouth design. Moreover, if there is a control group involving the use of any of the standard LDD agent viz. chlorhexidine the results and comparisons would have been more thorough in case of a parallel armed study.

CONCLUSION

Within the limits of this study and on the basis of the *in -vitro* results it can be concluded that –

Release of Neem constituents from collagen thread at 72 hours was 38.33% which there after remained constant when checked at 5^{th} and 7^{th} day. Approximately 76% curcumin was released from collagen thread within 5 days. Hence, therapeutic range of neem and curcumin will be administered before degradation of collagen starts.

Within the limits of this study and on the basis of the clinical study results it can be concluded that –

1. Plaque score showed significant ($P < 0.001$) change/decrease in PI score of both groups(group A and group B) at day 14 and 21 as compared to pre treatment (day 0). On comparing both the groups the net mean decrease (i.e. mean change from day 0 to day 21) of Group A (47.3%) was found 20.9% higher as compared to Group B (26.4%); Although this intergroup difference was not significant (P>0.05).

2. Gingival score showed significant ($P < 0.01$ or $P < 0.001$) change/decrease in GI score of both groups at all post periods (day 7, 14 and 21) as compared to pre treatment (day 0). On comparing both the groups the net mean decrease (i.e. mean change from day 0 to day 21) of Group B (39.7%) was found 10.9% higher as compared to Group A (28.8%); Although this intergroup difference was also not significant (P>0.05).

3. PPD score showed significant ($P < 0.001$) change/decrease in PPD score of both groups at day 21 as compared to pre treatment (day 0). On comparing both the groups the net mean decrease (i.e. mean change from day 0 to day 21) of Group A (48.2%) was found 17.8% higher as compared to Group B (30.4%). This intergroup difference was significant (P<0.05).

Hence, from this study it can be concluded that Neem incorporated collagen fibers and Curcumin incorporated collagen fibers can be used as an adjunct to SRP, for the added benefits in the control of periodontal disease.

The design of the study was parallel armed which reduces the power of the study and is a shortcoming of this report which can be overcome by using a split-mouth design.

Moreover, if there is a control group involving the use of any of the standard LDD agent viz. chlorhexidine the results and comparisons would have been more thorough in case of a parallel armed study.

More longitudinal research with larger sample size can be considered in future to strengthen the result of this study.

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APPENDICES

ANNEXURE -1

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

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Comparative Clinical Evaluation of The Efficacy of Azadirachta Indica Incorporated Collagen Fibers with Curcumin Incorporated Collagen Fibers- A Randomised Clinical Study."** submitted by **Dr Ekta Dwivedi** Post graduate student from the **Department of Periodontology** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **04th January, 2021** at BBDCODS.

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

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE -2

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

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

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

IEC Code: 26 (Revised) **BBDCODS/01/2021**

Title of the Project: Comparative Clinical Evaluation of the Efficacy of Azadirachta Indica Incorporated Collagen Fibers with Curcumin Incorporated Collagen Fibers- A Randomised Clinical Study.

Principal Investigator: Dr. Ekta Dwivedi **Department:** Periodontology

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

Type of Submission: Revised, MDS Project Protocol

Dear Dr. Ekta Dwivedi,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 07th January 2021.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

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

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

Forwarded by:

Lakshmi Bala 07/01/21

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ANNEXURE -3

सीएसआईआर - राष्ट्रीय विज्ञान संचार एवं सूचना स्रोत संस्थान
CSIR - National Institute of Science Communication and Information Resources
वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद् Council of Scientific & Industrial Research
(विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार Ministry of Science & Technology, Govt. of India)
NISCAIR

RAW MATERIAL HERBARIUM AND MUSEUM, DELHI (RHMD)

Ref. No.-NISCAIR/RHMD/Consult/2020/3720-21-1 22/12/2020

CERTIFICATE FOR CRUDE DRUGS AUTHENTICATION

This is to certify that leaves sample of *Azadirachta indica*, received from Ms. Ekta Dwivedi, vide letter No. Nil, Dated 10th December 2020 has been **found correct as dried leaves of *Azadirachta indica* A. Juss.** syn. *Melia azadirachta* L. **which is commonly known as Neem Tree, Margosa Tree.** The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic samples deposited in the Raw Material Herbarium and Museum, Delhi (RHMD).

Identification pertains to the quantity/quality of specimen/sample(s) received in RHMD. This certificate is not issued for any judicial purpose.

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वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद् Council of Scientific & Industrial Research
(विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार Ministry of Science & Technology, Govt. of India)

RAW MATERIAL HERBARIUM AND MUSEUM, DELHI (RHMD)

Ref. No.-NISCAIR/RHMD/Consult/2020/3720-21-2 22/12/2020

CERTIFICATE FOR CRUDE DRUGS AUTHENTICATION

This is to certify that rhizome, stolon sample of *Curcuma longa*, received from Ms. Ekta Dwivedi, vide letter No. Nil, Dated 10th December 2020 has been **found correct as rhizomes of *Curcuma longa* L. which is commonly known as Haldi, Haridra, Turmeric.** The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic samples deposited in the Raw Material Herbarium and Museum, Delhi (RHMD).

Identification pertains to the quantity/quality of specimen/sample(s) received in RHMD. This certificate is not issued for any judicial purpose.

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ANNEXURE -4

Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

सहमति पत्र

अध्ययन शीर्षक.....
अध्ययन संख्या.....
प्रतिभागी के पूर्ण नाम.....
जन्म तिथि / आयु.....
प्रतिभागी का पता .....
फोन नं. और ई-मेल पता .....
योग्यता .....
व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी .....
अन्य (उचित रुप मे टिक करें) .....
प्रतिभागी की वार्षिक आय .....
प्रत्याशीयो के नाम और प्रतिभागी से संबंध...(परीक्षण से संबंधित मौत के मामले मे मुआवजे के प्रयोजन के लिए)

1. मेरी पुष्टि है कि मैने अध्ययन हेतु सुचना पत्र दिनांक ..... को पढ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।

2. मैंने यहाँ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना , मेरे इलाज या कानूनी अधिकारो को प्रभावित किए बिना , अध्ययन में भाग न लेने के लिए स्वतंत्र हुँ ।

3. मैंने यह समझ लिया है कि अध्ययन के प्रायोजक , प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नही है, चाहे मैने इस अध्ययन से नाम वापस ले लिया है। हॉलाकि मै यह समझता हुँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नही दी जायेगी।

4. मै इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नही है।
5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक / रक्त) पर अध्ययन के लिए अपनी सहमति देता हुँ।

हॉ [ ] नही [ ] अनउपयुक्त [ ]

6. मै परीक्षण की अनुमति देता हुँ। मुझे इसके द्वारा यदि कोई परेशानी होती है, इसके बारे में जानकारी दे दी गई है। मैने रोगी जानकारी सूचना पत्र को पढ तथा समझ लिया है।

प्रतिभागी / कानूनी तौर पर स्वीकार्य प्रतिनिधि का हस्ताक्षर (या अंगूठे का निशान.....

हस्ताक्षरकर्ता का नाम..... दिनांक .....अन्वेषक के

हस्ताक्षर ..... दिनांक .....

अध्ययन अन्वेषक का नाम .....

गवाह के हस्ताक्षर ..... दिनांक .....गवाह के

नाम .....

मैनें पीआईडी और विधिवत भरे सहमति फार्म का एक हस्ताक्षर की नकल प्राप्त की.

प्रतिभागी कानूनी तौर पर प्रतिनिधि का हस्ताक्षर/ अंगूठे का निशान ..... दिनांक.....

BBDCODS

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Consent Form (English)

Title of the Study .....

Study Number.....
Subject's Full Name.....
Date of Birth/Age .....
Address of the Subject.....
Phone no. and e-mail address.....
Qualification .....
Occupation: Student / Self Employed / Service / Housewife/
Other (Please tick as appropriate)
Annual income of the Subject.....
Name and of the nominees(s) and his relation to the subject..... (For the purpose of compensation in case of trial related death).

1. I confirm that I have read and understood the Participant Information Document dated .....for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [ ] No [ ] Not Applicable [ ]**
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:.....
Signatory's Name..... Date .....
Signature of the Investigator..... Date.....
Study Investigator's Name..... Date.....
Signature of the witness..... Date.....
Name of the witness.....
Received a signed copy of the PID and duly filled consent form
Signature/thumb impression of the subject or legally Date.....

ANNEXURE -5

बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज
(बाबू बनारसी दास विश्वविद्यालय का एक घटक संस्थान)
बीबीडी सिटी, फैजाबाद रोड, लखनऊ - 227105 (भारत)

प्रतिभागी सूचना दस्तावेज (पीआईडी)

1- अध्ययन शीर्षक?

नीम में शामिल कोलैजन फाइबर और हल्दी में शामिल कोलैजन फाइबर की प्रभाविकता का चिकित्सकीय मूल्यांकन !

2- आमंत्रण अनुच्छेद?

आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित किया जा रहा है, इसलिए यह समझना आपके लिए महत्वपूर्ण है कि अध्ययन क्यों किया जा रहा है और इसमें क्या शामिल होगा। कृपया निम्नलिखित जानकारी को ध्यान से पढ़ने के लिए समय दें। किसी भी स्पष्टीकरण या आगे की जानकारी के लिए हमसे पूछें। चाहे आप भाग लेना चाहते हैं या नहीं, आपका निर्णय है।

3- अध्ययन का उद्देश्य क्या है?

नीम में शामिल कोलैजन फाइबर और हल्दी में शामिल कोलैजन फाइबर की प्रभाविकता का चिकित्सकीय मूल्यांकन !

4- मुझे क्यों चुना गया है?

इस अध्ययन के लिए आपको चुना गया है क्योंकि आप इस अध्ययन के लिए आवश्यक मानदंडों को पूरा कर रहे हैं।

5- क्या मुझे भाग लेना है?

शोध में आपकी भागीदारी पूरी तरह से स्वैच्छिक है। यदि आप करते हैं, तो आपको यह जानकारी पत्र दिया जाएगा और सहमति फॉर्म पर हस्ताक्षर करने के लिए कहा जाएगा। अध्ययन के दौरान आप अभी भी किसी भी समय बिना किसी कारण के वापस लेने के लिए स्वतंत्र हैं।

6- अगर मैं भाग लेता हूं तो मेरे साथ क्या होगा?

आप अध्ययन में नामांकित लोगों में से एक होंगे, नीम में शामिल कोलेजन फाइबर अथवा हल्दी में शामिल कोलेजन फाइबर, एक या एक से अधिक पेरिओडोन्टल पॉकेट्स में डाला जायेगा !आपका चिकित्सकीय मूल्यांकन सात दिन, चौदह दिन और इक्कीस दिन में किया जायेगा !

7- मुझे क्या करना है?

अध्ययन की जांच के लिए आपको अपने नियमित जीवन शैली को बदलने की ज़रूरत नहीं है।

8- परीक्षण की जा रही प्रक्रिया क्या है?

इस प्रक्रिया में प्लाक इंडेक्स, जिन्जाइवल इंडेक्स और पेरिओडोन्टल पॉकेट डेप्थ का मूल्यांकन किया जायेगा

9- अध्ययन के लिए हस्तक्षेप क्या हैं?

मरीज जिनमे मोडरेट क्रोनिक पेरिओडोन्टिटिस होगी वो अध्ययन के लिए चुने जायेंगे नीम में शामिल कोलेजन फाइबर अथवा हल्दी में शामिल कोलेजन फाइबर एक या एक से अधिक पेरिओडोन्टल पॉकेट में डाला जायेगा !

10- भाग लेने के दुष्प्रभाव क्या हैं?

इस अध्ययन के कोई दुष्प्रभाव नहीं हैं।

11- भाग लेने के संभावित नुकसान और जोखिम क्या हैं?

इस अध्ययन में कोई जोखिम शामिल नहीं है।

12- भाग लेने के संभावित लाभ क्या हैं?

यह अध्ययन नीम में शामिल कोलेजन फाइबर और हल्दी में शामिल कोलेजन फाइबर की तुलनात्मक प्रभाविकता को जानने में हमारी मदद करेगा, इसमें शामिल मरीजों में प्लाक इंडेक्स,जिन्जाइवल इंडेक्स और पेरिओडोन्टल पॉकेट डेप्थ कम होगी ।

13- क्या होगा अगर नई जानकारी उपलब्ध हो जाए?

यदि शोध के दौरान अतिरिक्त जानकारी उपलब्ध हो जाती है तो आपको इनके बारे में बताया जाएगा और आप अपने शोधकर्ता के साथ चर्चा करने के लिए स्वतंत्र हैं, आपका शोधकर्ता आपको बताएगा कि आप अध्ययन में जारी रखना चाहते हैं या नहीं। यदि आप वापस लेने का निर्णय लेते हैं, तो आपका शोधकर्ता आपके वापसी के लिए व्यवस्था करेगा। यदि आप अध्ययन में जारी रखने का निर्णय लेते हैं, तो आपको एक अद्यतन सहमति फॉर्म पर हस्ताक्षर करने के लिए कहा जा सकता है।

14- शोध अध्ययन बंद होने पर क्या होता है?

यदि अध्ययन निर्धारित समय से पहले समाप्त / खत्म हो जाता है, तो यह रोगी / स्वयंसेवक को समझाया जाएगा।

15- क्या होगा अगर कुछ गलत हो जाए?

यदि कोई गंभीर प्रतिकूल घटना होती है, या अध्ययन के दौरान कुछ गलत हो जाता है, तो शिकायतों को संस्था (ओं), और संस्थागत नैतिक समुदाय को रिपोर्ट करके संभाला जाएगा।

16- क्या इस अध्ययन में मेरा हिस्सा गोपनीय रखा जाएगा?

हां इसे गोपनीय रखा जाएगा।

17- शोध अध्ययन के नतीजों का क्या होगा?

अध्यन के नतीजो का आकलन किया जायेगा

18- शोध का आयोजन कौन कर रहा है?

यह शोध अध्ययन अकादमिक संस्थान द्वारा आयोजित किया जाता है। आपको शामिल किसी भी प्रक्रिया के लिए भुगतान नहीं करना है।

20- अध्ययन की समीक्षा किसने की है?

इस अध्ययन की समीक्षा विभाग के प्रमुख और संस्थान के आईईसी / आईआरसी द्वारा की गई और अनुमोदित की गई है।

21- अधिक जानकारी के लिए संपर्क करें

डॉ एकता द्विवेदी

पीरियोडोंटोलॉजी विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-227,105

मोब-8573879863

डॉ वंदना ए पंत (HOD)
पीरियोडोंटोलॉजी विभाग
बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।
लखनऊ-227,105
मोब- 9935957775

डॉ लक्ष्मी बाला

सदस्य सचिव

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज

लखनऊ

bbdcods.iec@gmail.com

पीआई का हस्ताक्षर .....

नाम .....

दिनांक .....

Babu Banarasi Das College of Dental Sciences

(A constituent institution of Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Participant Information Document (PID)

Study title: Comparative clinical evaluation of the efficacy of *Azadirachta indica* incorporated collagen fibers with curcumin incorporated collagen fibers- A randomised clinical study

1. Invitation paragraph

You are being invited to take part in a research study, it is therefore important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully. Ask us for any clarifications or further information. Whether or not you wish to take part is your decision.

2. What is the purpose of the study?

In vivo comparative clinical evaluation of the efficacy of *Azadirachta indica* incorporated collagen fibers with curcumin incorporated collagen fibers.

3. Why have I been chosen?

You have been chosen for this study as you are fulfilling the required criteria for this study.

4. Do I have to take part?

Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

5. What will happen to me if I take part?

You will be one of the subjects, enrolled in the study. *Azadirachta indica* incorporated

6. What do I have to do?

You do not have to change your regular lifestyles for the investigation of the study.

7. What is the procedure that is being tested?

The procedure will involve to assess the Plaque index, Gingival index, and Probing pocket depth.

8. What are the interventions for the study?

Patient with moderate chronic periodontitis (Localised or generalised) will be selected for the study. Collagen fibers incorporated with Azadirachta indica or collagen fibers incorporated with curcumin will be place in one or more sites.

9. What are the side effects of taking part?

There are no side effects on patients of this study.

10. What are the possible disadvantages and risks of taking part?

There are no risks or disadvantages of taking part in this study.

11. What are the possible benefits of taking part?

This study will help us to compare the efficacy of both *Azadirachta indica* and curcumin incorporated collagen fibers. It will reduce plaque index, gingival index, and periodontal pocket depth .

12. What if new information becomes available?

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you whether you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consentform.

13. What happens when the research study stops?

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

14. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and Institutional ethical community.

15. Will my taking part in this study be kept confidential?

Yes it will be kept confidential.

16. What will happen to the results of the research study?

The results of the study will be to assess plaque index, gingival index, and periodontal probing depth.

17. Who is organizing theresearch?

This research study is organized by the academic institution (BBDCODS).

18. Will the results of the study be made available after study is over?

Yes.

19. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Dept, and the IEC/IRC of the institution.

20. Contact for further information

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Babu Banarasi College of Dental Sciences.
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Dr. Laxmi Bala,
Member Secretary,
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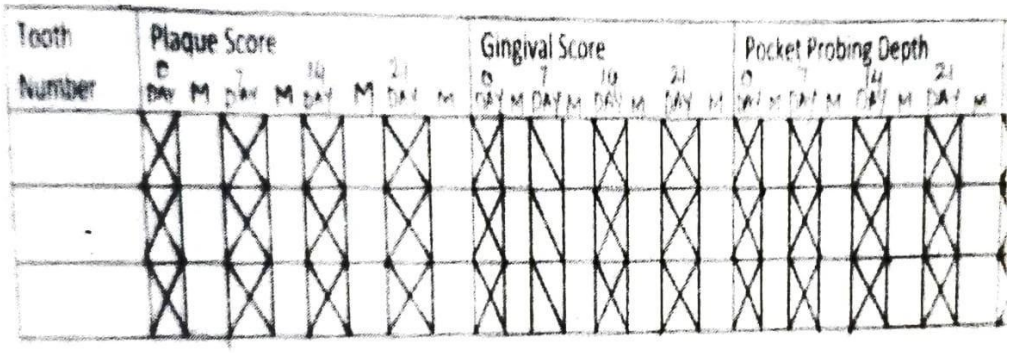
Lucknow
bbdcods.iec@gmail.com

ANNEXURE -6

Name of the Patient:

Tetracycline/Neem incorporated Fibers



ANNEXURE- 7

Formula used for the analysis

Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Standard deviation and standard error

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

and SE (standard error of the mean) is calculated as

$$SE = \frac{SD}{\sqrt{n}}$$

where, n= no. of observations

Analysis of Variance

Analysis of variance (ANOVA) is used when we compare more than two groups simultaneously. The purpose of one-way ANOVA is to find out whether data from several groups have a common mean. That is, to determine whether the groups are actually different in the measured characteristic. One way ANOVA is a simple special case of the linear model. For more than two independent groups, simple parametric ANOVA is used when variables under consideration follows Continuous exercise group distribution and groups variances are homogeneous otherwise non parametric alternative Kruskal-Wallis (H) ANOVA by ranks is used. The one way ANOVA form of the model is

$$Y_{ij} = \alpha_{.j} + \varepsilon_{ij}$$

where;

- Y_{ij} is a matrix of observations in which each column represents a different group.
- $\alpha_{.j}$ is a matrix whose columns are the group means (the "dot j" notation means that α applies to all rows of the j^{th} column i.e. the value α_{ij} is the same for all i).
- ε_{ij} is a matrix of random disturbances.

  The model posits that the columns of Y are a constant plus a random disturbance. We want to know if the constants are all the same.

Tukey multiple comparison Test

After performing ANOVA, Tukey HSD (honestly significant difference) post hoc test is generally used to calculate differences between group means as

$$q = \frac{\bar{X}_1 - \bar{X}_2}{SE}$$

where,

$$SE = \sqrt{\frac{S^2}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

S^2 is the error mean square from the analysis of variance and n_1 and n_2 are number of data in group 1 and 2 respectively.

Level of significance "*P*" is the probability signifies level of significance. The mentioned p in the text indicates the following:

P > 0.05- Not significant (ns)

P < 0.05- Just significant (*)

P < 0.01- Moderate significant (**)

P < 0.001- Highly significant (***)

ANNEXURE- 8

Group A: SRP + *AZADIRACHTA INDICA* (NEEM)

S.NO.	Tooth No.	Pocket probing depth (PPD) (mm)			Gingival index (GI)				Plaque index (PI)		
		0 Days	21 days	0 days	7 days	14 days	21 days	0 days	7 days	14 days	21 days
1	13	6	4	2	1.5	1.25	1.25	2.5	2	1.75	1.5
2	14	5	3	1.5	1	1	1	2.5	2.5	2	1.75
3	15	5	2	2.5	2	2	2	2	1.75	1.75	1
4	26	6	3	1.25	1.25	1	1	2	1.75	1.5	1.5
5	34	6	3	1.5	1.5	1	1	1.25	1	1	0.75
6	35	5	2	1.25	1.25	1	1	2	1.75	1.5	1
7	15	5	2	1.5	1.25	1.25	1	1.5	1.25	1.25	0.75
8	26	5	3	1.5	1	0.75	0.75	2	1.75	1.5	1
9	36	5	3	1.5	1.25	1.25	1	0.75	0.5	0.5	0.25
10	45	6	3	1.5	1.25	1	1	2.5	1.5	1	1
11	46	8	4	1.5	1.25	1.5	1.5	2.5	2	1.5	1.5
12	13	4	2	0.75	0.5	0.5	0.5	0.75	0.75	0.5	0.5
13	36	6	3	1.5	1.5	1.5	1.25	2	1.75	1.5	1
14	45	6	3	1.25	1.25	1.25	1	2	1.75	0.75	0.5
15	46	7	4	2.5	2	2	1.75	2.5	2	1.5	1.5
16	24	5	2	2	2	1.5	1.25	2	1	0.75	0.75
17	35	6	3	1	1	1	0.75	2	1.75	1.5	1.5
18	36	6	3	1.5	1	1	0.75	2	1.5	1.5	1
19	46	7	4	2	1.75	1.75	1.5	1.5	1	1	0.5
20	47	5	3	1.25	1.25	1	1	0.75	0.5	0.5	0.25

Group B: SRP + *CURCUMA LONGA* (CURCUMIN)

S.NO.	Tooth No.	Pocket probing depth (PPD) (mm)			Gingival index (GI)				Plaque index		
		0 Days	21 days	0 days	7 days	14 days	21 days	0 days	7 days	14 days	21 days
1	16	5	4	1.5	1.25	1.25	1	2.5	2.5	2	2
2	17	5	3	1.5	1.5	0.75	0.75	2.5	2.5	2.5	2.5
3	15	5	3	2.5	2	1.75	1.75	2	2	1.75	1.75
4	25	6	4	1.25	1	1	1.25	2	2	1.5	1.5
5	36	5	4	1.5	1	1	0.5	1.5	1.5	1.5	1.25
6	33	5	4	1.25	1	0.75	0.75	2.5	2.5	2	1.75
7	14	6	4	1.5	1.25	1	0.75	1.5	1.5	1.25	1
8	24	5	3	1.5	1	0.75	0.75	2	1.75	1.5	1.5
9	26	6	4	2.5	1.25	1.25	1.25	0.75	0.5	0.5	0.5
10	15	6	4	1.25	1	0.75	0.75	2	2	1.5	1.5
11	16	8	6	1.25	1.25	1	1	2.5	2.5	1.75	1.75
12	14	4	3	0.75	1	0.75	0.75	0.75	0.75	0.5	0.5
13	34	6	4	2	1.5	1.25	1.25	2.5	1.75	1.75	1.5
14	46	6	5	1.25	1.25	1	1	1	1	0.75	0.75
15	47	6	4	2.5	2	1.75	1.5	2.5	2	2	1.75
16	26	5	3	2	2	1.5	1	1.25	1.25	1	0.75
17	31	6	4	1	1	0.5	0.5	2	1.75	1.5	1.5
18	32	5	4	1.5	1	0.75	0.5	2	1.75	1.5	1.5
19	36	7	5	2	1.5	1.5	1.5	1.5	1.5	1	1
20	46	5	3	1	1	0.5	0.5	0.75	0.5	0.5	0.25

Curiginal

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