COMPARATIVE EVALUATION OF *Azadirachta indica* (NEEM) FIBER WITH TETRACYCLIINE FIBER AS A LOCAL DRUG DELIVERY AGENT – A RANDOMISED CONTROL STUDY

Dissertation

Submitted to

BABU BANARASI DAS UNIVERSITY, LUCKNOW,

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In the partial fulfilment of the requirements for the degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTOLOGY

By

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Under the guidance of

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Reader

Department of periodontology

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

LUCKNOW

(Faculty of Babu Banarasi Das University)

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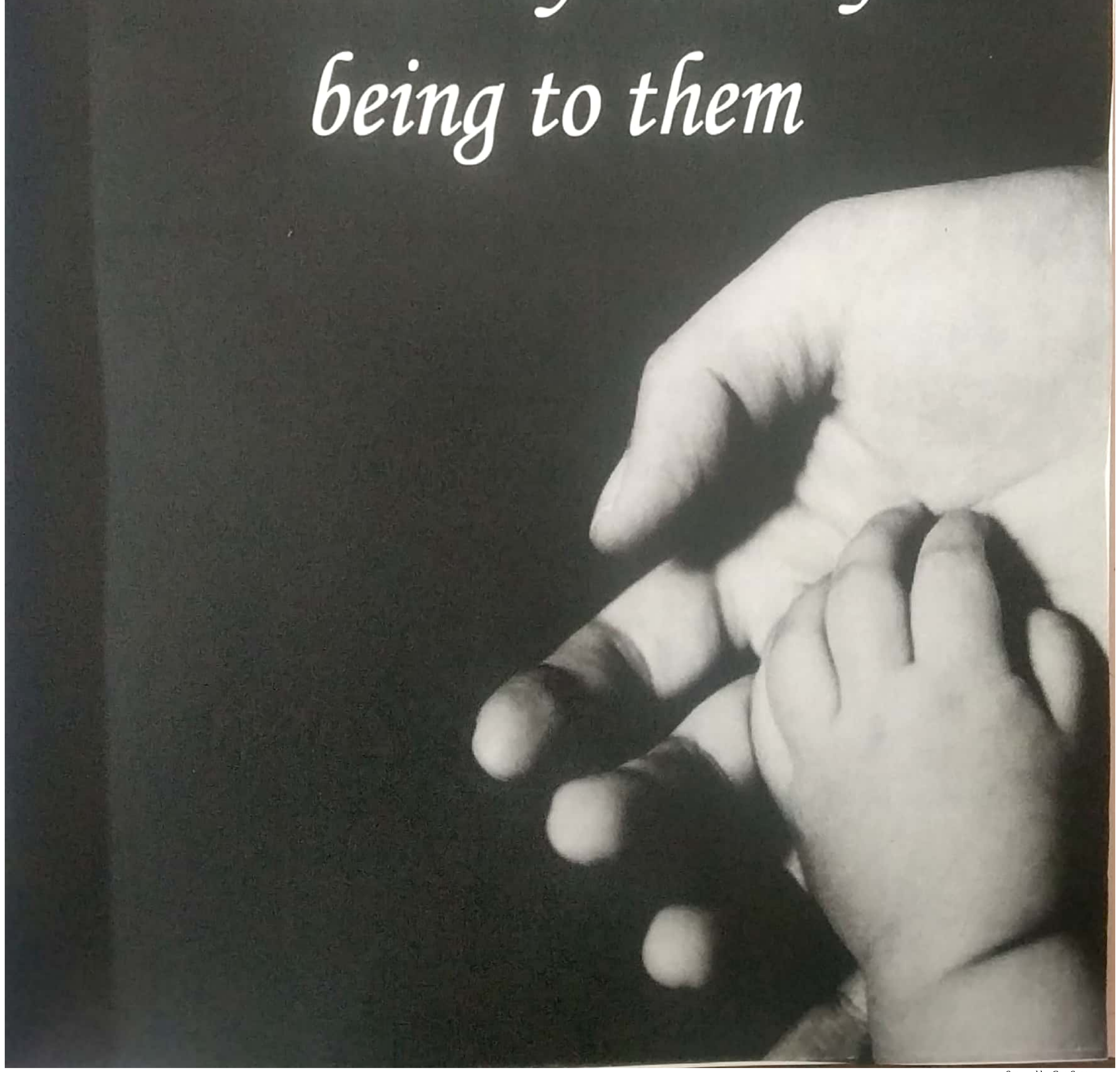
Dedicated To My Mumma, Papa Vismay

I Owe my sense of being to them



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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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Words cannot describe my emotions for my beloved ***Parents*** *and* ***sister*** *who always stood by me and gave me unconditional love and support in times of joy and distress and have given me the strength to face the world. Last but not the least I thank the almighty and ever loving "GOD".*

Dr.Vaanchha Sharma

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LIST OF ABBREVIATIONS

A.*actinomycetemcomitans*	Actinobacillus *actinomycetemcomitans*
ALN	Alendronate
AZA	Azadirachta
B.*gingivalis*	Bacteroides *gingivalis*
B.*intermedius*	Bacteroides *intermedius*
BPRs	Black Pigmented Rods
BOP	Bleeding On Probing
CCL_4	carbontetrachloride
CHX	Chlorhexidine
ELISA	Enzyme Linked Immuno Sorbent Assay
GC-MS	gas chromatography–mass spectrometry
FACS	flow cytometry
hGF	human gingival fibroblasts
Ig G	immunoglobulin G

IL	Interleukin
IgM	Immunoglobulin M
LDD	Local drug delivery
MMP	Matrix metallo Proteinases
MTT	3-(4,5Dimethylthiazol-2-YI)-2,5-Diphenyltetrazolium Bromide
MIC	minimum inhibitory concentration
MGI	modified gingival index
NVC	Neem Vehicle Concentration
P. *gingivalis*	Porphyromonas *gingivalis,*
P. *intermedia*	Prevotella *intermedius*
PCR	Polymerase chain reaction
RAL	Relative attachment level
SRP	Scaling and Root planing
Spp	Species
TC	Tetracycline Therapy
(v/v)	volume by volume

ABSTRACT

ABSTRACT

Anti- microbial therapy is essential along with conventional therapy in the management of periodontal disease. Instead of systemic chemical agents, herbal products could be used as antimicrobial agents. Herbal local drug delivery systems are effective alternative for systemic therapy in managing the chronic periodontal disease. In this study, clinical parameters regarding the efficacy and time release pattern of *Azadirachta indica* (Neem) placed in chronic periodontitis patients compared with tetracycline fibers was evaluated. Neem leaves were used to prepare an 37.8 % extract using Soxhlet apparatus and an *in vitro drug* drug diffusion study was carried out to establish the release pattern of neem. Drug release from collagen thread and into the tissues simulated on KC Diffusion Cell was 23.85% and 38.33% respectively. To assess the clinical efficacy, 45 sites with 5-6 mm of pockets were divided into 2 groups i.e tetracycline group (control) and Neem incorporated collagen fibers(experimental group). Clinical parameters including Plaque score Gingivitis score, PPD ,CAL were recorded at baseline , 7 day, 14 day and 21 day. After fiber placement at baseline coe-pack was given which was removed on the 7 day. Results showed that the Plaque score and gingivitis score reduced significantly (p value 0.002) at 21 days compared with baseline for tetracycline group. PPD reduced significantly (p value <0.001) at 7 days whereas CAL reduced at 14 day significantly for tetracycline group. Plaque score reduced significantly (p value 0.001) at 14 days whreas gingivitis score reduced significantly (p value <0.001) at 21 days compared with baseline for neem group. PPD and CAL reduced significantly (p value <0.001) from 7 days compared with baseline for neem group. Mean % variation observed in plaque score for tetracycline group and neem group was 6.25% and 9.22% respectively. Mean % variation observed in gingivitis score for tetracycline group and neem group was 6.62 % and 9.09% respectively. Mean % improvement observed in Pocket probing depth for tetracycline group and neem group was 35 % and 47.04% respectively. Mean % improvement observed in clinical attachment level for tetracycline group and neem group was 32.89 % and 42.68% respectively. Hence, Neem group proves to be more efficacious when compared with tetracycline fibers on all clinical parameters i.e neem has higher mean % variation from baseline to 21 days .

INTRODUCTION

INTRODUCTION

Destructive periodontal diseases are characterized by loss of the supporting tissues of teeth, and they are recognized as major public health problem worldwide. Periodontal disease results from host microbial interaction with anaerobic bacteria pivotally contributing to supporting tissue destruction. These pathogenic bacteria by colonizing on the tooth surface and sub gingival areas, liberate toxins (by products and enzymes) producing inflammatory disease; by breaking extracellular matrices and host cell membranes to produce nutrients for their growth.

Non surgical treatment of periodontal diseases primarily involves scaling and root planing. Scaling and root planing fails to completely remove all pathogens due to their ability to penetrate deeper tissue or because of inappropriate instrumentation often leaving behind significant number of bacteria to recolonize within 42 after single debridement session.[1] For the management of periodontal diseases, antimicrobial agent may be used adjunctively to scaling and root planing which can be conveyed into periodontal pockets by systemic antimicrobial therapy, rinsing, irrigation, local application using sustained and controlled delivery devices.

The antimicrobials administered via serum, readily reaches the microorganism at the depth of the diseased sites. Systemic administration include higher risk of toxicity with prolonged or frequent courses enabling superinfecting organisms to persist as the subgingival microbiota over extended time periods sustaining the periodontal pathology and may even give rise to systemic complications.

The local route of antimicrobial administration can accomplish many folds higher therapeutic doses in sub gingival sites than those possible by systemic therapy, better patient compliance. It may be especially useful for individuals with superinfections or the ones displaying gastrointestinal or other side effects after systemic antimicrobial therapy.[2]

Five main anti microbial agents used are tetracycline, minocycline, doxycycline, chlorhexidine[3], metronidazole. The tetracycline are broad spectrum antimicrobials affecting anaerobes and facultative organisms, bacteriostatic at concentration found in gingival crevicular fluid after systemic administration, and local delivery providing bactericidal concentrations [4,5] with associated minimal adverse effects. Tetracycline has a distinct property which can be used in benefit for the periodontal therapy is its ability

to concentrate in gingival crevicular fluid.[6] The disadvantage of tetracycline is their ability to kill benign organisms associated with health as well as pathogens.[7] Tetracycline may cause super infections diarrhea and pseudo membranous colitis. Gastrointestinal side effects are most common adverse effects. Tetracyclines are contraindicated during pregnancy due to the risk of fetal tooth enamel dysplasia and irregularities in the fetal bone growth. Treatment of young children (< 8 years) with tetracycline may cause dentition abnormalities. High doses of tetracycline may cause hepatic necrosis especially in pregnant females. Tetracyclines may exacerbate pre-existing renal dysfunction although not being directly nephrotoxic. [8-10]

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. According to the WHO , as many as 80% of the world's people depend on traditional medicine (herbal) for their primary healthcare needs[11]. Neem has been used in India and south Asia for thousands of years as the preferred tool for maintaining healthy teeth and gums. Brushing with Neem twigs and chewing Neem leaves and seeds after a meal has been the traditional dental care practice in this area. Neem (*Azadirachta indica*) is a tree from the Meliaceae family originated from India. The Sanskrit name of the Neem tree is 'Arishtha' meaning 'reliever of sickness' in India the tree is considered as "sarbaroganibarini" which means reliever of all diseases. Over 700 herbal preparations based on the Neem are found in the ayureda, siddha unani and other local health traditions.[11] The Neem tree has been defined by De Jussieu in 1830.[12]

Azadirachtin (AZA, **1**), the main active component of this plant present in smaller quantities in leaves, is a tetranortriterpenoid . Other active substances are salanin, 14-epoxiazadiradione, meliantrol, melianone, gedunin, nimboline, nimbin, deacetilasalanin, azadiractol, azadirone, vilosinin, meliacarpine.[13,14] Extracts of leaf, oil and seed kernels are effective against certain human fungi, including *Trichophyton*, *Epidermophyton*, *Microsporum*, *Trichosporon*, *Geotricum* and *Candida.* Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis, Vibrio cholerae, Klebsiella pneumoniae, M. tuberculosis, M. pyogenes, Streptococcus mutans and S. faecalis.*[15]

Therefore to utilize the beneficial effects of Neem for the treatment of periodontitis local drug delivery of Neem is utilized to treat the same. Few clinical studies were conducted to see the effect on human gingival fibroblast on root surface as an effect of Neem. The cytoprotective, oral friendly quality of NE emphasize the superiority of Neem over chlorhexidine.[16, 17]

Since the systemic toxicity of antimicrobials is well reported , therefore local drug delivery can be a better alternative for the treatment of periodontal pockets. For this study Neem *(A. indica)* being indigenous material with above said properties, has been taken in the form of fibers for the treatment of periodontitis and further comparing it with tetracycline, considered as gold standard.

AIM AND OBJECTIVES

1. AIM

The aim of the study is to evaluate the clinical parameters regarding the efficacy and time release pattern of the *Azadirachta indica* (Neem) family Meliaceae placed in chronic periodontitis patients compared with tetracycline fibers.

2. OBJECTIVES

- To evaluate the time release pattern of *Azadirachta indica* (Neem) incorporated fiber – *in vitro*.
- To assess the clinical efficacy of *Azadirachta indica* (Neem) incorporated fiber in chronic periodontitis.
- To assess the clinical efficacy of tetracycline fiber in chronic periodontitis.
- To compare the clinical efficacy of Neem incorporated fiber with tetracycline fibers.

MATERIALS AND METHOD

A comparative evaluation of *Azadirachta indica* (Neem) fiber with tetracycline fiber as a local drug delivery agent was conducted in the Department of Periodontology, Babu Banarasi Das College of Dental Sciences, Lucknow, Uttar Pradesh in collaboration with School of Pharmacy, Babu Banarasi Das University. The approval for the experimental protocol was taken from ethics committee, Babu Banarasi Das College of Dental Sciences, Lucknow. (ANNEXURE 1, ANNEXURE 2)

MATERIALS –

The time release pattern of *Azadirachta indica* (Neem) incorporated fibers were evaluated *in vitro* in the School of Pharmacy, BBDU. Commercially available vial each containing 25mg of gamma sterilized samples of sterile type -I fibrillar collagen were procured from EUCARE PHARMA Pvt Ltd., Chennai.

Materials required for *in vitro* analysis –

Grinder

Sieve

Flask

Micro pipette

Soxhlet apparatus

K C Diffusion cell

Spectrophotometer (1700 UV –VIS- SHIMADZU)

SUBJECTS :

Inclusion and exclusion criteria

As this part of study was in vitro, therefore no human subjects were involved.

PLANT :

The present study was undertaken to evaluate the clinical parameters regarding the efficacy and time release pattern of the Neem.

NEEM-

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Sapindales

Family : Meliaceae

Genus : Azadirachta

Species : *indica*

Scientific Name : *Azadirachta indica*

Common names : neem, miracle tree, arishtha (sanskrit), margosa tree, indian liliac.

Part used : leaf

Chemical composition of Neem :

Azadirachtin (AZA, **1**), the main active component of this plant, is a tetranortriterpenoid. Other active substances are salanin, 14-epoxiazadiradione, meliantrol, melianone, gedunin, nimboline, nimbin, deacetilasalanin, azadiractol, azadirone, vilosinin, meliacarpine. [43, 44]

COLLECTION AND VALIDATION OF PLANT MATERIAL (PLATE I)

Neem leaves were collected 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 leaves (7 Kgs) were communited 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 air tight light resistant containers for further studies. A small pack of silica gel was also placed in container to prevent any moisture. (Plate II)

Neem (leaf) collected was collaborated and validated by CSIR- NATIONAL INSTITUTE OF SCIENCE COMMUNICATION AND INFORMATION RESOURCES.(ANNEXURE 3)

(Ref. No. – NISCAIR/ RHMD/ Consult/2018/3170-19)

PREPARATION OF EXTRACT

Azadirachta indica leaves were collected from the medicinal garden of the School Of Pharmacy BBDU and were dried in cool, dry shady place. (Plate I) Taxonomic

Neem leaves

Uniform sized powder of Neem

Plate -1

identification of Neem leaves was performed by CSIR-NISCAIR, Delhi; where voucher specimens were deposited.

(Ref. No. – NISCAIR/ RHMD/ Consult/2018/3170-19)

The leaves were dried under controlled conditions, then using soxhlet apparatus extraction process was carried out.

Soxhlet apparatus (FIGURE 1, PLATE II)

It was originally designed for the extraction of a lipid from a 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.

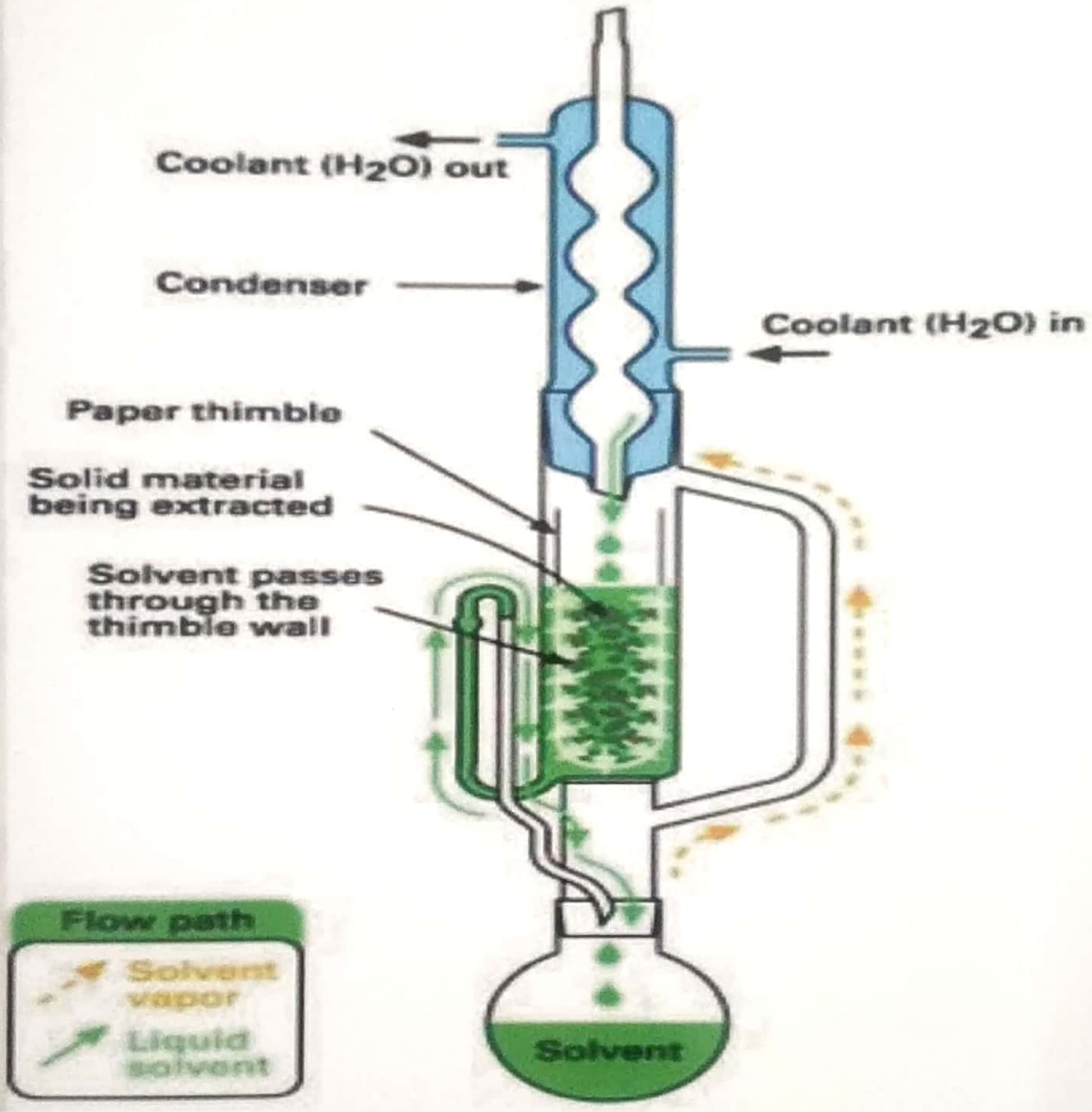
The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material 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, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Approximately 1 Kg of dried leaves was taken for the study and was extracted using Soxhlet apparatus. The percentage yield was 37.8 %. The phytochemical nature of the extract was ascertained by established tests and the results were matched by standard

FIGURE 1: PRINCIPLE OF SOXHLET APPARATUS



Soxhlet Apparatus

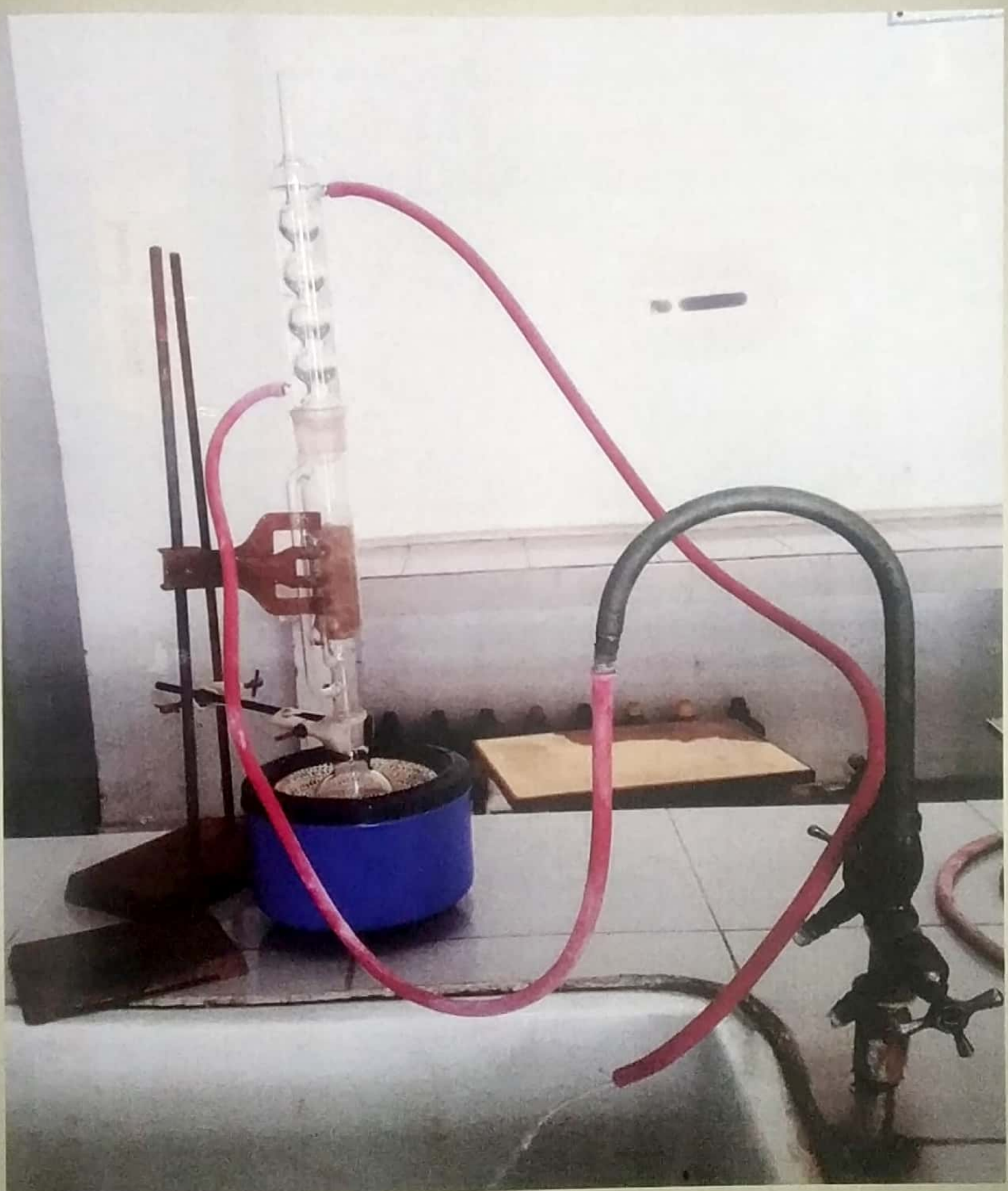


Plate -II

tests.The extract was then filtered through filter paper, and was kept in an air tight amber coloured container.

The final extract contained 37.8 % Neem.

IN VITRO METHODOLOGY

Spectrophotometric analysis (FIGURE 2, PLATE III)

A spectrophotometer is an instrument measuring the amount of photons (the intensity of light) absorbed after it passes through sample solution to determine the concentration. In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed color.

The amount of photons that goes through the cuvette and into the detector is dependent on the length of the cuvette and the concentration of the sample. Once you know the intensity of light after it passes through the cuvette, you can relate it to transmittance (T). Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation:

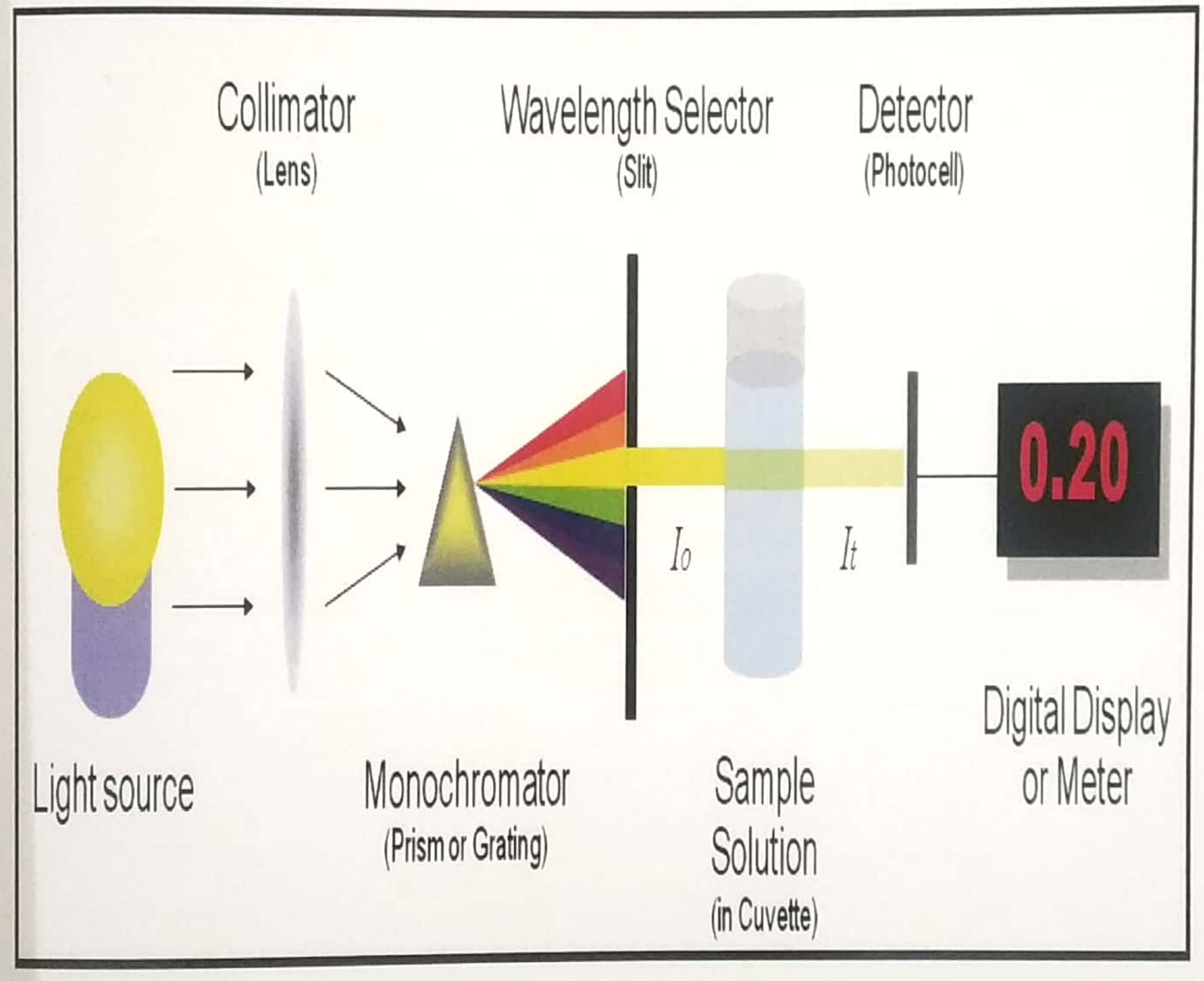
$$Transmittance(T) = \frac{I_t}{I_o}$$

Where I_t is the light intensity after the beam of light passes through the cuvette and I_o is the light intensity before the beam of light passes through the cuvette. Transmittance is related to absorption by the expression:

$$Absorbance(A) = -log(T) = -log(\frac{I_t}{I_o})$$

With the amount of absorbance known from the above equation,the unknown concentration of the sample by using Beer-Lambert Law can be determined.UV spectrum of the isolated Neem extract was obtained using UV visible spectrophotometer ranging in wavelength from 200- 400 nm.

FIGURE 2: PRINCIPLE OF PHOTOSPECTROMETER



Spectrophotometer

K C Diffusion Cell

Plate -111

***In vitro* DRUG DIFFUSION STUDY-**

In vitro drug diffusion study was performed using KC Diffusion Cell ; with capacity of 50 mL. **KC Diffusion Cell**: (FIGURE 2, PLATE III)

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 membrane which was isolated by dipping hen egg in concentrated Hydrochloric acid solution. This HCl solution dissolved outer $CaCo_3$ 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° C and the temperature was regulated by controlling the water flow inside KC diffusion cell.

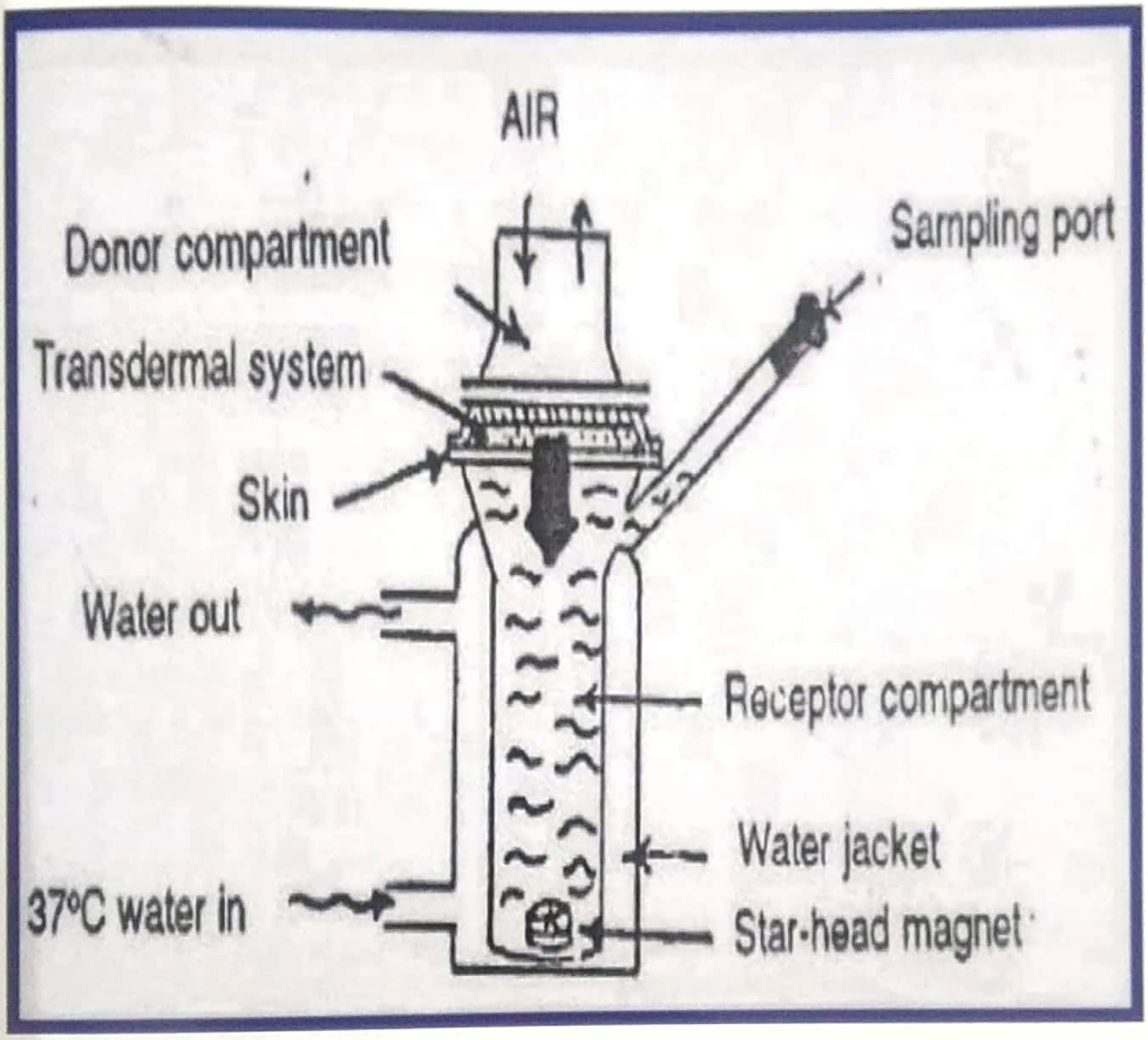
Neem extract was filled in donor chamber above the semi permeable membrane (egg membrane). Samples were collected at regular interval of 1 hour for 7 hours. To get the overall release of components from the extract through semi permeable membrane; a sample were collected at 72 hours, 5^{th} day, 7^{th} day.

A fixed volume of sample (1 ml) 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 constituents from the extract into tissues (through egg membrane)

INCORPORATION OF NEEM INTO STERILE COLLAGEN FIBERS -

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

FIGURE 3: KC DIFFUSION CELL



IN VIVO STUDY

A total of 45 sites were selected including both males and females in the age group 30-60 years .

Inclusion criteria

1) A total of 30 patients including both males and females in the age group of 30 – 60 years.
2) Patients diagnosed to have chronic periodontitis with probing pocket depth > 5mm
3) Free from any systemic disease and who had not undergone any form of periodontal therapy in the last 6 months.

Exclusion Criteria

1) Pregnant ladies or lactating mothers
2) Patients having systemic diseases
3) Smokers
4) Patients who had received any topical or systemic antibiotic treatment for any purpose in the past 3 months including the use of mouth wash or currently on systemic antibiotic.
5) Drug allergies
6) Teeth with traumatic occlusion

CLINICAL STUDY DESIGN

On the basis of inclusion and exclusion criteria, 45 sites in 15 patients were randomly selected and treatment protocol for the selected site weres decided randomly by lottery method. These sites were divided into 2 groups - SRP+ TTC and SRP+ Neem according to the treatment to be given.

ARMAMENTARIUM (Plate IV)

Diagnostic Instruments

- Mouth mirror
- Explorer
- UNC-15 Probe
- Tweezer

Materials and instruments required for fiber insertion

- Cumine scaler, (Hu-Friedy USA)
- Gracey curettes (Hu-Friedy USA)
- Tetracycline fibers - A B Plus Periodontal fibers
- Neem incorporated collagen fibers
- Beaker
- 1 ml syringe
- Dapen dish
- Cheek retractor
- Coe- pack

The nature and design of the clinical trial was explained to the patients and consent was obtained. Oral hygiene instructions for supra gingival plaque control were given. The individual sites with probing depth of 5-6 mm at baseline were chosen and randomly assigned for the study. A total 45 sites were selected for this study.

The selected sites were grouped as-

Control group : these sites received scaling and root planing along with tetracycline fibers (Plate V-VII)

Armamentarium

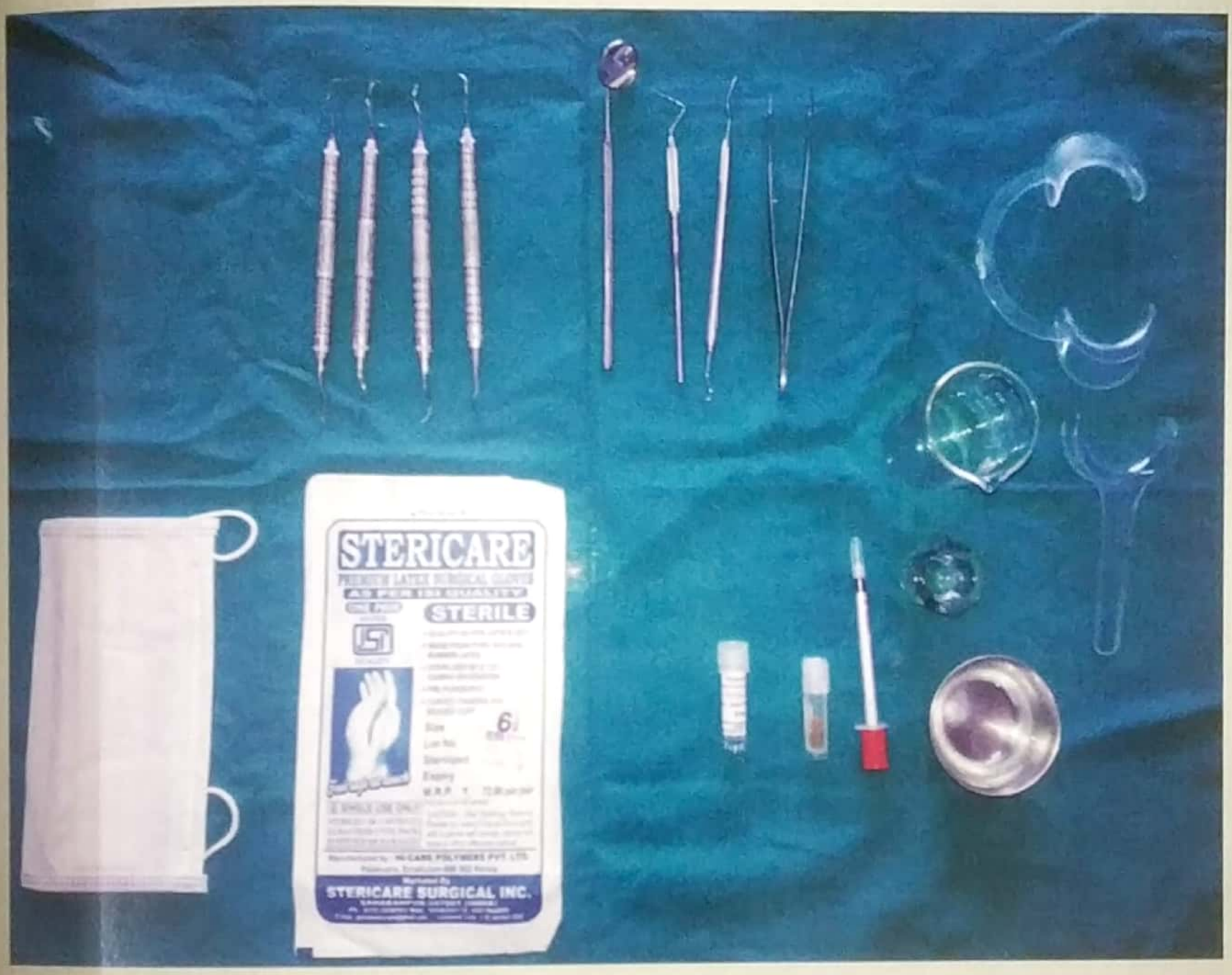
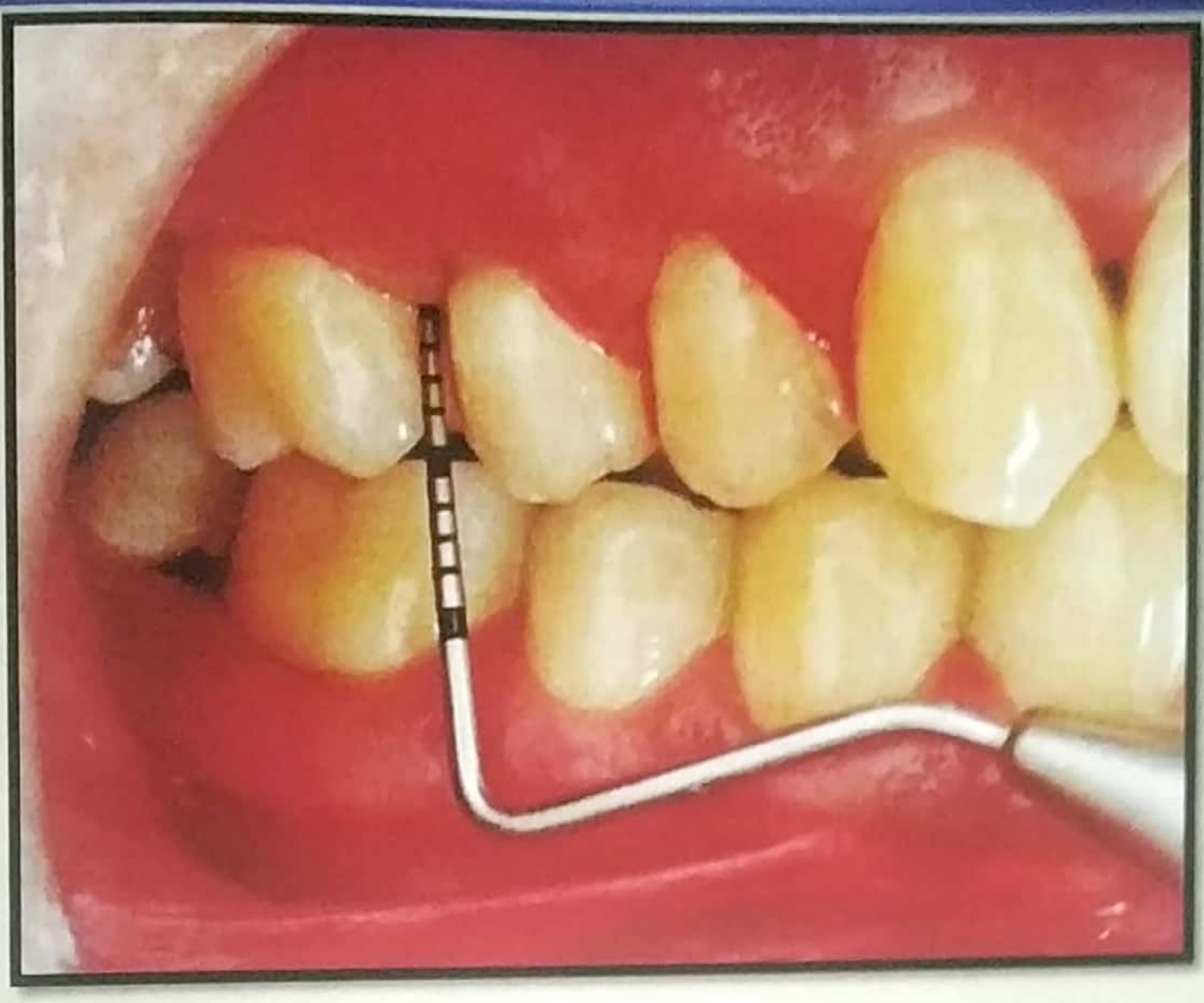


Plate -IV

Baseline evaluation for Tetracycline group



Placement of Tetracycline fibers

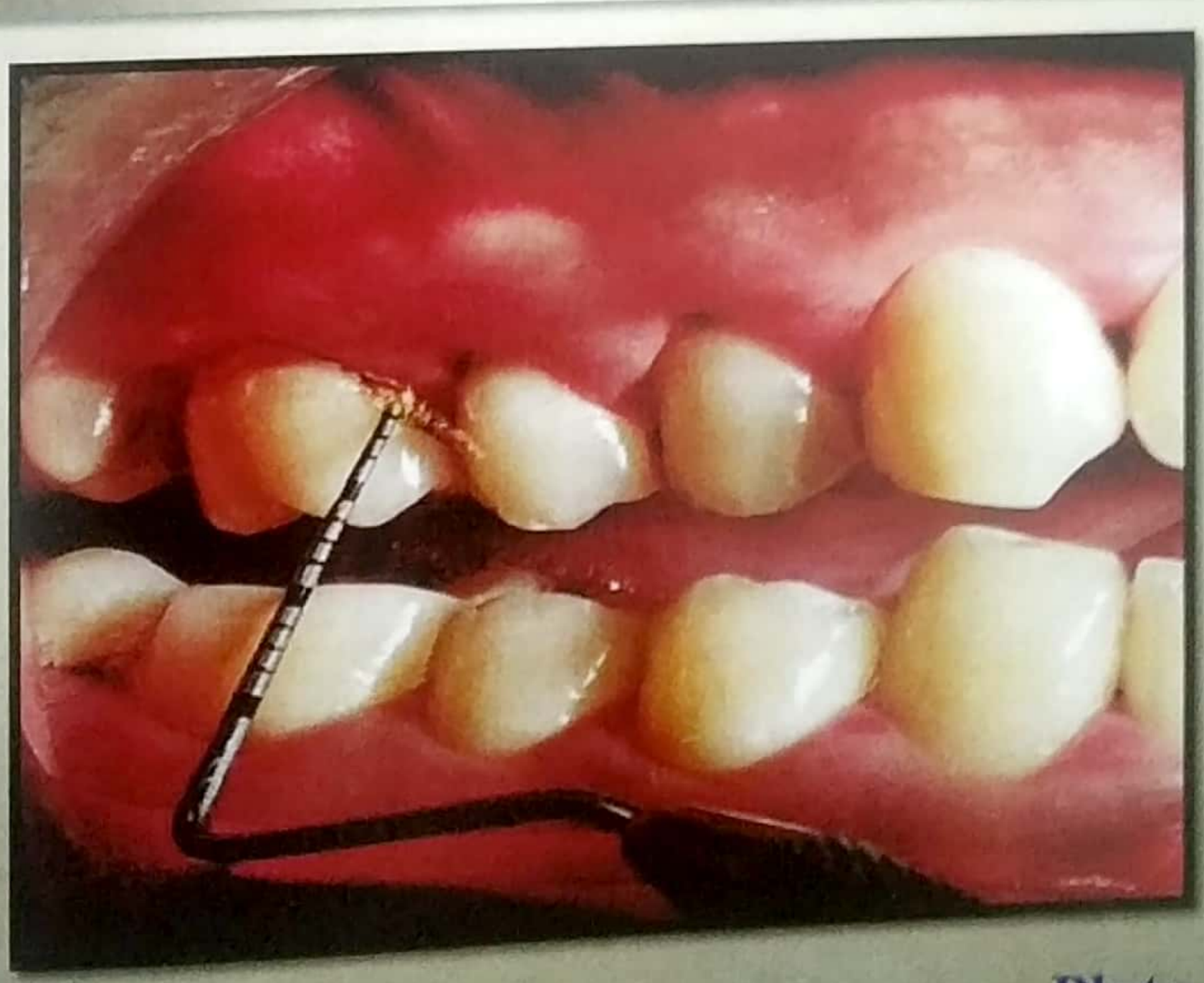
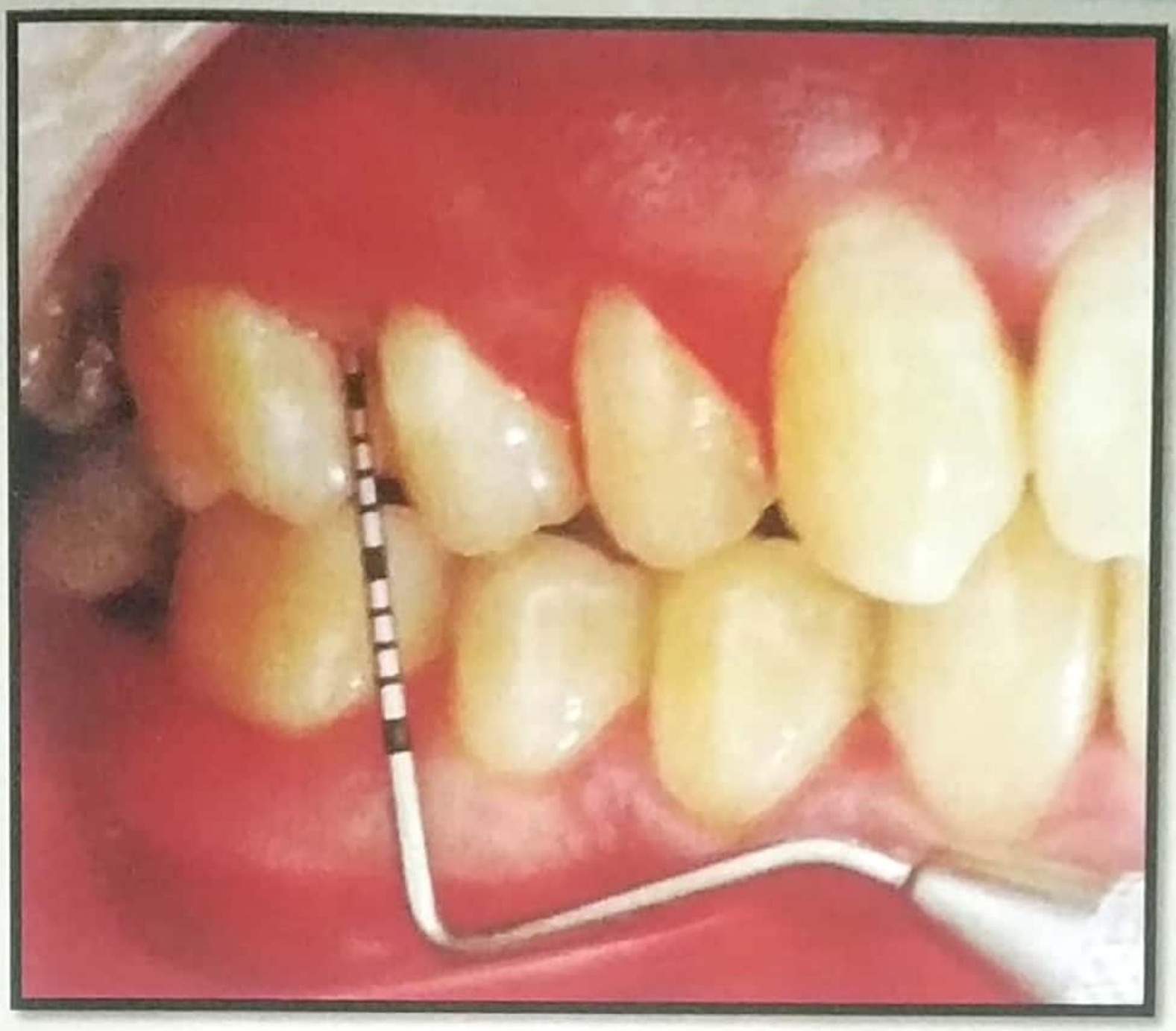


Plate -V

Evaluation on 7th day for Tetracycline fibers



Evaluation on 14th day for Tetracycline fibers

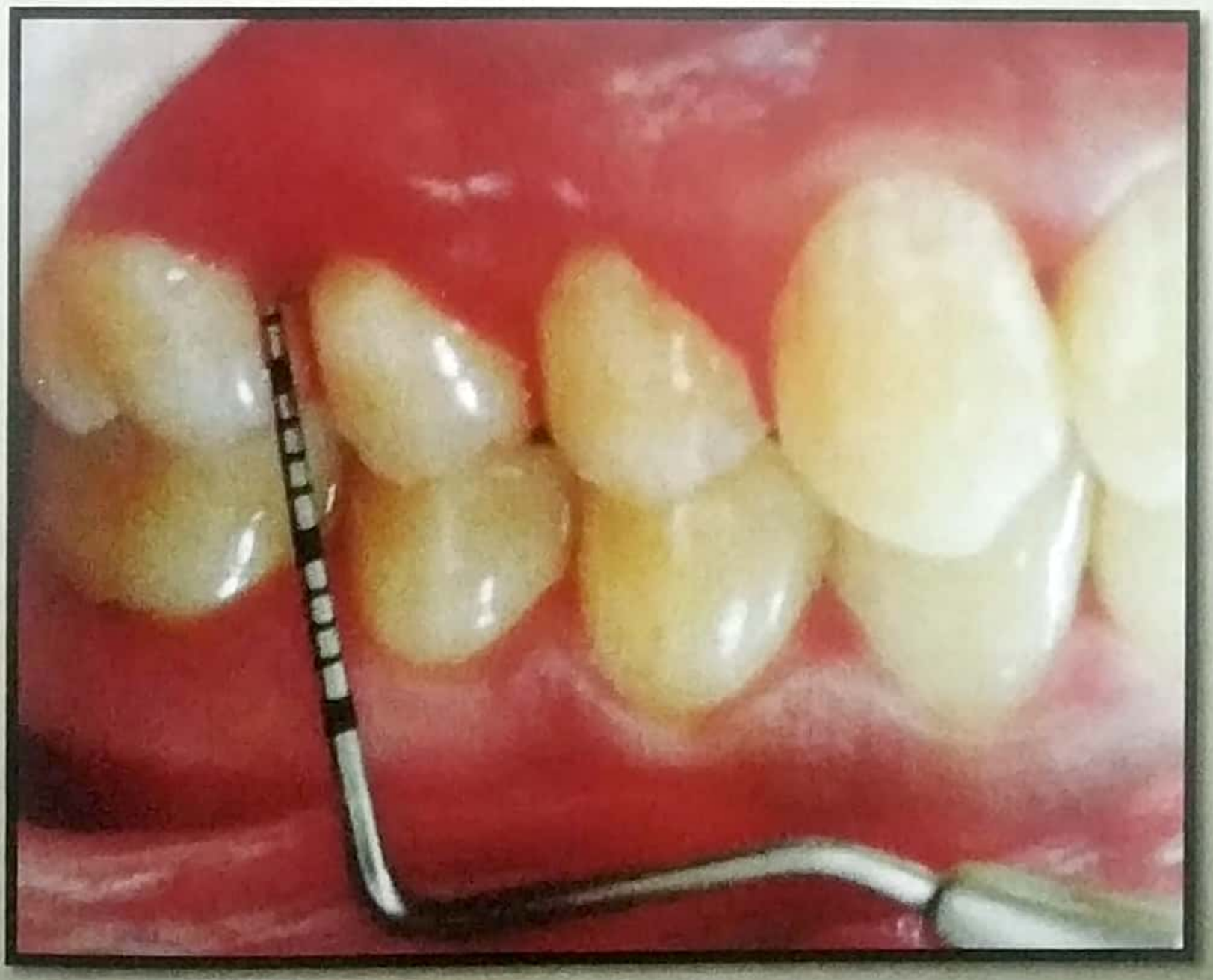


Plate -VI

Evaluation on 21st day for Tetracycline fibers

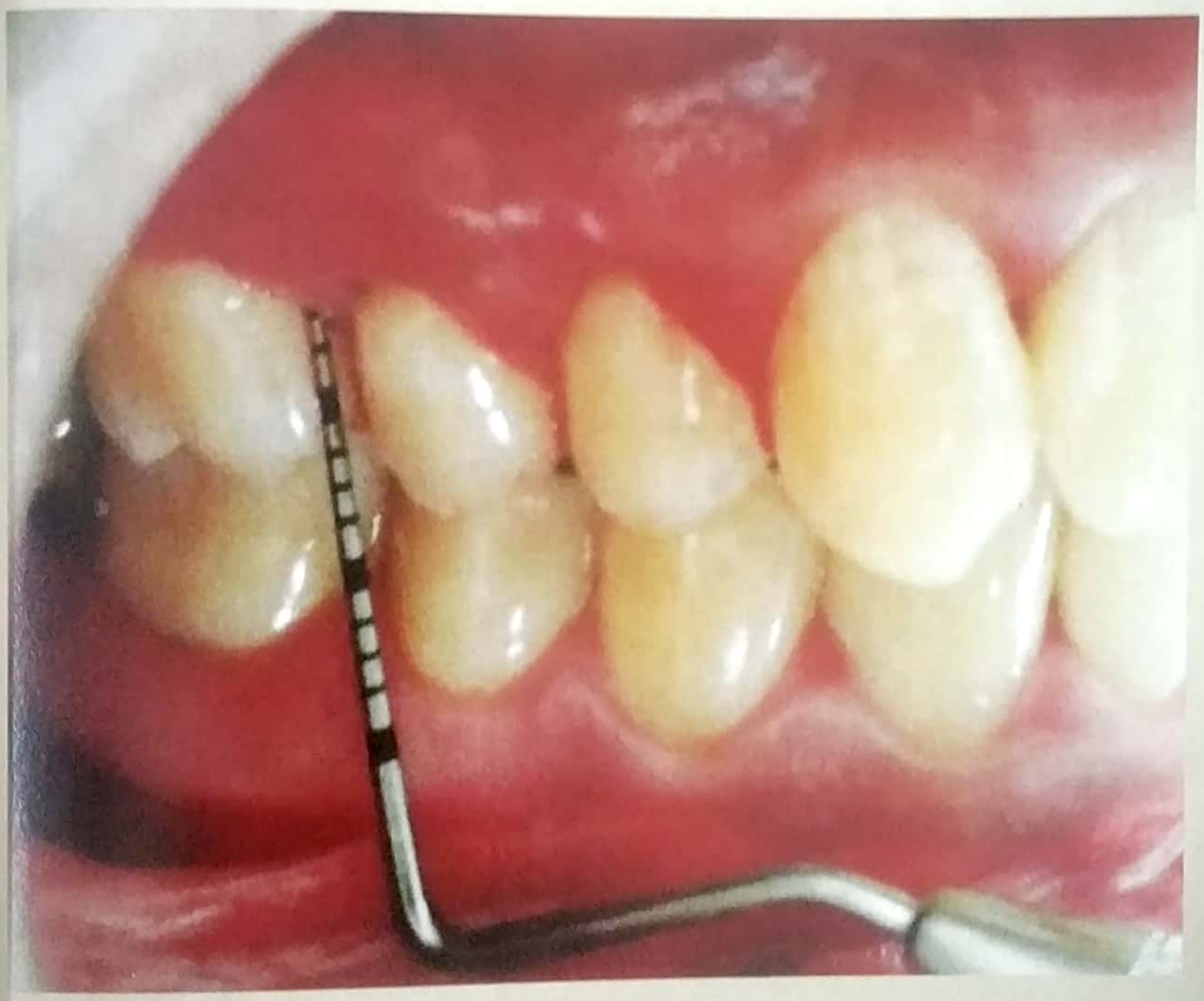


Plate - VII

Experimental group : these sites received scaling and root planing along with Neem incorporated collagen fibers.(Plate VIII-X)

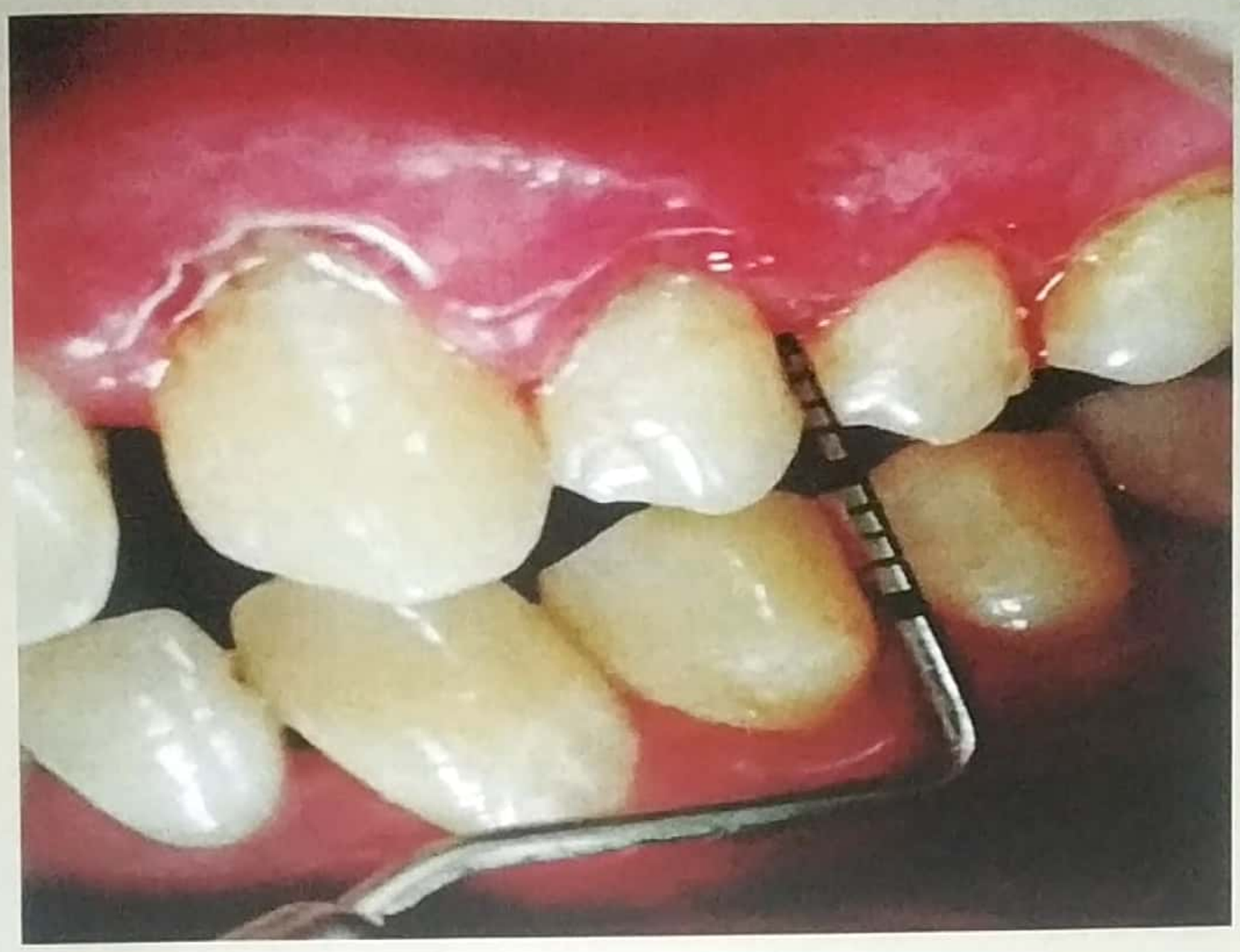
To wet the fibers before placement, 2 drops of Neem extract was added by 1 ml syringe to the collagen fibers 2 hours and few drops of sterile saline solution was added on the tetracycline fibers and 2 drops before placement in periodontal pockets. Required portion of the wet fibers were taken and placed into the pocket site with periodontal probe gently. The gingiva was subsequently adapted to close the entrance of the site and hand pressure was applied for just a few minutes to encourage haemostasis.

After the placement of fibers in control and experimental groups, the treated sites were given Coe-pack. The clinical parameters of the selected target areas were undertaken before placement of the fibers at baseline, 7 days, 14 days, 21 days follow up at recall visits only supra gingival scaling was done.

The following clinical parameters were used to assess the periodontal status:

- Plaque index – (silness and Loe 1964)[74]
- Gingival index(Loe and silness 1963)[75]
- Probing pocket depth(PPD)[76]
- Clinical attachment level(CAL)[76]

Baseline evaluation for Neem fibers



Placement of Neem fibers

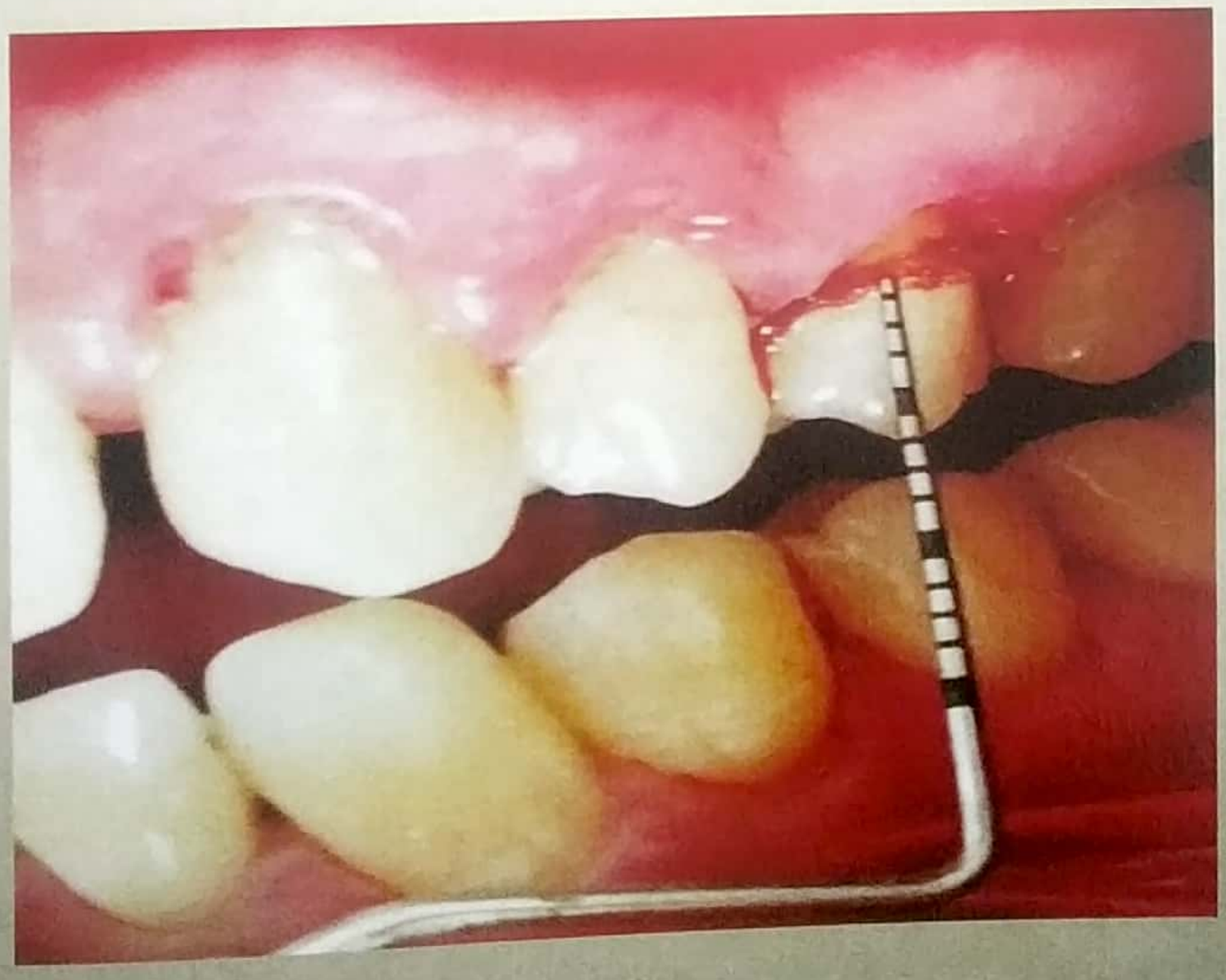
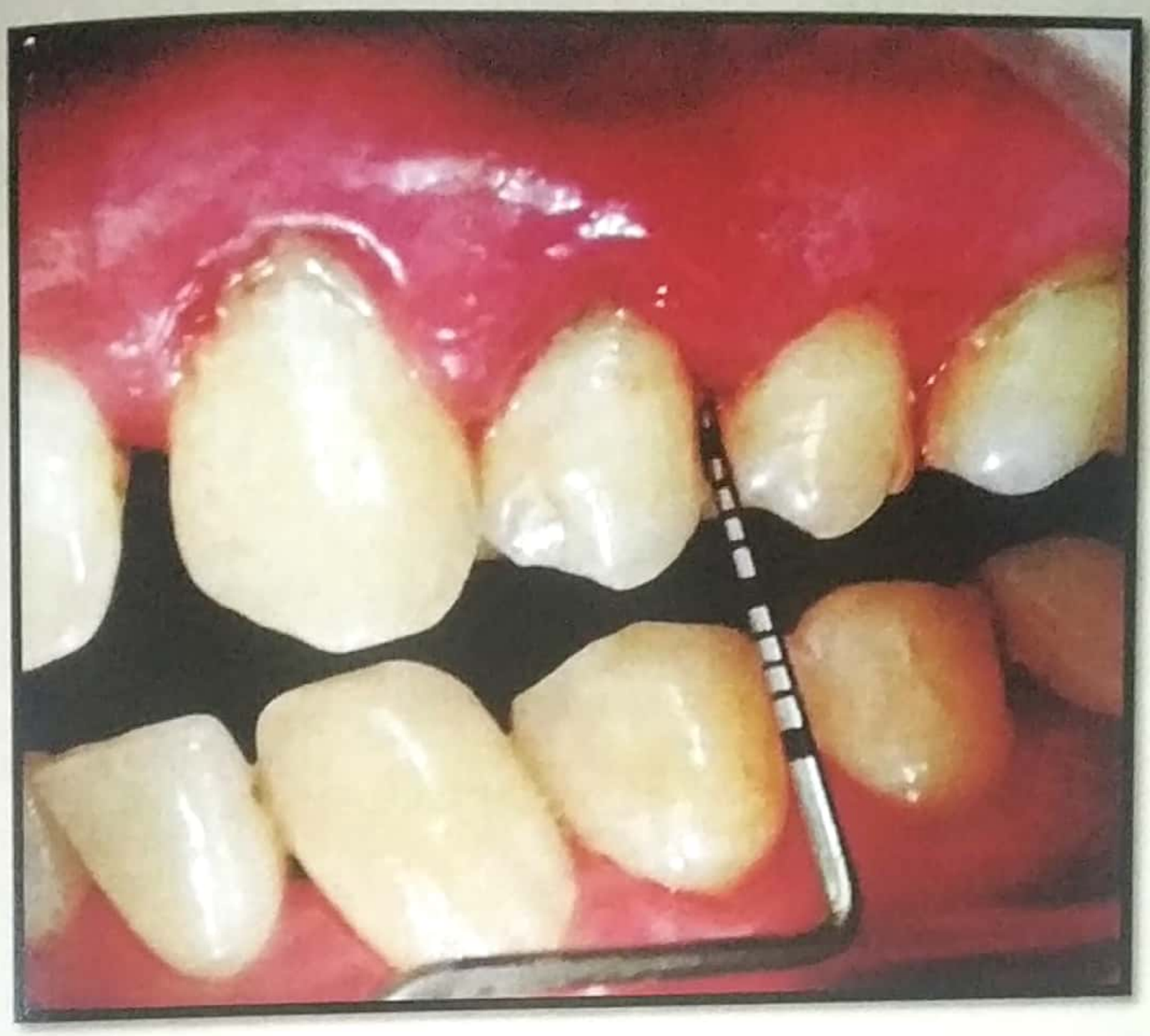


Plate - VIII

Evaluation On 7th day for Neem fibers



Evaluation on 14th day of Neem fibers

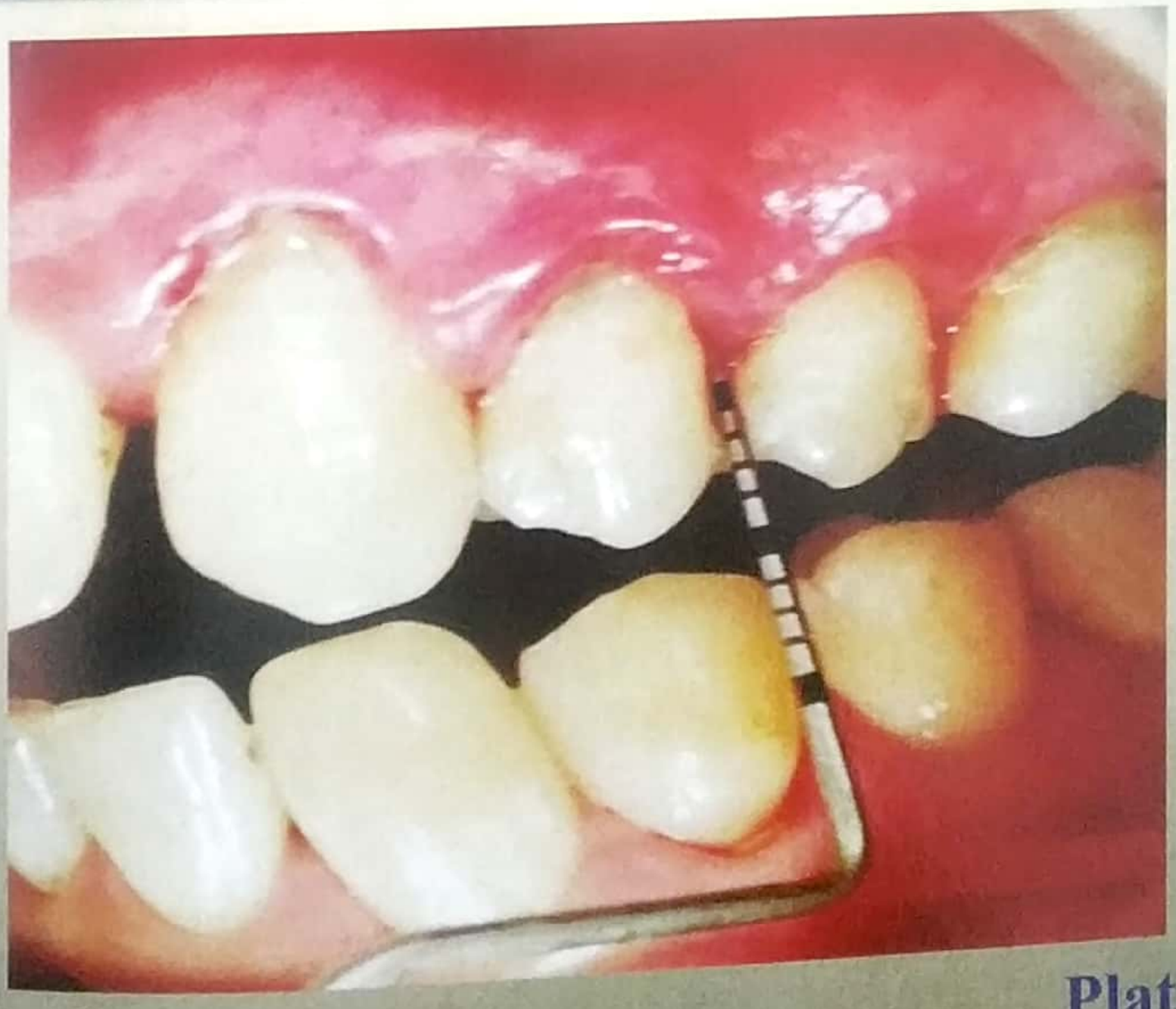


Plate - IX

Evaluation on 21st day for Neem fibers

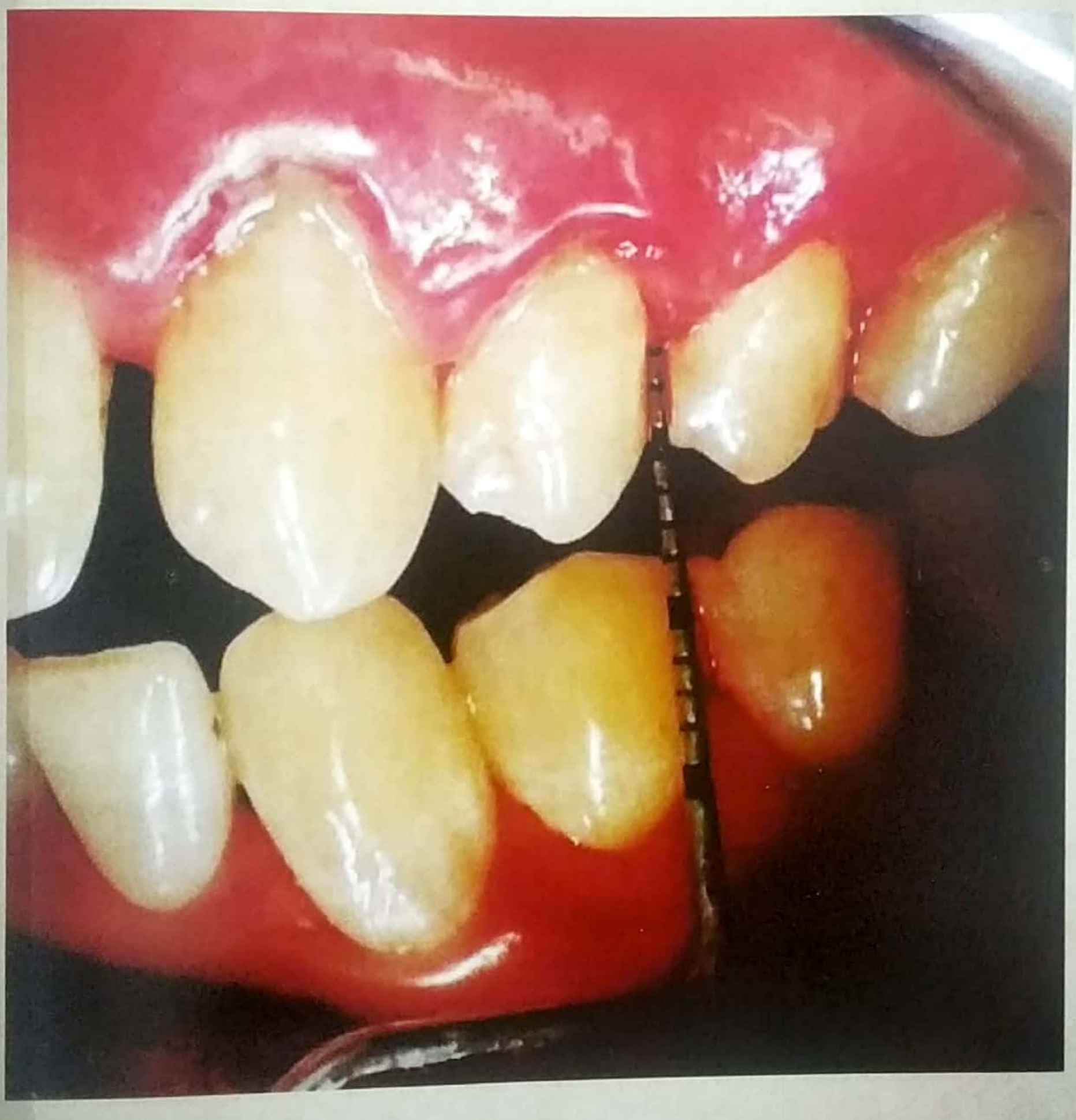


Plate - X

REVIEW OF LITERATURE

Various animal and human studies have proved Neem's efficacy and safety for its use in humans. Some of the important studies are reviewed here-

Lisgarten MA (1976)[18] in a light and electron microscopic study stated that different microbiological flora is associated with periodontal health, as well as disease. He further concluded that the sub gingival flora associated with periodontal disease consisted of relatively fewer cells adherent to the root surface. There was a concomitant increase in the population of gram- negative and flagellate cells, as well as spirochetes.

Lantz MS, Switalski LM, Kornman KS, Hook M (1985)[19] suggested by conducting a study that fibrinogen binding by *Bacteroides intermedius* might represent a mechanism of bacterial tissue adherence rendering them to attach to and detach from a fibrinous substratum. The presence of such substratum may modulate tissue colonization by providing specific attachment sites only for those bacteria possessing the ability to bind fibrinogen. The result concluded that the composition and distribution of the host protein along the tissue wall of the sulcus or periodontal pocket determines the composition of sub gingival micro flora.

Slots J, Bragd L, Wikstrom M, Dahlen G (1986)[20] assesssed total of 235 subgingival sites, for *A. actinomycetemcomitans, B. gingivalis* and *B. intermedius.* On the basis of radiographic changes in the crestal alveolar bone level, the disease progression was determined. *A. actinomycetemcomitans* isolation was carried out using the selective TSBV medium and *B. gingivalis* and *B. intermedius* isolations were performed using a nonselective blood agar medium. *A. actinomycetemcomitans* appeared in significantly higher prevalence in treated-progressive lesions (80.8%)) than in nontreated-progressive lesions (42.3%). The result concluded that *A. actinomycetemcomitans, B. gingivalis* and *B. intermedius* are closely related to disease-active periodontitis.

Slots J, Listgarten MA (1988)[21] evaluated in their study that *P.gingivalis, P.intermedius* and *A.actinomycetemcomitans* seem to be major pathogens in advancing periodontitis. These organisms are recovered in higher prevalence and proportions from progressive periodontitis lesions than from quiescent periodontal sites. Secondly, elevated antibody levels against P. *gingivalis* and *A.*

actinomycetemcomitans were found in serum and gingival crevice fluid of periodontitis patients compared to normal controls. Third *B. gingivalis* and *B. intermedius* express potent proteases and *A- actinomycetemcomitans* various noxious substances which have the potential to perturb important host defenses and to disintegrate key constituents of the periodontal tissues. Monitoring these bacteria may assist the assessment of treatment efficacy and risk of further.

Maeda N et al (1998)[22] examined the incidence of black- pigmented rods (BPRs), especially *P.intermedia* and *P.nigrescens*, in periodontal health and disease by collecting the samples from gingival sulcus or periodontal pocket. The degradative enzyme activities of *P.intermedia* were compared among the strains from periodontal health and disease. BRPs were found in 71% of healthy examined sites, 87%of the active disease sites. *P.gingivalis* was detected only in active sites of periodontally diseased patients (17.8% of 180 strains). *P.intermedia* was the predominant BPR in both healthy and active sites (37.3 and 41.7%, respectively) of the patients. However, *P. nigrescens* was the predominant BPR (70.5% of 173 strains) in periodontally healthy subjects. The enzyme activities of esterase,esterase- lipase, acid- phosphatase and α- fucosidase of *P.intermedia* strains isolated from active sites in patients were significantly higher than those of healthy subjects. Hence, concluding that *P. intermedia* might increase the activity of degradative enzymes under a certain condition and support the progression of periodontitis.

EFFECT OF SRP

Mousques T, Lisgarten MA, Philips W (1980)[1] investigated under dark field microscopy the effect of single session of SRP on the sub gingival periodontal flora of 14 adult humans. The result suggested that single session of SRP is capable of disturbing certain bacterial forms, requiring 42 days to for the proportion to return to baseline levels.

Hinrichs JE, Wolff LF, Pihlstrom B, Schaffer EM, Liljemark WF, Bandt CL (1984)[23] investigated the changes in sub gingival microflora following root planing and relates shift in microflora with changes in clinical measurements. Author

concluded Spirochetes, total motile organisms, Fusobacterium spp, and dark pigmented Bacteroides spp, decreased and coccoid forms increased.

Sbordone L, Ramaglia L, f Gulletta E, Iacono V (1990)[24] under dark field microscopy and cultural analysis assessed the sub gingival recolonization patterns of the adult periodontitis patients after a single session of SRP. Clinical indices and microbial parameters were reassessed. A significant improvement in probing depth was noted for up to 60 days after treatment, while the gingival index did not change markedly during the course of the study. The microbial composition as per cultural and dark field data of treated sites was similar to that of periodontally healthy sites 7 days after SRP, differences between them became apparent at the 21 day sampling point. The darkfield data showed that the sites consisted of cocci with few spirochetes. Cultural data demonstrated that the majority of the cocci were anaerobic, namely *Streptococcus intermedius, Veillonella párvula*, and *Peptostreptococcus micros*.The most prevalent anaerobic rods prior to and 60 days after therapy were *Fusobacterium nucleatum, Bacteroides gingivalis, and B. intermedius*. These results indicate that the results suggessted that a single session of SRP is insufficient to maintain a healthy subgingival microflora.

Haffajee AD, Cugini MA, Dibart S, Smith C, Kent Jr. RL. Socransky SS(1997)[25] examined the clinical and microbiological differences between subjects who responded well or poorly to SRP. Clinical assessment of plaque, redness, suppuration, BOP, pocket depth and attachment level were made prior to and 3 months post-SRP in 57 subjects with periodontitis. Sites that gained more than 2 mm of attachment post therapy showed a significant decrease in the counts of *P.gingivalis*, *T. denticola* and *B.forsythus*. This study indicates that SRP is most effective in subjects and sites with high levels of the sub gingival species.

Cobb C M(2002)[26] presented his perspective based on the evidence on the clinical significance of non-surgical periodontal therapy. Sub-gingival debridement and SRP are the traditional methods of controlling sub-gingival microflora. The primary objective of SRP is the removal of both calculus and contaminated cementum. Evidence from clinical trials reveals a consistency of clinical response in the treatment of chronic periodontitis by SRP using manual, sonic, or ultrasonic

instrumentation. The effectiveness of either procedure decreases with increasing probing depth, especially when probing depths exceed 5mm. Each method of instrumentation appears to yield the same degree of sub-gingival calculus removal and control of sub gingival plaque, and both provoke a similar healing response. The conflicting studies suggest that supra gingival plaque control is effective in early and moderate disease but not advanced periodontal disease.

Hung H-C, Douglass CW (2002)[27] conducted a meta-analysis to investigate the effect of SRP on periodontal probing depth and attachment loss. The results showed that periodontal probing depth and gain of attachment level do not improve significantly following root planing and scaling for patients with shallow initial periodontal probing depths. There was about a 1-mm reduction for medium initial periodontal probing depths and a 2-mm reduction for deep initial periodontal probing depths. There was about a 0.50-mm gain in attachment for medium initial periodontal probing depth measurements and slightly more than a 1-mm gain in attachment for deep initial periodontal probing depth measurements. Surgical therapy for patients with deep initial probing depths showed better results than SRP in reducing probing depths.

Konopka L, Pietrzak A, Brzezinska-Blaszczyk E (2012)[28] assessed the influence of scaling and root planing on amounts of interleukin IL-1β, IL-8 and MMP-8 in gingival crevicular fluid from patients with chronic periodontitis. 30 patients with generalized advanced chronic periodontitis and 21 periodontally healthy subjects were recruited for the control group. Periodontal parameters as well as gingival crevicular fluid humoral factor , and the amounts of IL-1b, IL-8 and MMP-8 in gingival crevicular fluid ; measured by ELISA were evaluated in both the groups at baseline and at 1 and 4 wk after scaling and root planing treatment. Result indicated that short-term nonsurgical therapy resulted in a significant improvement in periodontal indices and in a marked decrease of IL-1b, IL-8 and MMP-8 gingival crevicular fluid levels. Nevertheless, no significant correlations were found between clinical parameters and amounts of humoral factors after therapy.

STUDIES ON LOCAL DRUG DELIVERY SYSTEM-

MacAlpine RM, Magnusson I, Kiger R, Crigger M, Garrett S, Egelberg J (1985)[29] investigated to determine whether sub gingival irrigation would enhance the healing effect of supra- and subgingival debridement of ≥6mm of periodontal pockets in 64 sites with plaque control measure implemented and 1 episode of root planing done. Irrigation immediately followed by instrumentation, and was repeated every 2 weeks for 24 weeks with either chlorhexidine, tetracycline, saline or served as non-irrigated control sites. Results showed that the bleeding sites decreased, spiroehetes decreased, probing pocket depths decreased from 7.6 to 4.7 mm, probing attachment levels showed a gain of 1.2 mm. Improvement of the chlorhexidine and tetracycline irrigated sites was similar to that of the saline irrigated and non-irrigated control sites. Biweekly chlorhexidine, tetracycline or saline irrigation of deep pockets did not appear to augment the effects of non-surgical periodontal therapy.

Soskolne WA, Heasman PA, Stabholz A, Smart GA, Flashner PM, Newman HN (1997)[30] evaluated in a randomized, blinded, multi-center study split mouth design comparing SRP with, sub gingivally placed drug delivery system containing 2.5 mg CHX in 118 patients with moderate Periodontitis.SRP was performed at baseline only, while CHX mouthwash was used both at baseline and at 3 months. Clinical and safety measurements including PD, CAL, and BOP as well gingivitis, plaque, and staining indices were recorded at baseline, and at 1, 3, and 6 months. Result revealed significantly greater reduction in PD and CAL of 7-8mm pockets treated with CHX compared with SRP both at 3 and 6 months.

Greenstein G and Polson A (1998)[7] reviewed the role of local drug delivery systems in the management of periodontal diseases. Tetracycline fibers, metronidazole and minocycline gels, Chlorhexidine chips, and doxycycline polymer were assessed. with regard to their functional characteristics, effectiveness as a monotherapy, as compared to scaling and root planing, and ability to enhance conventional therapy. Finally, it was concluded that the local drug delivery systems with controlled release properties have the potential to be used as a therapeutic component in the management of periodontal diseases.

Garrette S etal (1999)[31] conducted a two multi centre studies on 411 patients with moderate to severe periodontitis evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene, scaling and root planing in the treatment of chronic periodontitis. Results proved sub gingivally delivered doxycyline hyclate to be equally effective to SRP and superior to control and oral hygiene groups. Study also suggested that subjects are not on any additional risks following doxycyline therapy compared to conventional periodontal therapy.

Shah JC, Sadhale Y, Chilukuri DM (2001)[32] reviewed that the cubic phases have been shown to deliver small molecule drugs and large proteins by oral and parenteral routes in addition to local delivery in vaginal and periodontal cavity. Biodegradability, phase behavior, ability to deliver drugs 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 extremely high viscosity may limit its use to specific applications such as periodontal, mucosal, vaginal and short acting oral and parenteral drug delivery.

Persson GR, Salvi GE, Lisa J.A, Mayfield JA, Lang NP (2006)[33] assessed the microbiological outcome by DNA- DNA checkerboard hybridization method of local administration of minocycline hydrochloride microspheres 1mg (Arestins) in cases with peri-implantitis and with a follow up period of 12 months. The impact of Arestins on *A.actinomycetemcomitans* was greater than the impact on other pathogens. Up to 180 Day reductions in levels of *Tannerella forsythia*, *P. gingivalis*, and *Treponema denticola* were also found.

Renvert S, Lessem J, Dahlen G, Renvert H, Lindahl C (2008)[34] conducted a study to assess the clinical and microbiologic outcome of local administration of minocycline microspheres CHX gel , in patients of peri-implantitis.32 patients with probing depth $\geq$ 4 mm with bleeding and /or exudation on probing .patients were randomly allocated to give either of the two. Result concluded use of minocycline significantly reduced probing depths but treatment needs to be repeated.

Jain N, Jain N, Jain GK, Javed S, Iqbal Z, Talegaonkar S, Ahmad FJ (2008)[35] stated that the GCF provides a leaching medium for the release of a drug from the

dosage form and for its distribution throughout the pocket. These features, together with the fact that the periodontal diseases are localized to the immediate environment of the pocket, make the periodontal pocket a natural site for treatment with local delivery systems.

Dodwad et al. (2012)[36] reviewed that in conjunction with SRP, the adjunctive use of local drug delivery may enhance 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.

Sharma A, Pradeep AR (2012)[37] studied the efficacy of a 1% ALN gel compared to a placebo gel as a local drug delivery system in adjunct to SRP (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, 2 and 6 months, and radiographic parameters at baseline and 6 months. Results suggested that the 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.

Balappanavar AY, Sardana V, Singh M (2013)[38] evaluated and compared the effectiveness of 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health. 30 healthy subjects were selected and randomly assigned into 3 groups i.e., group A - 0.2% chlorhexidine gluconate (bench mark control), Group B - 2% neem, and group C - 0.5% tea of 10 subjects per group. Mean plaque and gingival scores were reduced over the 3 week trial period for experimental and control groups. Anti- plaque 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.

Kudalkar MD, Nayak A, Bhat KS1, Nayak RN (2014)[39] examined the anti-inflammatory effect of *Neem* and Aloevera by way of its inhibitory effect on MMP-2 and MMP-9 activity in cases of chronic periodontitis and compared it with doxycycline. The results showed the *Neem* achieved inhibition of MMP-2 and

MMP-9 at 1500µg/ml concentration, Aloevera had an inhibitory effect on MMP-2 and MMP-9 at 2000 g/ml concentration and doxycycline had an inhibitory effect on MMP-2 and MMP-9 at 300 g/ml concentration; thus implying lower concentrations are required for doxycycline.

Christopher J. (2015)[40] Conducted a meta-analysis on 72 articles on the effectiveness of SRP with or without the systemic antimicrobials, a systemic host modulator (subantimicrobial-dose doxycycline), locally delivered antimicrobials (chlorhexidine chips, doxycycline hyclate gel, and minocycline microspheres), and a variety of nonsurgical lasers (photodynamic therapy with a diode laser, a diode laser, neodymium:yttrium-aluminum-garnet lasers, and erbium lasers). The panel judged 4 adjunctive therapies as beneficial with a moderate level of certainty: systemic subantimicrobial-dose doxycycline, systemic antimicrobials, chlorhexidine chips, and photodynamic therapy with a diode laser.

Gupta A, Govila V, Pant VA, Gupta R, Verma UP, Ahmad H, et al (2018)[41] examined the efficacy of local drug delivery system of ZLN gel as an adjunct to SRP for the treatment of human periodontal intrabony defects clinically and radiographically. In moderate to severely affected forty 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 and 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.

NEEM-

PHYTOCHEMICAL CONSTITUENTS-

Sinha S, Murthy PSN, Rao CVN, Ramprasab G, Sitarahmaiha S, Kumar DG, Savant KS (1999)[42]. examined the extraction of Azadirachtin in a study by simpler chromatographic methods by additionally adding CCl_4 partition before final extraction which removes most of the nimbin salanin and triterpenoids. Azadirachtin present in 0.3 – 0.6 % is the principle active constituent of *Azadirachta indica*.Enriched product contained high concentration of pure form of azadirachtin.

Pricila D, Alves, Brandao MGL, Nunan EA, Vianna CD (2009)[43] evaluated the chromatographic and antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and a mold fungus of Neem leaves hydroalcoholic extracts; in 96% ethanol percolation at different concentrations (50%, 60%, 70%, 80% and 90% (v/v)to detect dose dependent relationship of the activity. The extracts were tested in different increasing concentrations, in order to detect a dose-dependent relationship of the activity. Result concluded that extracts do not have AZA in a quantifiable amount, indicating its absence in the hydroalcoholic extracts tested, due to crop location, plants young age or soil condition. Despite the absence of AZA, the 70% and 80% (v/v) ethanol extracts showed activity against *Staphylococcus aureus* Although AZA could not be detected in the Neem leaves extracts, antimicrobial activity detected against *S. aureus* may be due to the presence of several substances, other than AZA, indicating that the leaves extract can be used against this bacterium, a very important pathogenic microorganism.

Susmitha S, Vidyamol KK, Ranganayak P, Vijayaragavan R (2013)[44] qualitatively analysed the extract of neem plant by TLC for the detection of alkaloids flavonoids, lipids with different anti microbial activity *in vitro* against human pathogenic *Escherichia coli* and Salmonella sp by cup diffusion method. A TLC was also performed by using different solvent system .Minimum Bactericidal Concentration value of 5mg/l was obtained against *Escherichia coli and Salmonella sp* were found to be resistant with all the solvent extracts except water..The result concluded that the separated active compounds alkaloid, flavonoids, lipid from TLC

were found to be more effective against all tested organisms in shade dried sample in fresh neem, lipids were ineffective against the tested organisms.

MEDICINAL PROPERTIES

Pillai NR, Santhakumari G (1981)[45] compared the anti inflammatory and anti arthritic effect of nimbidin with two standard anti-inflammatory agents, phenylbutazone, and prednisolone, against various experimental animal models of inflammation. It was found to significantly reduce experimentally induced acute paw edema in rats induced by phlogistic agents, carrageenin and kaolin. The test drug significantly suppressed the formalin-induced arthritis of ankle joint and the fluid exudation in croton oil-induced granuloma in rats. In acute phase of inflammation, nimbidin (40 mg/kg) was found to possess significant activity as compared to phenylbutazone (100 mg/kg).The study concluded that the drug was found to be effective in both acute and chronic phases of inflammation it can be considered as a general anti-inflammatory agent.

Pillai NR, Santhakumari G (1984)[46] investigated the effect of nimbidin in different types of acute and chronic gastroduodenal lesions and ulcers in a few experimental models .Anti-ulcer studies (preventive tests) revealed very significant protective effect of the test drug in doses of 20 to 40 mg/kg (p.o.) in acetylsalicylic acid, stress, serotonin and indomethacm induced gastric lesions in rats. The test drug also afforded remarkable protection in both types of chemically induced duodenal lesions in rodents. In ulcer healing tests, nimbidin significantly enhanced the healing process in acetic acid induced chronic gastric lesions in albino rats and dogs.

Khalid SA, Farouk A, Geary TG, Jensen JB (1986)[47] examined in vitro for antimalarial activity against *Plasmodium falciparum* on twenty-one compounds isolated from nine medicinal plants used in traditional medicine which includes alkaloids, lignans, triterpenes, coumarins, limonoids and flavonoids. Most were relatively inactive; one limonoid, gedunin, had an ICsO value roughly equivalent to quinine.

Van der nati JM, Klerx JPAM', Van H, Silvac KTDD, Labadif RP (1987)[48] examined the immunomodulatory activity *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 leukocytes was observed and a dose-dependent increase in the production of migration inhibition factor by lymphocytes.

Ray A, Banerjee BD, Sen P (1996)[49] studied the effects of *A. indica* on the tests of humoral and cell mediated immune response after 3 weeks of oral Azadirachta indica (aqueous 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 response, there is enhancement of macrophage migration inhibition and foot pad thickness after *Azadirachta indica* treatment. These results suggest possible immunopotentiating effects of *Azadirachta indica.*

Biswas K, Chattopadhyay I, Banerjee RK, Bandhopadhyay U (2005)[50] reviewed that neem is the most useful traditional plant with medicinal properties. Nimbidin, bitter crude extract has anti- inflammatory, anti pyretic, hypoglycemic, 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, hypoglycemic, anti ulcer, anti fertility, anti malarial, anti carcinogenic, antioxidant, antiviral effects.

Paul R, Prasad M, Sah NK (2011)[51] 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, protective, tumor-suppressive, immunomodulatory and apoptotic effects against various types of cancer and their molecular mechanisms.

Verma UP, Dixit J (2012)[16] examined the influence of CHX and NE on Cultured hGF through morphological and biochemical assays. Fibroblasts were derived from healthy gingival biopsy specimens harvested aseptically. The effects of CHX and NE were evaluated on cultured hGF. Morphological studies with hGF exposed for 1 min to CHX and NE each, individually at conc. ranging from 1%-100% indicate altered

morphology beyond 1% CHX. However, NE shows similar results at 75% concentrations. SRB assays are the benchmarks for ascertaining the cytostatic/proliferative/toxic effect of any drug or ligand such as CHX and NE. CHX shows dose-dependent inhibition while NE gradually increases inhibition at 10% followed by the attainment of steady state at subsequent concentrations. The cytoprotective, oral friendly quality of NE emphasize the superiority of NE over CHX.

Hossain MA, Al-Toubi WAS, Weli AM, Al-Riyami QA, Al-Sabahi JN (2013)[52] inestigated *in vitro* antioxidant activity and characterize the chemical con-stituents 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 the order of chloroform > butanol > ethyl acetate extract > hexane extract > methanol extract.

Verma UP, Gupta A, Yadav RK, Tiwari R, Sharma R , Balapure AK (2018)[17] 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.

EFFICACY IN PERIODONTAL DISEASES

Wolinsky LE, Mania S, Nachnani S, Ling S (1996)[53] examined the inhibitory effects of aqueous extracts derived from the bark-containing sticks (Neem stick) of *Azadirachta indica* upon bacterial aggregation, growth, adhesion to hydroxyapatite, and production of insoluble glucan, which may affect in vitro plaque formation. Neem stick extracts screened for MIC against a streptococci by means of a broth dilution assay. Pre-treatment of saliva-conditioned hydroxyapatite with the neem stick or gallotannin-rich extract prior to exposure to bacteria yielded significant reductions in bacterial adhesion. Incubation of oral streptococci with the Neem stick

extract resulted in a microscopically observable bacterial aggregation. The result suggested that Neem stick extract can reduce the ability of some streptococci to colonize tooth surfaces.

Saha S, Jagannath G, Kumari M, Mohamed S, Singh P (2002)[54] to evaluate the clinical efficacy of the Neem mouthwash on common microbial flora of mouth. Ninety subjects were randomly assigned in to three groups. (30-Neem, 30-Chlorhexidine and 30-Distilled water). Interventions consisted of a 15 days therapy of the 25% Neem mouthwash, 0.12% chlorhexidine gluconate (positive control) and distilled water (negative control) respectively. 4 strains were used i.e. *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus* and *Streptococcus sanguis.* Microbial analysis indicates that 25% Neem mouth wash has significant role in reducing the common microbial flora.

Pai MR, Acharaya LD (2004)[55] conducted a study to evaluate the effectiveness of neem leaf extract against plaque formation in males over 6 weeks and salivary bacterial count was checked for *Streptococcus mutans* and lactobacilli species. A mucoadhesive dental gel containing 25mg/g of leaf extract was formulated and efficacy was checked for 6 weeks with commercially available chlorhexidine gluconate (0.2% w/v). The result suggested that neem extract gel reduced plaque index and bacterial count than control group.

Botelho MA, Santos RAD, Martins JG, Caravhlo CO, Paz MC, Ruela RS et al (2008)[56] studied the comparative short-term efficacy and safety of a *Azadirachta indica* mouthrinse on gingival inflammation and microbial plaque, compared to 0.12% CHX. Plaque index, gingival index and gingival bleeding index obtained at baseline, one and four weeks. Additionally, the count of cariogenic bacteria (*Streptococcus mutans*) in the saliva was assessed before and after treatment. All clinical index scores were reduced in both groups seven and 30 days after treatment. *A. indica*-based mouth rinse is highly efficacious and that it may be used as an alternative therapy in the treatment of periodontal.

Jain S, Kaur H, Brar S (2012)[57] 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, 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.

Abhishek KN (2015)[58] studied the effect of neem toothpaste on plaque and gingivitis. 30 students divided into 2 group were included in the study. A washout phase of 2 ½ days were carried out for both the groups. Following prophylaxis subjects were randomly allocated into 2 groups. Statistically significant difference was observed with the use of neem toothpaste in terms of plaque index and gingival index.

Dhingra K, Vandana KL (2016)[59] studied the systematic review was to evaluate the effectiveness of Azadirachta indica (neem)-based herbal mouthrinse in improving plaque control and gingival health. These studies reported that Neem mouthrinse was as effective as CHX mouthrinse when used as an adjunct to toothbrushing in reducing plaque and gingival inflammation in gingivitis patients

Vennila K, Elanchezhiyan S, Ilavarasu S (2016)[60] examined the 10% neem oil chip as a local drug delivery system to evaluate the efficacy in the periodontal disease management. After scaling and root planning,10% nonabsorbable neem chip was placed in the pocket in one side of the arch with other side treated with SRP only. Clinical parameters checked on the baseline,7th day, and 21st day and plaque samples for *P.gingivalis* strains on the baseline and 21st day. Clinical parameters showed statistically improved on the neem chip sites and presence of *P. gingivalis* strains were significantly reduced on the neem chip sites seen using PCR.

TETRACYCLINE AS A LOCAL DRUG DELIVERY AGENT

Goodson JM, Haffajee A, and Socransky SS (1979)[61] used local drug delivery for the first time for treatment of chronic periodontitis using tetracycline filled hollow fibers which were placed in the gingival sulcus showed effect both on the periodontal mifcroflora and clinical manifestations of disease. They also concluded that virtual elimination of spirochetes from the gingival sulcus was possible by a single placement and spirochetes, ones eliminated from a site, do not rapidly recolonize.

Lindhe J, Heijl, Goodson JM, Socransky SS (1979)[62] assessed the microbial effect on periodontal pathogens of locally administered hollow tetracycline fibre devices, in 5 patients with each having 4 pair of contralateral teeth involved with advanced periodontal disease (PPD $\geq$ 6mm). Clinical parameters i.e PI score, GI score, PPD were measured on 7, 14, 28 and 37 day. For microbial assessment samples from deep sub gingival sites were taken and analysed in dark field microscope. The experiment showed that the use of tetracycline fibre at diseased sites markedly alter the microbial composition initially simultaneously improving clinical symptoms and signs of periodontitis.

Gomes BC, Golub LM, Ramamurthy NS (1984)[63] on their study on tetracycline found that level of tetracycline approximating physiologic concentrations, were found to inhibit parathyroid hormone-induced bone resorption in organ culture; the specificity of this effect was demonstrated by comparison with other types of antibiotics. These antibiotics can inhibit mammalian collagenolytic enzymes by a mechanism unrelated to the drug's antibacterial efficacy.

Golub LM, Goodson JM, Lee HM, Vidal AM, McNamara TF, Ramamurthy NS (1985)[64] in a series of experiment demonstrated that tetracycline can inhibit mammalian collagenases and proposed that this property could be useful in treating periodontal disease characterized by excessive collagen degradation. One effect was the dramatic reduction of tissue collagenase activity within the gingival crevicular fluid of periodontal pockets after administering a standard regimen of a tetracycline.

Slots J and Rams TE (1990)[2] in his article discussed about the disadvantages of tetracycline. These were discoloration and hyperplasia of teeth and depressed skeletal growth, Photosensitivity, Antibiotic resistance, superinfection and systemic complictions due to Decreased absorption of tetracycline due to chelation with antacids, alumunium, and bismuth. This further proved the importance of local drug delivery in periodontal disease.

Drury G and Yukna RA (1991)[65] did study on Histologic Evaluation of combining Tetracycline and Allogeneic Freeze Dried Bone on Bone Regeneration in Experimental Defects in Baboons. They concluded that Tetracycline in combination

with Freeze Dried Bone Allograft enhances new bone formation in experimental alveolar bone defects in comparison to use of Freeze Dried Bone Allografts alone.

Goodson JM etal (1991)[66] examined the clinical response of tetracycline fiber therapy selecting 4 sites in each patient with 6-10 mm of pocket and BOP present and divided patients into 4 groups- TC fiber therapy, scaling, control fibers(fibers without drug), and untreated sites. Result showed that TC fiber therapy significanlty decresed PPD, increased CAL, decresed BOP to greater extent than observed in all other test groups including scaling.

Kazakos GM, Cabb CM, Manisan SL, Barker BF, Killay WJ (1993)[67] studied and characterized the soft tissue wall of periodontal pockets after a 10-day in vivo exposure to monolithic tetracycline-impregnated fibers, with and without root planing. Ten days after initial therapy, all teeth were extracted with associated soft tissue pocket walls intact. Results indicated that use of tetracycline-impregnated fibers did not adversely affect the epithelial lining and no significant effect on the density or character of the inflammatory response present in adjacent soft tissue and confirmed the antimicrobial effects of the fibers.

Newman MG, Kornman KS, Doherty FM (1994)[68] evaluated the clinical parameters i.e PPD,BOP, CAL at baseline , 1 ,3,6 months using TTC fiber therapy as an adjunct to SRP in maintainence patients with 5-8 mm of pockets and h/o BOP. Result showed PPD and BOP started to decrease at 3 months and continued till 6 months whereas CAL showed significant gain at 6 months.

Wilson TG, McGuire MK, Greenstein G, Nunn M (1997)[69] presented 5 years data pertaining to a sub group of patients from previous study who were treated with scaling and root planing plus tetracycline fibers. This study assessed GR, BOP, PPD at baseline , 1, 3 , 6 months. There was no significant difference between the treatment at 5 years.

Seymour RA and Heasman PA (1995)[6] in their systemic review on use of Tetracycline fibers in chronic periodontitis patient stated about the effects of tetracycline apart from their anti-bacterial effect. These include collagenase inhibition, anti-inflammatory actions, inhibition of bone resorbtion, and their ability

to attach fibroblasts to root surfaces. Consequently tetracycline has also been used for root conditioning purpose.

Haffajee AD, Socransky SS, Dibart S, Kent Jr. RL (1996)[70] compared the effect of systemically administered tetracycline and augmentin in periodontitis patient and co-related its effect by high or low levels of P. gingivalis, P. intermedia, P nigrescens and B. forsythus. The greatest proportion of gaining sites was seen at pockets >6 mm. particularly in subjects receiving adjunctive tetracycline. The 4 test species were decreased more in subjects receiving tetracycline.

Radvar M, Pourtaghi N, Kinane DF (1996)[71] conducted a study on 54 patients with pockets >5mm and bleeding on probing present randomised into 4 groups i.e SRP + 25% tetracycline, SRP+ 2% Minocycline, SRP+ metronidazole, SRP and measured PPD, CAL,BOP, MGI score on baseline and at the end of the treatment i.e 6 weeks. Result showed that TTC +SRP gave the greatest advantage in the treatment of the persistent periodontal pockets at least during 6 weeks.

Moses O, Nemcovsky CE, Tal H, Zohar R (2001)[72] reported that Collagen membranes are degraded by matrix metalloproteinases. Their degradation rate can be altered either by enhancing structural integrity or by delaying the degradation process using MMP inhibitor and TC presents inhibitory effects on matrix MMP. So by immersing membranes in tetracycline solution before implantation can delay the degradation.

Gurha S,Chandarashekhar KT, Mishra R, Tripathi VD (2016)[73] compared the efficacy of LDD of tetracycline hydrochloride fibers (Periocol- TC) on the levels of *P.gingivalis* used as an adjunct to scaling and root planing in the treatment of chronic generalized periodontitis.100 periodontitis sites were randomly assigned to experimental or control group (50 sites in each group). Experimental sites (Group A) were treated with SRP and Periocol-TC. Controls (Group B) were treated with SRP alone. Plaque samples and clinical parameters were recorded on baseline, 15th and 45th day for quantitative and qualitative analysis of P g. In experimental group reduction in PI, GI, PPD and colonies of *P.gingivalis* and gain terms of RAL, were statistically more as compared to control group. Hence, concluding Periocol – TC as a valuable adjunct to SRP in the Treatment Of Chronic Periodontitis.

OBSERVATIONS AND RESULTS

Results and Observations

The present study deals with evaluation of anti- microbial therapy as an adjunctive to the conventional therapy in the management of periodontal disease. Instead of systemic antibiotics, herbal products could be used as antimicrobial agents. Herbal local drug delivery systems are effective alternative for systemic therapy in managing the chronic periodontal disease. The above clinical and microbiological parameters were recorded on the baseline, 7th day, 14th day, 21st day. The clinical parameters were assessed at the baseline at selected sites. The local drug delivery system containing Neem fibers were placed. Subjects were recalled after 7 days; coe-pack was removed Oral hygiene maintenance instructions were given. Clinical parameters were repeated on the 14th day and 21st day.

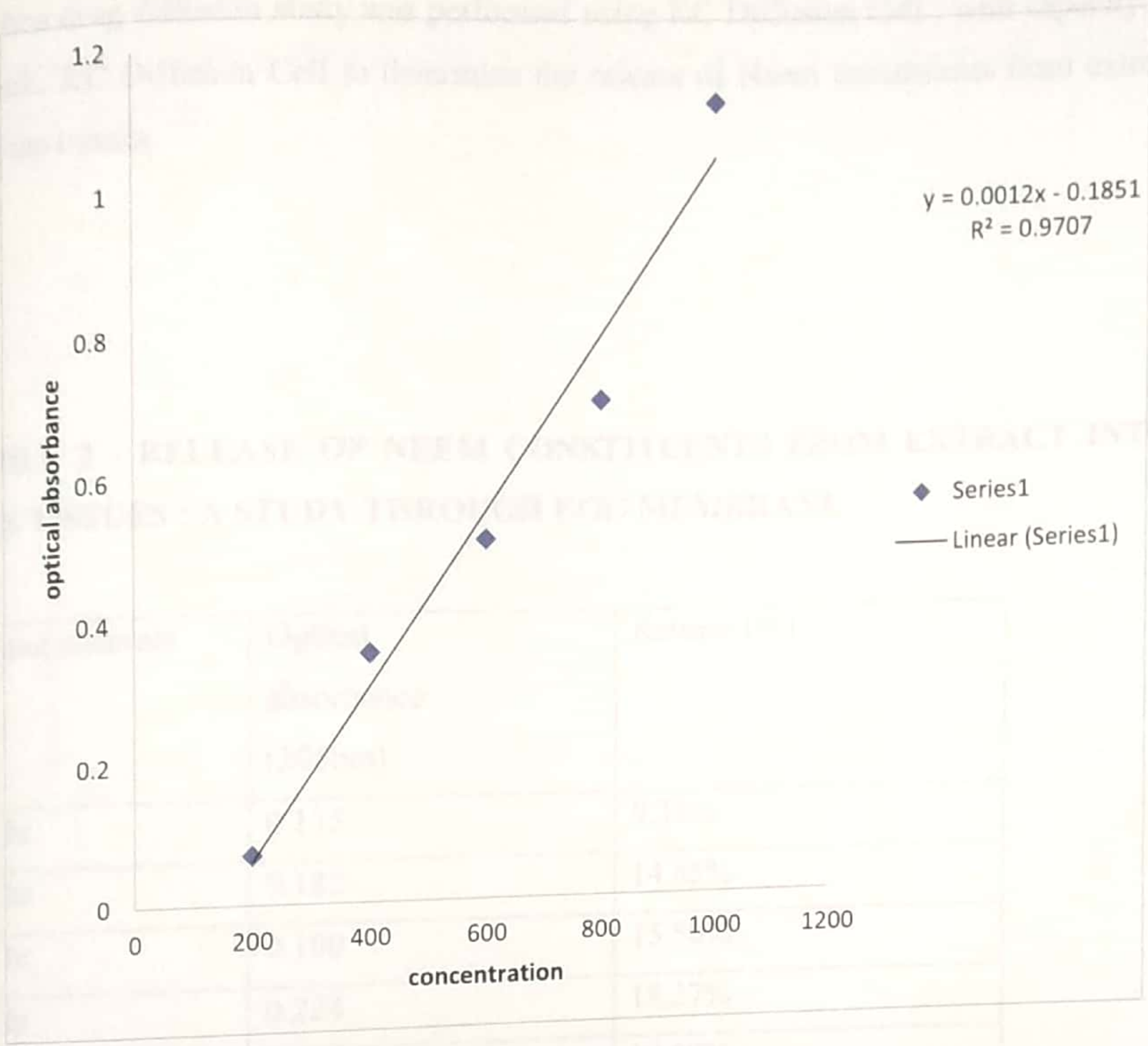
IN VITRO STUDY

Time release pattern of *Azadirachta indica*

UV spectrum of the isolated Neem extract was obtained using UV visible spectrophotometer ranging in wavelength from 200- 400 nm. A distinct peak was obtained at 305 nm which was selected as λ max. .(TABLE 1, GRAPH 1)

TABLE 1 : Determination of concentration of isolated Neem extract

CONCENTRATION (mg)	OPTICAL ABSORBANCE
200	0.70
400	0.355
600	0.514
800	0.710
1000	1.126



GRAPH 1- STANDARD GRAPH TO DETERMINE CONCENTRATION OF ISOLATED NEEM EXTRACT

DRUG DIFFUSION STUDY

In vitro drug diffusion study was performed using KC Diffusion Cell ; with capacity of 50 mL. KC Diffusion Cell to determine the release of Neem constituents from extract into the tissues.

TABLE 2 - RELEASE OF NEEM CONSTITUENTS FROM EXTRACT INTO THE TISSUES : A STUDY THROUGH EGG MEMBRANE

Time intervals	Optical absorbance (305nm)	Release (%)
1 hr	0.115	9.38%
2 hr	0.182	14.85%
3 hr	0.190	15.50%
4 hr	0.224	18.27%
5 hr	0.232	18.92%
6 hr	0.235	19.16%
7 hr	0.236	19.24%

Release after 72 hrs was 38.33% which remained constant on 5^{th} day and ^{7th} day. (GRAPH 2)

GRAPH 2 – RELEASE OF NEEM CONSTITUENTS FROM EXTRACT INTO THE TISSUES.

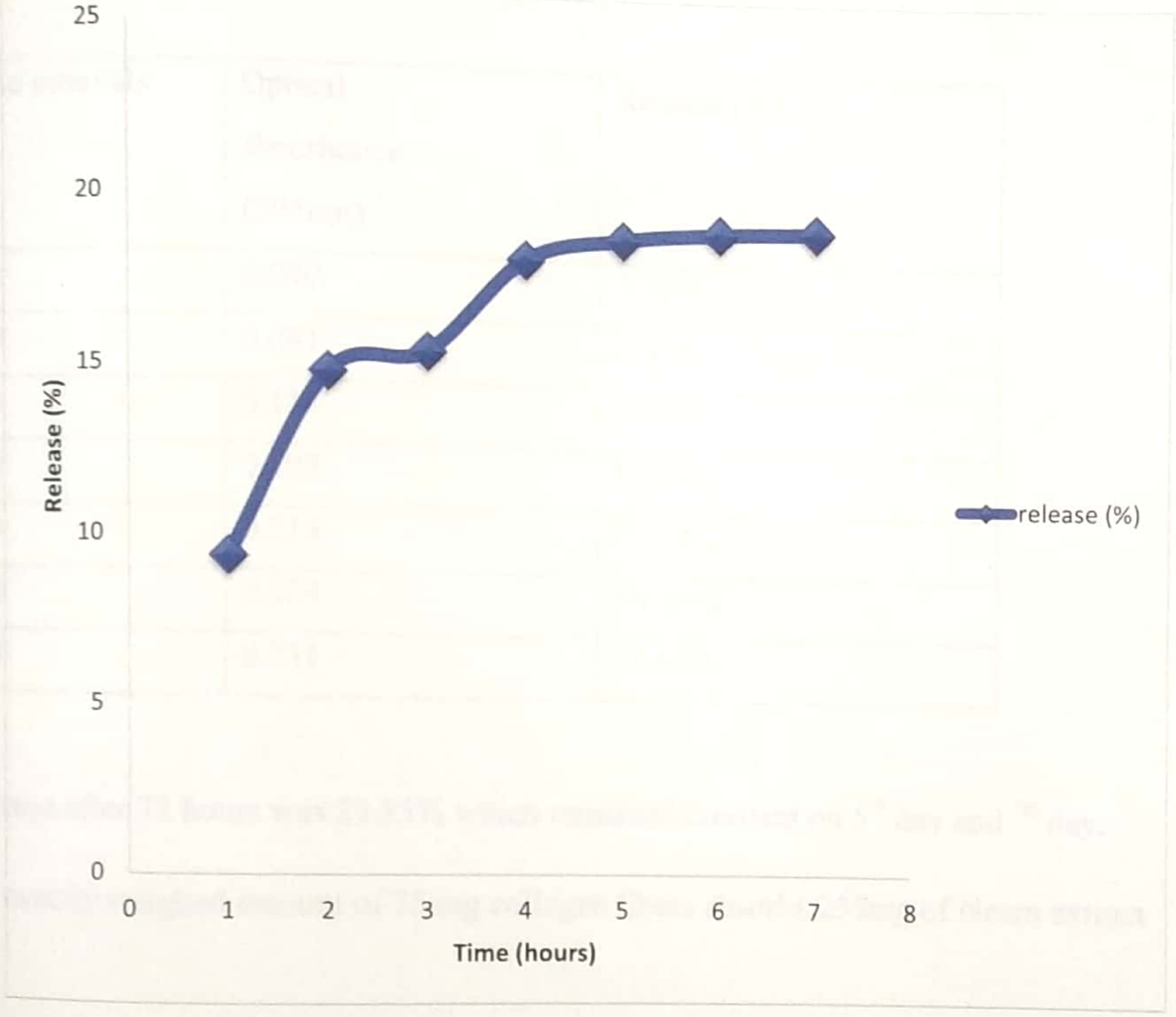
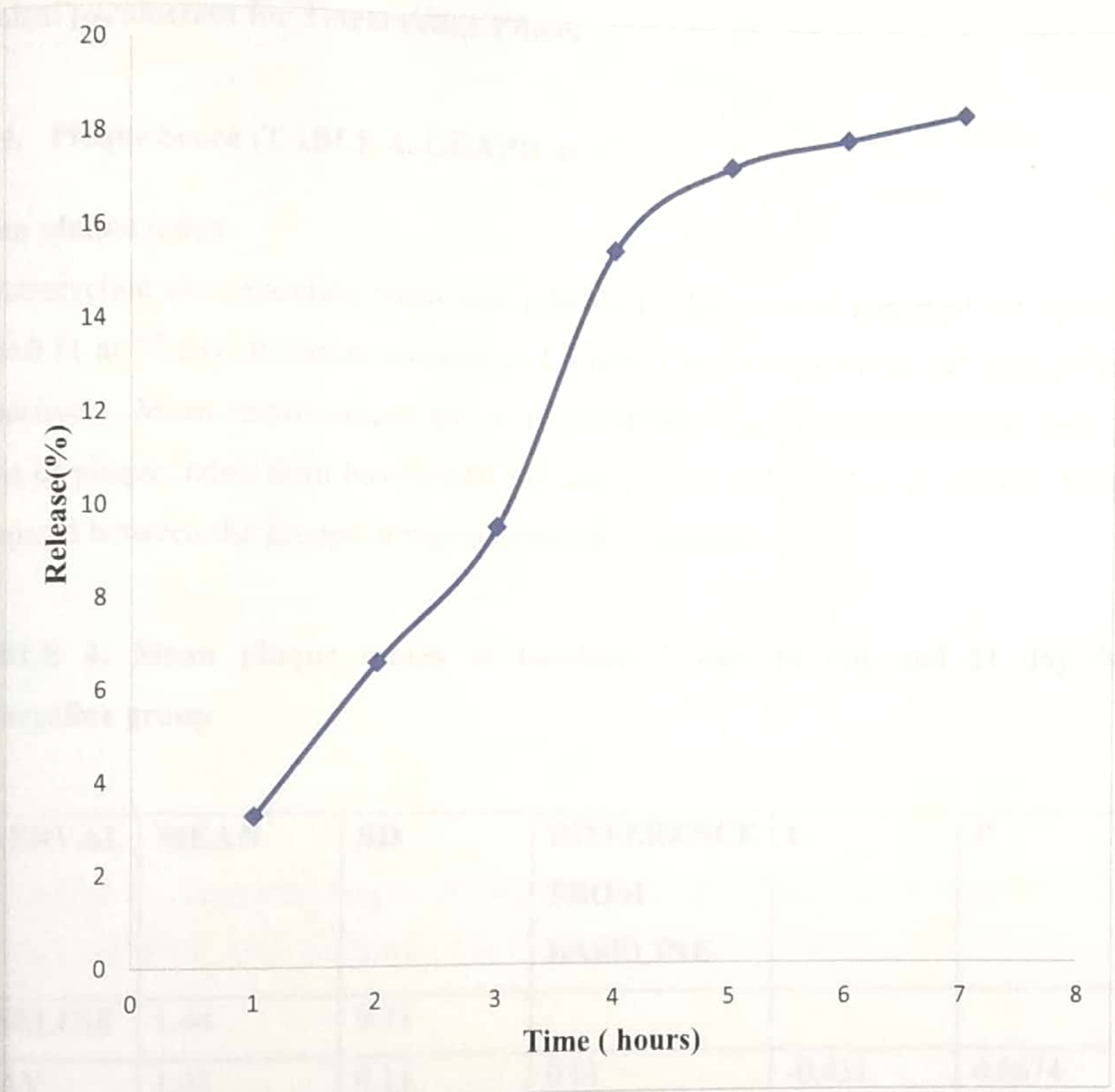


TABLE 3 – RELEASE OF NEEM CONSTITUENTS FROM COLLAGEN THREAD : A STUDY THROUGH EGG MEMBRANE

Time intervals	Optical absorbance (305nm)	Release (%)
1 hr	0.040	3.26%
2 hr	0.081	6.61%
3 hr	0.118	9.62%
4 hr	0.193	15.74%
5 hr	0.216	17.62%
6 hr	0.224	18.27%
7 hr	0.231	18.84%

Release after 72 hours was 23.85% which remained constant on 5^{th} day and ^{7th} day.

An exactly weighed amount of 25 mg collagen fibers absorbs 255mg of Neem extract



GRAPH 3 - RELEASE OF NEEM CONSTITUENTS FROM COLLAGEN THREAD : A STUDY THROUGH EGG MEMBRANE

Clinical evaluation

No adverse reaction was observed in any subject, and no patient reported any discomfort. Healing was uneventful. All subjects tolerated the drug very well and without any postoperative complications.

Clinical parameters for Tetracycline Fibers

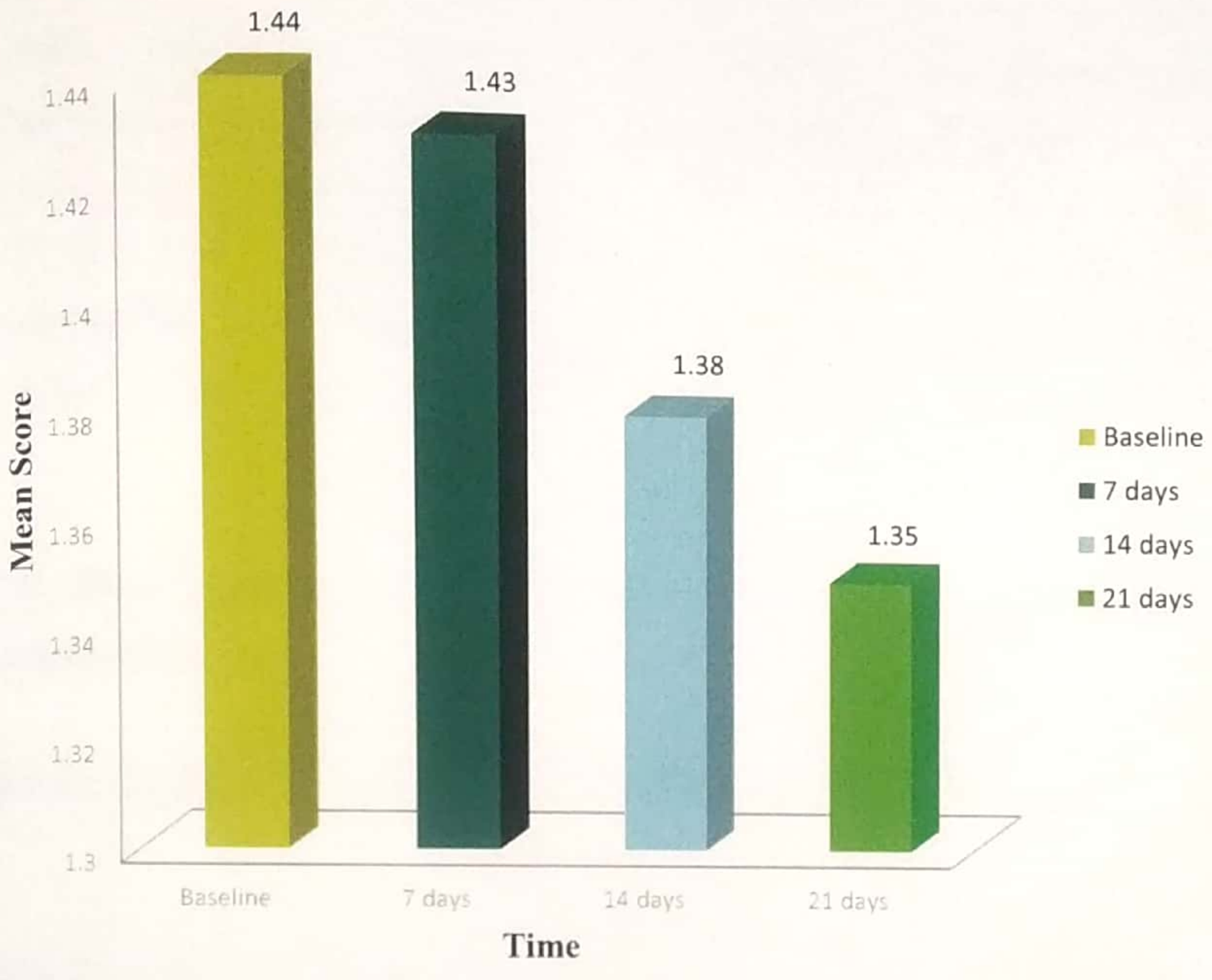
1. **Plaque Score (TABLE 4, GRAPH 4)**

Mean plaque index

At tetracycline sites, baseline mean was 1.44±0.11 which almost remained the same to 1.43±0.11 at 7th day. It further reduced to 1.38±0.11 and 1.35±0.11 on 14th and 21thday respectively. Mean improvement which is statistically significant (0.0113) is seen in terms of plaque index from baseline to 14th day (0.013) and 21st day i.e. 0.0002. When compared between the groups, it was statistically significant.

TABLE 4: Mean plaque scores at baseline, 7 day, 14 day and 21 day for tetracycline group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	P
BASELINE	1.44	0.11			
7 DAY	1.43	0.11	0.01	-0.431	0.6674
14 DAY	1.38	0.11	0.06	-2.587	0.0113
21 DAY	1.35	0.11	0.09	- 3.881	0.0002



GRAPH 4 - DISTRIBUTION OF MEAN PLAQUE SCORES AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR TETRACYCLINE GROUP.

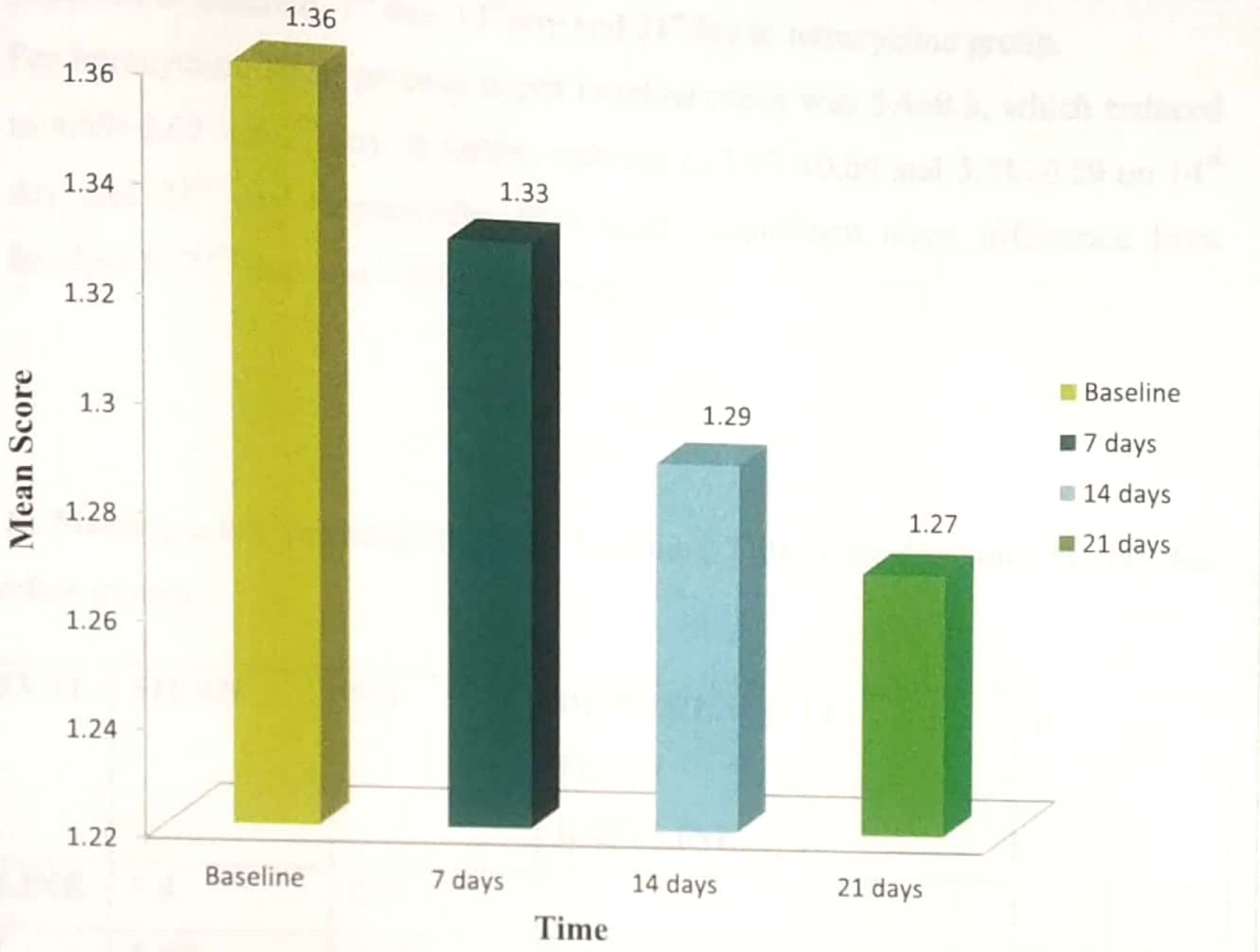
2. Gingivitis score (TABLE 5, GRAPH 5)

Table 5 and Graph 5 - represents the mean gingivitis score values and changes observed at Baseline, 7^{th} day, 14^{th} day, 21^{st} day in tetracycline group.

At tetracycline sites, baseline mean was 1.36±0.1 which reduced to 1.33±0.1 at 7^{th} day. Later it reduced to 1.29±0.1 and 1.27±0.1 on 14^{th} and 21^{th} day respectively. Mean improvement which is statistically significant(<0.0001) is seen in terms of gingivitis score from baseline to 21^{st} day i.e 0.009.

Table 5: Mean gingivitis score at baseline , 7 days 14 day and 21 day for tetracycline group.

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	P
BASELINE	1.36	0.1			
7 DAY	1.33	0.1	0.03	-1.423	0.1583
14 DAY	1.29	0.1	0.07	-3.332	0.0013
21 DAY	1.27	0.09	0.09	-4.488	<0.0001



GRAPH 5 - DISTRIBUTION OF MEAN GINGIVITIS SCORES AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR TETRACYCLINE GROUP.

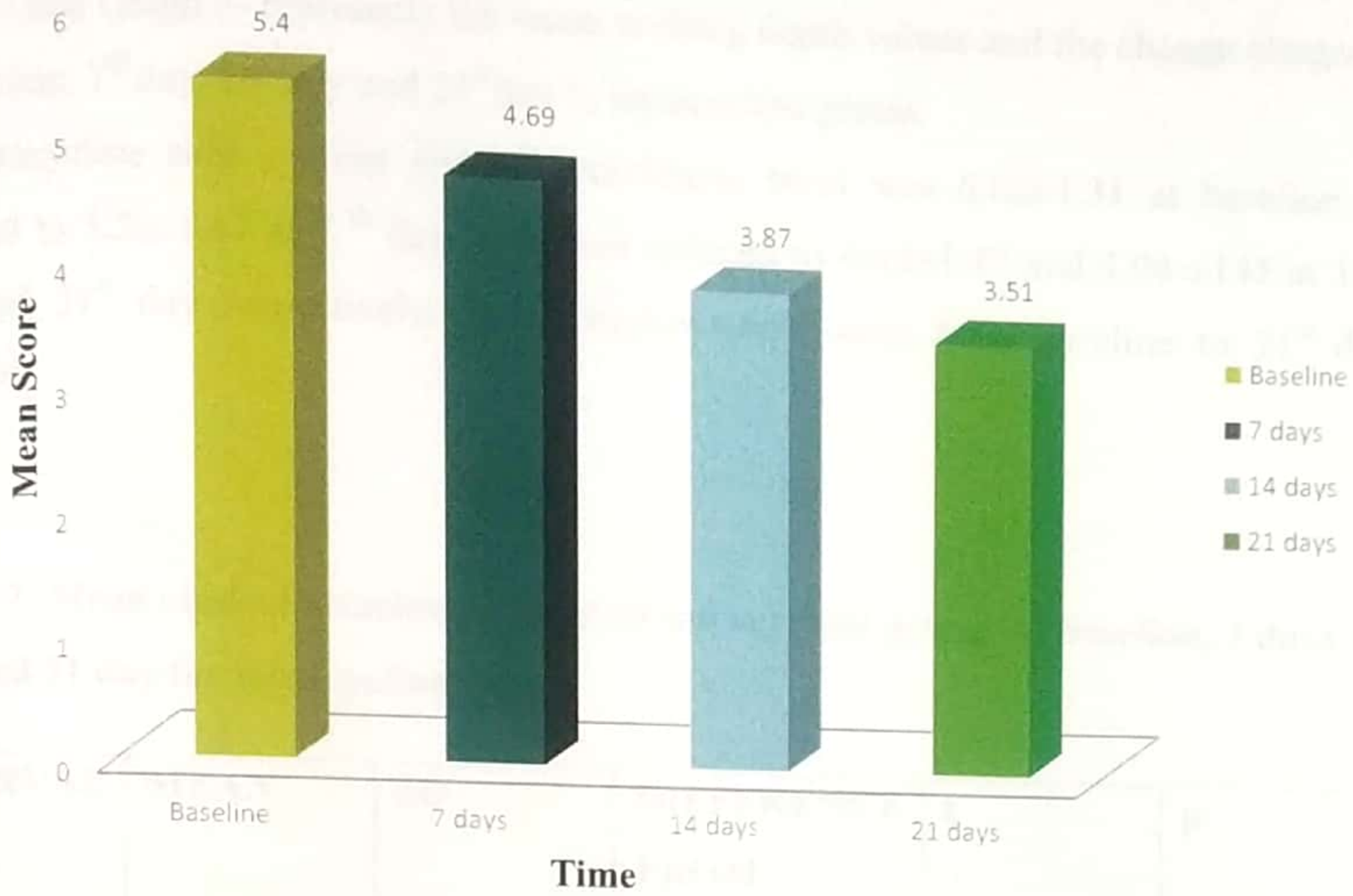
3. Pocket Probing Depth

Table 6 and Graph 6 - represents the mean probing depth values and the change observed at baseline, 7th day, 14th day and 21st day in tetracycline group.

For tetracycline sites, probing depth baseline mean was 5.4±0.5, which reduced to 4.69±0.67 on 7th day. It further reduced to 3.87 ±0.69 and 3.51±0.59 on 14th day and 21st day respectively. Statistically significant mean difference from baseline to 21st day was 1.89 is observed.

Table 6: Mean pocket probing depth at baseline, 7 days 14 day and 21 day for tetracycline group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	p
BASELINE	5.4	0.5			
7 DAY	4.69	0.67	0.71	-5.697	<0.0001
14 DAY	3.87	0.69	1.53	-12.045	<0.0001
21 DAY	3.51	0.59	1.89	-16.394	<0.0001



GRAPH 6 - DISTRIBUTION OF MEAN POCKET PROBING DEPTH AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR TETRACYCLINE GROUP

4. Clinical Attachment Level

Table 7 and Graph 7- represents the mean probing depth values and the change observed at baseline, 7th day, 14thday and 21stday in tetracycline group.

At tetracycline sites , mean clinical attachment level was 6.02±1.31 at baseline. It reduced to 5.29±1.47 at 7 th day. It further reduced to 4.44±1.47 and 4.04 ±145 at 14th day and 21st day respectively. Mean improvement seen from baseline to 21st day was1.98.

Table 7: Mean clinical attachment level of tetracycline group at baseline, 7 days 14 day and 21 day for tetracycline group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	p
BASELINE	6.02	1.31			
7 DAY	5.29	1.47	0.73	-2.487	0.0148
14 DAY	4.44	1.47	1.58	-5.383	<0.0001
21 DAY	4.04	1.45	1.98	-6.797	<0.0001

Clinical parameters for Neem Fibers

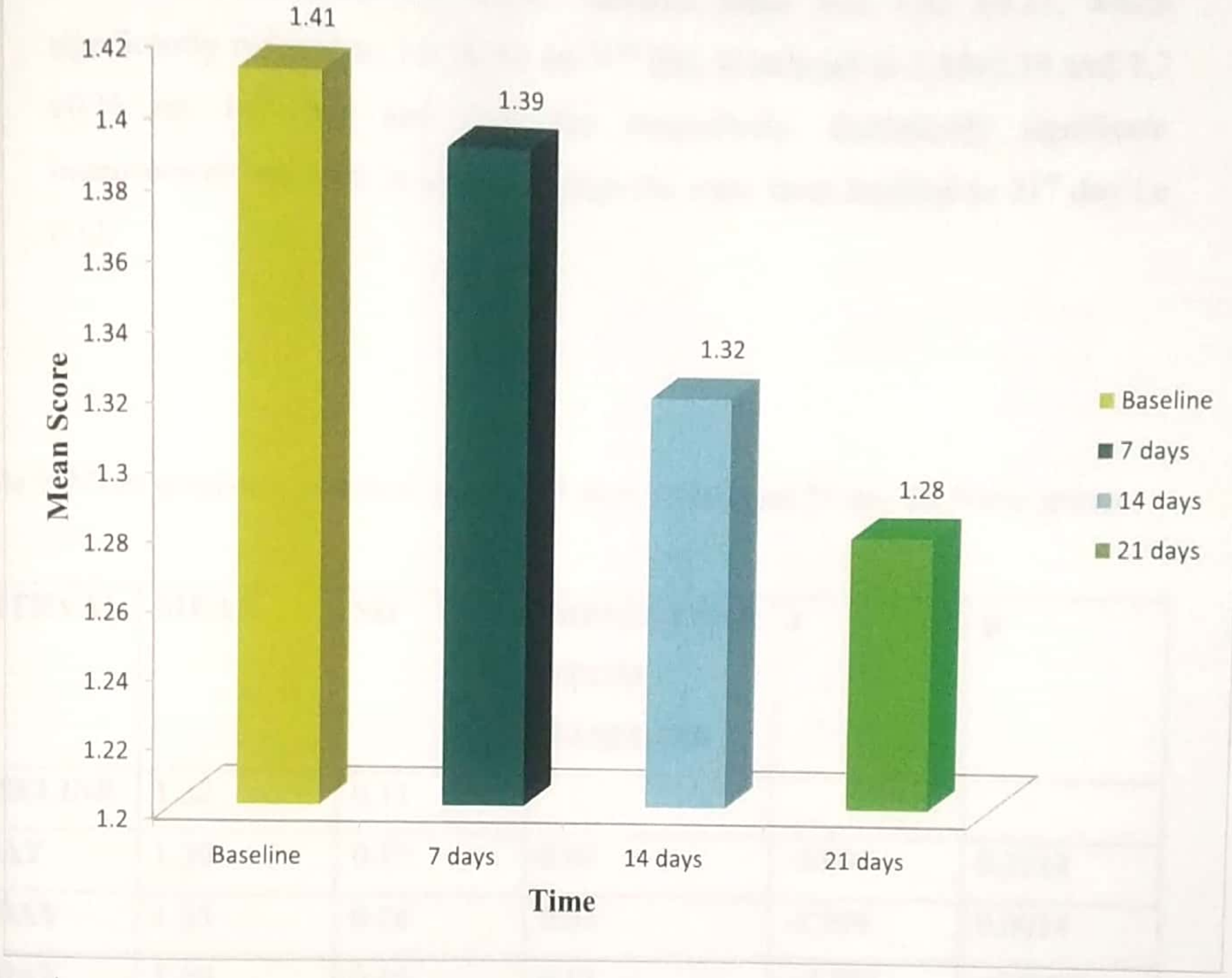
1. **Plaque index score**

Table 8 and Graph 8 - represents the mean plaque score values and changes observed at Baseline, 7^{th} day, 14^{th} day, 21^{st} day in Neem group.

At Neem sites, the mean plaque score at baseline was 1.41±0.12, which reduced to 1.39± 0.12 on 7^{th} day. It further reduced to 1.32 ± 0.12 on 14^{th} day and 1.28± 0.12 on 21^{st} day respectively . Mean improvement of statistical significance is seen from baseline to 21^{st} day i.e 0.13.

Table 8: Mean plaque index scores at baseline, 7 days 14 day and 21 day for Neem group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	p
BASELINE	1.41	0.12			
7 DAY	1.39	0.12	0.02	-0.898	0.3713
14 DAY	1.32	0.12	0.09	-4.039	0.0001
21 DAY	1.28	0.12	0.13	- 5.834	<0.0001



GRAPH 8 - DISTRIBUTION OF MEAN PLAQUE SCORE AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR NEEM GROUP

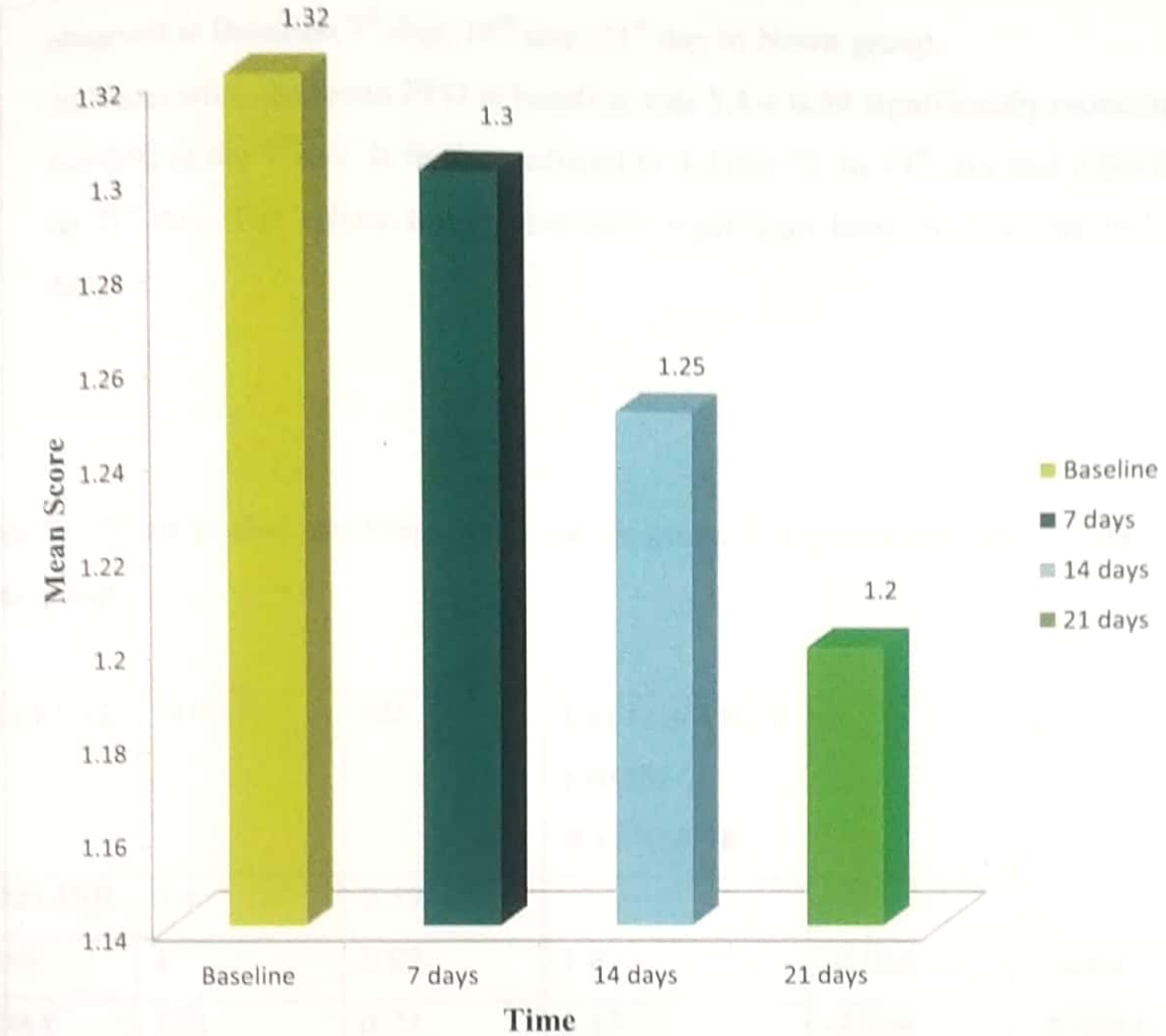
2. Gingivitis Score

Table 9 and Graph 9 - represents the mean plaque score values and changes observed at Baseline,7th day, 14th day, 21st day in Neem group.

At Neem sites, Gingivitis score baseline mean was 1.32 ±0.11, which significantly reduced to 1.3 ±0.12 on 7 th day. It reduced to 1.25±0.14 and 1.2 ±0.16 on 14th day and 21st day respectively. Statistically significant improvement was seen in terms of gingivitis score from baseline to 21st day i.e 0.12.

Table 9: Mean gingivitis scores at baseline, 7 days 14 day and 21 day for Neem group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	p
BASELINE	1.32	0.11			
7 DAY	1.30	0.12	0.02	-0.936	0.3514
14 DAY	1.25	0.14	0.07	-2.994	0.0034
21 DAY	1.20	0.16	0.12	- 4.707	<0.0001



GRAPH 9 - DISTRIBUTION OF MEAN GINGIVITIS SCORE AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR NEEM GROUP

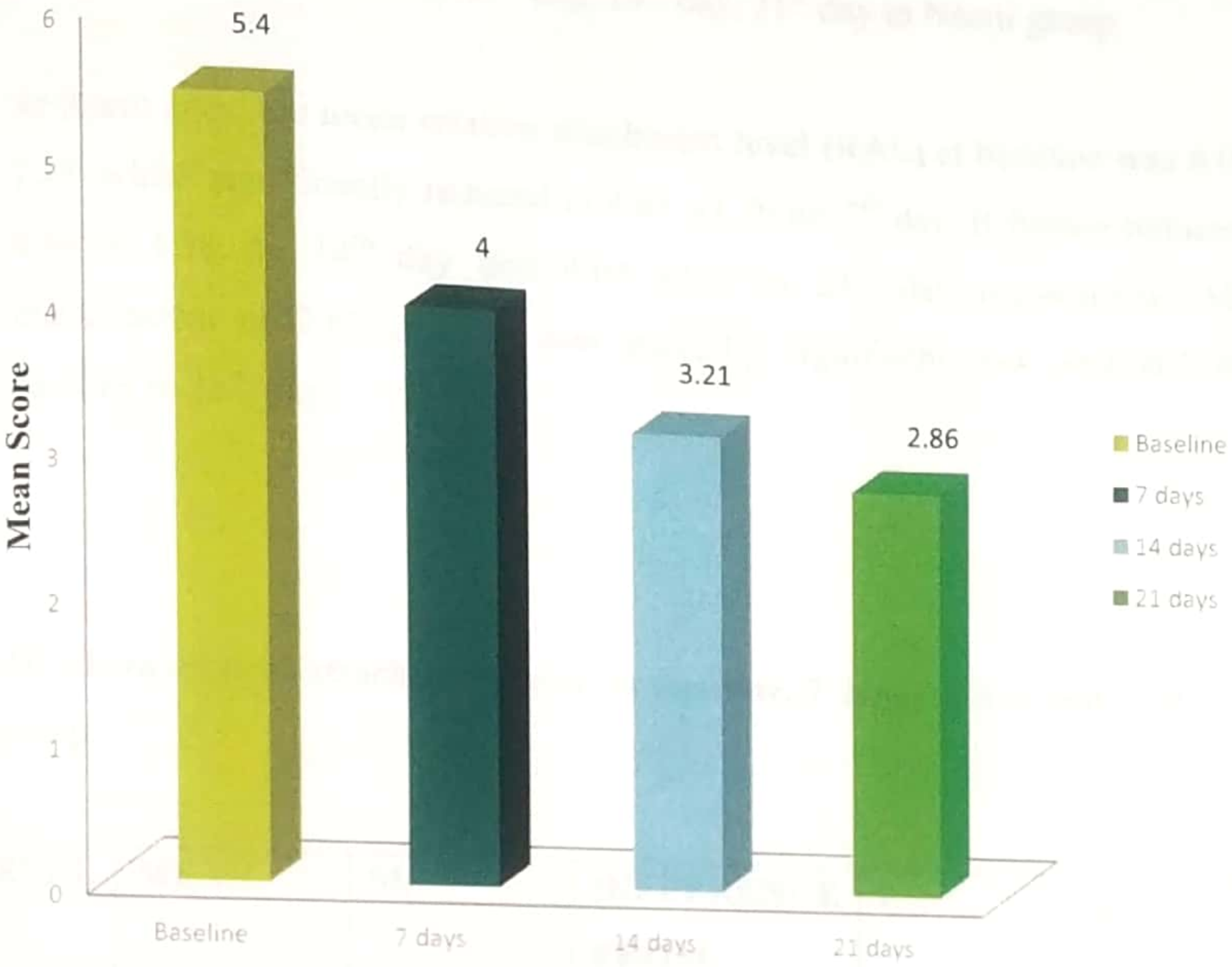
3. Pocket Probing Depth

Table 10 and Graph 10 - represents the mean plaque score values and changes observed at Baseline, 7th day, 14th day, 21st day in Neem group.

At Neem sites, the mean PPD at baseline was 5.4 ± 0.59 significantly reducing to 4 ± 0.92 at the 7thday. It further reduced to 3.21±0.72 on 14th day and 2.86±0.63 on 21st day. The values were statistically significant between baseline and 21st day.

Table 10: Mean pocket probing depth at baseline, 7 days 14 day and 21 day for neem group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	p
BASELINE	5.4	0.59			
7 DAY	4	0.92	1.4	-0.936	0.0001
14 DAY	3.21	0.72	2.19	-2.994	<0.0001
21 DAY	2.86	0.63	2.54	- 22.411	<0.0001



GRAPH 10 - DISTRIBUTION OF MEAN POCKET PROBING DEPTH AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR NEEM GROUP

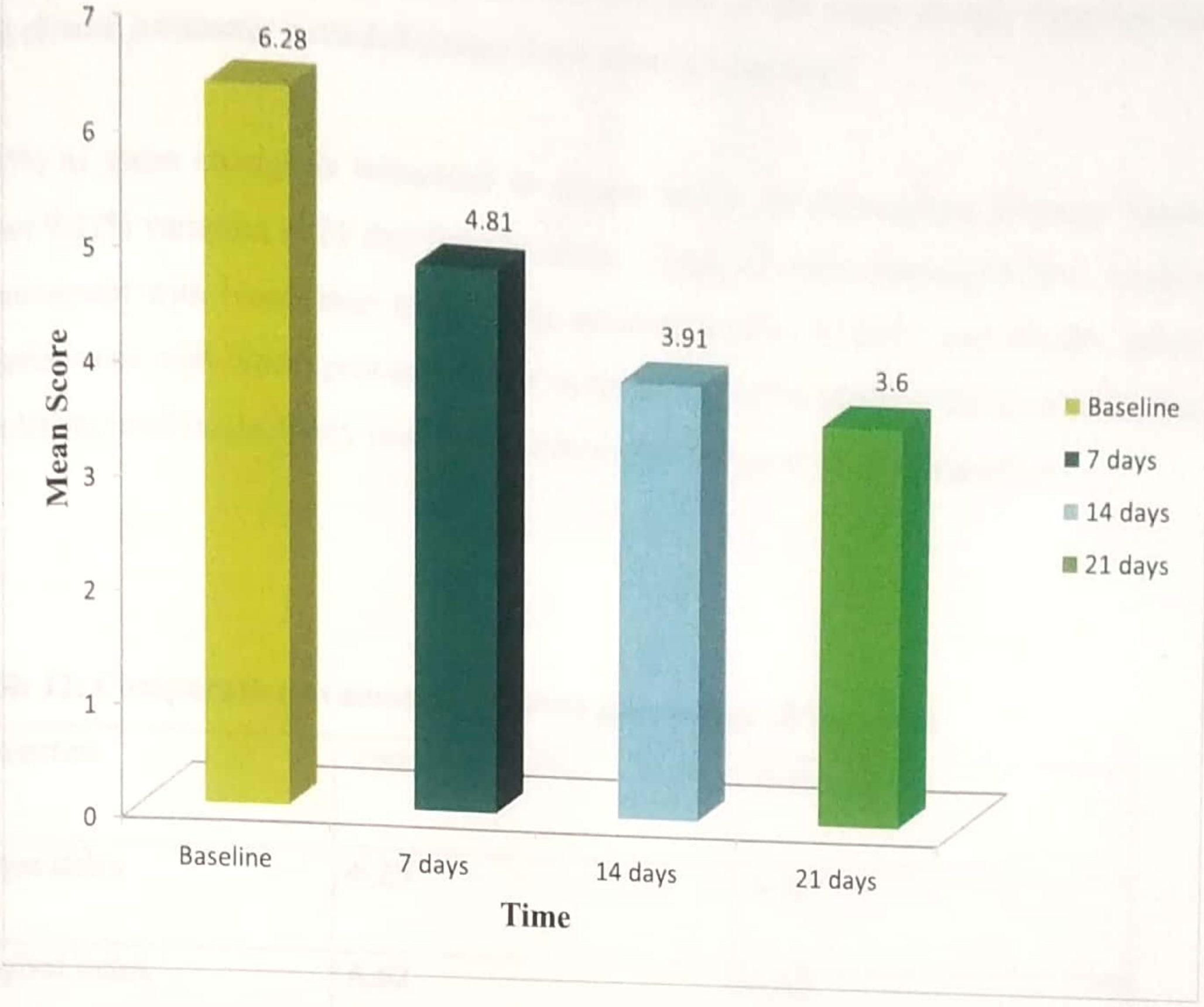
4. Clinical Attachment Level

Table 11 and Graph 11 - represents the mean clinical attachment level values and changes observed at baseline,7th day, 14th day, 21st day in Neem group.

At Neem sites, the mean relative attachment level (RAL) at baseline was 6.02 ± 1.37 which significantly reduced to 4.81 ±1.56 on 7th day. It further reduced to 4.44 ± 1.38 on 14th day and 4.04 ±1.3 on 21st day respectively. Mean improvement of 2.68 , which was statically significant was observed from baseline to 21st day.

Table 11: Mean clinical attachment level at baseline, 7 days 14 day and 21 day for neem group

INTERVAL	MEAN	SD	DIFFERENCE FROM BASELINE	t	p
BASELINE	1.32	0.11			
7 DAY	1.30	0.12	0.02	-0.936	0.3514
14 DAY	1.25	0.14	0.07	-2.994	0.0034
21 DAY	1.20	0.16	0.12	- 4.707	<0.0001



GRAPH 11 - DISTRIBUTION OF MEAN CLINICAL ATTACHMENT LEVEL AT BASELINE, 7th DAY, 14th DAY AND 21st DAY FOR NEEM GROUP

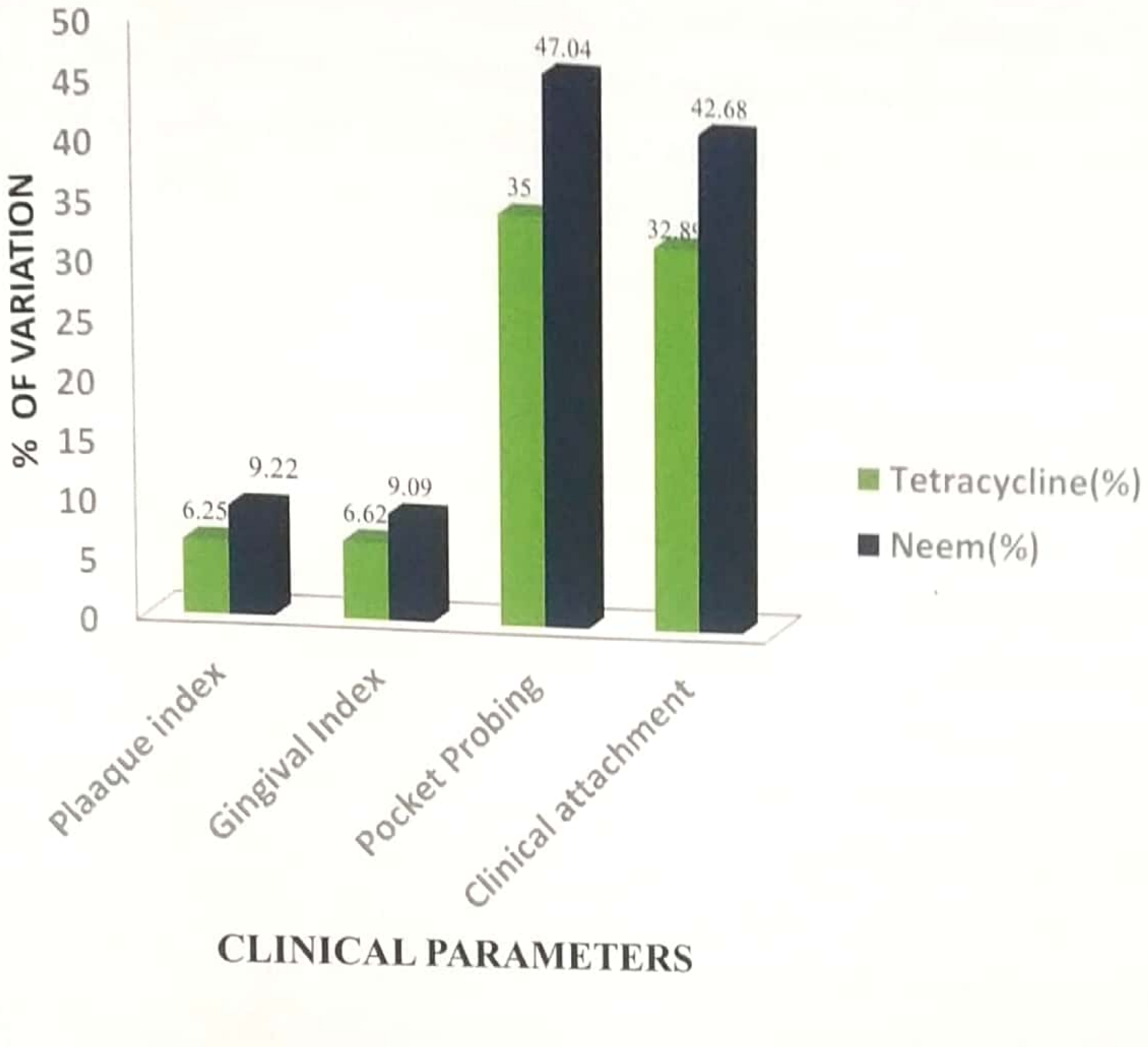
9. Comparative evaluation of mean percentage of variation observed

Table 12 and Graphs 12 represents the comparison of the mean change observed for each clinical parameter recorded during the course of treatment.

6.25% of mean change is witnessed in plaque index for tetracycline whereas Neem shows 9.22% variation at 21 day from baseline. Gingival index showing 9.09% greater improvement with Neem than tetracycline showing 6.62%. 47.04% and 42.68% mean variation seen with Neem proves it better in terms of pocket probing depth and clinical attachment level respectively over tetracycline with 35% and 32.89% variation.

Table 12: Comparative evaluation of mean percentage of variation

Parameters	Tetracycline (%)	Neem(%)
Plaque index	6.25	9.22
Gingival index	6.62	9.09
Pocket probing depth	35	47.04
Clinical attachment level	32.89	42.68



GRAPH 12 – COMPARISION OF MEAN % CHANGE BETWEEN NEEM AND TETRACYCLINE GROUP.

DISCUSSION

Periodontitis is among the most prevalent oral diseases. It is a multifactorial polymicrobial infection, induced primarily by oral anaerobic bacteria such as P.*gingivalis* and F.*nucleatum*. The adherence of bacteria to mucosal cells is a critical step in the development of periodontal infection. *P.gingivalis* is able to adhere to cellular and acellular surfaces[77], form a biofilm[78], and function as a keystone pathogen, by elevating the virulence of the entire microbial community and interfering with host immunity[79]. F.*nucleatum* is capable of invading epithelial cells[80], stimulating proinflammatory cytokine expression[81], and expressing a variety of surface adhesins, allowing coaggregation with most oral bacteria. As P. *gingivalis* and F. *nucleatum* are strongly associated with periodontitis, their suppression may be important in controlling the disease. Majority of the treatment for elimination of pathogenic bacteria relies on mechanical removal of as much as bacterial deposits as possible. SRP proved to be equally effective in reducing PI, GI, PPD and CAL to modified widman flap surgery ranging between 4-6 mm in a longitudinal study.[82] Another study proved that SRP reduced PPD by more than 1 mm for sites with medium initial PPD and by 2mm for sites with deep initial PPD.[83]

With the realization that pocket bacteria accumulate as biofilms, studies are now directed towards eliminating microbes in the biofilm with the help of antimicrobials. It can be either given systemically or through local route of administration. Systemic anti microbial therapy inhibits the growth of the majority of the periodontal pathogens in periodontal pockets, including furcation areas and may affect bacteria within the pocket epithelia and connective tissue. It also eliminates the bacteria from non- dental sites, thus reducing the risk for bacterial repopulation.[2] Limitations of systemic administration includes adverse drug reactions, development of bacterial resistance [84,] uncertain patient compliance [85,86] and lower concentration of the drug at sub gingival sites.[87] However, local drug delivery helps overcome these disadvantages and it can attain many folds higher concentration of an antimicrobial agent in sub gingival sites compared with a systemic drug regimen. Since 1979, attention has been laid down to deliver an anti microbial agent to specific sites of periodontal infection. Both SRP and LDD, work on different principle, SRP acts by disrupting and removing bacteria and calculus deposits whereas LDD acts by its bactericidal and bacteriostatic action delivered in sub gingival

pockets. Therefore, adjunctive use of local drug delivery along with SRP gives cumulative effect in treatment of periodontitis.[30, 32, 35,38,42]

Tetracycline is one of the most widely used antimicrobial in the treatment of periodontal disease. Evidence from the studies has led to approval of tetracycline as LDD treatment modality by the US Food and Drug Administration and by the European Union Regulatory Bodies It is a broad spectrum antibiotic which acts on gram positive and gram negative bacteria. It has also anti inflammatory properties [88,89] , anti- collagenase action [90], and bone resorption [91]. It helps in periodontal regeneration by means of smear layer removal. Also, when applied on dentin surface binding with fibronectin increases. The absorbed fibronectin stimulates fibroblast attachment and growth,while suppressing epithelial attachment and growth.[92] Besides, tetracycline has the ability among antibiotics to concentrate in the gingival crevicular fluid of the periodontal pocket at levels 2-10 times greater than those found in the serum[93]. Further, these antibiotics can bind to the tooth surface and then be slowly released as a still active anti microbial, a characteristic that could prolong their therapeutic effectiveness for at least sometimes after the patient stops taking the drug.

Owing to these tetracycline was the drug of choice as control; to be compared to our experimental drug.

As a model of local drug delivery, tetracycline is used in the form of fibers, strips, films, gels. In our study, commercially available tetracycline containing fibers (Periodontal AB Plus) was used as a control group. Its effectiveness has already been acknowledged as an adjunct to SRP.[66,68,94]

Although tetracycline being a major broad spectrum antibiotic with the above mentioned advantages, the developing microbial resistance in the microorganism has emerged to be an important issue globally. Various new synthetic substitutes developed encounter the same problem sooner or later. Hence, to overcome these issues we must utilize our natural heritage symbiotically. Medicinal plants appear to be an essential part of fight against a wide range of diseases. For thousands of years the beneficial properties of the Neem tree (*Azadirachta indica A. Juss*.) have been recognized in India and mentioned in Ayurveda, and perhaps it is the country's most useful traditional plant. It has been universally accepted as a wonder tree because of its diverse utility. Neem oil and the

bark and leaf extracts have been therapeutically used as folk medicine in the treatment and control of leprosy, intestinal helminthiasis, respiratory disorders, constipation, blood morbidity, rheumatism, biliary infections, itching, skin ulcers and many more along with a general health promoter.[95]

In our study we used Neem leaves extract which are extremely rich in a large variety of polyphenol agents, thus exhibits beneficial effects. Neem polyphenols can avidly bind to surfaces of both microbial and mammalian cells and to endow them with potent antioxidant properties.[96] Therefore, bacteria coated with polyphenols seem to be an excellent mode for achieving long-lasting antioxidant activity in the oral mucosa, with the bacteria, alive or killed by the polyphenols, serving as carriers of these molecules. Neem exhibits less toxic effects in comparision with chlorhexidine on cultured human fibroblasts thus emphasizing cytoprotective oral friendly quality superior to chlorhexidine.

Therefore, utilizing the beneficial properties of Neem to treat periodontitis, local drug delivery in the form of collagen fibers were administered to check its efficacy and time release pattern in periodontitis cases.

The split-mouth design has been the principal research tool in periodontal clinical trials to compare different treatment modalities. In this study to avoid mixing of neem and tetracycline which probably may leached out and bias the treatment effect specially in mandibular teeth, we chose whole mouth clinical design

Inclusion and exclusion criteria were considered as per the previous studies. [60,100]

***IN VITRO* PARAMETER-**

TIME RELEASE PATTERN OF *Azadirachta indica*

In vitro drug diffusion study was performed to check the time release pattern of *Azadirachta indica* using KC Diffusion Cell.

A semipermeable membrane is a type of biological or synthetic, polymeric membrane that will allow certain molecules or ions to pass through it by diffusion or occasionally by more specialized processes. An example of a biological semi-permeable membrane is the lipid layer on which is based on the plasma membrane that surrounds all biological cells. After dipping the eggshell in concentrated HCL solution, inner semi permeable

membrane can easily be obtained. The egg is surrounded by a semi permeable membrane. Hence, to simulate human body environment egg membrane was used.

Drug diffusion study was performed to evaluate the time release pattern of neem incorporated collagen fibers. Samples were collected and analysed to check release of neem extract into the tissues through egg membrane at 7 hours at 1 hour interval and later at 72 hour. At 1st hour 9.38% of extract was released which rapidly increased to 14.85% at 2 hours. It further increased to 18.92% at 5th hour. The neem was seen to release at a slower rate with increasing time i.e 19.16% and 19.24 %at 6th and 7th hour. Release was further evaluated at 72 hours which came to be 38.33% followed by a constant rate of release at 5th and 7th day. Hence, initial faster release of the drug can be attributed to the initial dissolution phenomenon.

The release of neem extract from collagen thread through egg membrane upto 7 hours at 1 hour interval and later at 72 hour. 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 5th 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.

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 Neem was already achieved as seen by the release pattern of Neem *in vitro*.

CLINICAL PARAMETERS-

The present study was designed to evaluate the clinical efficacy of newly introduced Neem incorporated collagen fibers versus tetracycline fibers as an adjunct to scaling and root planing in chronic periodontitis patients. A total of 50 sites were selected from 15 patients in each group and were enrolled in the study.

The selected patients had interproximal sites showing a probing pocket depth of 5-6mm. These sites were randomly divided into two groups-tetracycline fibers and neem fibers. Both the groups were evaluated for clinical parameters of plaque index, gingival index, probing pocket depth and clinical attachment level. These parameters were clinically evaluated at baseline, 7thday, 14th day and 21st day. The clinical parameters were recorded at baseline after scaling and root planing.

The plaque index by *Silness & Loe (1964)*[75] was included in the study as it reflects the oral hygiene status of the patient throughout the study. The *Loe & Silness (1963)*[74]gingival index was used to visually score the gingivitis on papillae and margins of facial and lingual gingiva on all natural teeth. Probing pocket depth and clinical attachment level measurements were taken using University of North Carolina-15 Probe. No adverse effects were reported by the patients at any given time during the study duration. All the patients showed statistically and clinically significant improvements in selected clinical parameters at both the follow up visits, when compared with the baseline.

In the present study, intragroup comparisons of all the groups were evaluated at baseline, 7thday, 14th day and 21st day.

Plaque score-

The plaque score of tetracycline group is summarized in table 1. On 7th day, PI remained almost same with mean difference of 0.01 and reduced slightly at 14th day showing a mean improvement of 0.06. At 21st day PI score reduced and a statistically significant mean improvement of 0.09 and p value 0.002 was seen.

The plaque score for Neem group is summarized in table 5. At 7th day, PI reduced slightly with mean difference of 0.02 . At 14th day, PI score reduced statistically significantly showing a mean improvement of 0.09 and p value 0.001. At 21st day, PI

score reduced and a statistically significant mean improvement of 0.13 and p value <0.001 was seen.

As summarized in table no.9, mean % change (reduction) in plaque score of Tetracycline is 6.25% , whereas Neem shows 9.22% mean % change (reduction) at 21 day from baseline. Thus, greater mean % change with Neem group as compared to tetracycline group is witnessed.

Hence, it can be inferred that both the groups show statistically significant improvement on 21st day. Since the Neem group showed better improvement from 14th day onwards, it can be said that Neem improved PI score faster than the control i.e tetracycline fibers. Lesser change observed in both the groups on 7th day can be attributed to the presence of slough tissue, debris beneath the Coe-pack which was removed on that day.

The reduction in plaque score in our study is in accordance with study conducted by Lindhe J etal in 1979[62] assessing the effect of locally administered tetracycline via hollow fiber devices measuring clinical parameters and microbial analysis. Plaque score which was measured on baseline , 7 thday, 14 thday , 28 thday and 37 thday. The result showed significant reduction in plaque score in tetracycline group from 14 thday till 37 thday.

Heijl etal in 1991[97] examined the efficacy of periodontal treatment using tetracycline containing fibers and the result showed that plaque score remained similar to the baseline i.e 1 and did not increase throughout the treatment.

Some of the bioactive components from the Neem plant may belong to the family of compounds known as gallotannins. The presence of gallotannins during the early stages of plaque formation 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. Secondly, the effective inhibition of glucosyltransferase activity and the reduced bacterial adhesion to salia coated hydroxyapitite, due to the presence of gallotannin extracts, suggest some potential anti-plaque Activity.[53]

Vennila etal in 2016[60] showed that 10% Neem chip when used as an adjunct to SRP significantly reduced the plaque score from 3.294 ± 0.519 at baseline to 0.784 ±0.277 at 21 day.

Saha S etal in 2017[54] reported in a study that *Actinomyces viscosus* are one of the initial colonizers of plaque formation and root caries. The count of *A. viscosus* was increased in patients suffering from gingivitis depicting pre-existing early periodontal disease. In neem group, all subjects showed significant reduction at 7 days and non significant reduction at 15 days.

M Raveendra Pai etal in 2003[55] evaluated the anti plaque activity of 25% neem gel on clinical evaluation of neem extract gel oer 6- week period showed $p<0.05$ significant reduction in the plaque score i.e 1.588 ±0.33 at baseline to 0.916 ±0.28 at 21 day and 0.423 ±0.48 at 42 day. This study stated that 25% neem extract had good antibacterial property by significantly reducing *Streptococcus mutans* and *Lactobacillus* species bacterial count i.e p value <0.05

In another study by Botelho etal 2008[56] assessed the efficacy of neem mouthwash in the treatment of chronic gingivitis. Results supported our observation of statistically significant plaque score reduction $p<0.001$ during the course of 4 weeks of study.

Wolinsky etal 1996[53] studied the inhibitory effects of aqueous extracts of neem bark upon bacterial aggregation, growth, adhesion to hydroxyapatite, and production of insoluble glucan, which may affect in vitro plaque formation. The Neem stick extract inhibited insoluble glucan synthesis. Incubation of oral streptococci with the Neem stick extract resulted in a microscopically observable bacterial aggregation.

Gingival score

The gingival score of tetracycline group is summarized in table 2. On 7thday, GI score slightly with a mean improvement of 0.03 and reduced at 14th day showing a mean improvement of 0.07.On 21st day GI score further reduced and a statistically significant improvement of 0.09 and p value <0.0001 was witnessed.

The gingival score for Neem group is summarized in table 6. On 7th day, GI score reduced with a mean improvement of 0.02 and reduced on 14th day showing a mean improvement of 0.07.On 21st day GI score further reduced and a statistically significant improvement of 0.12 and p value <0.0001 was seen.

Tetracycline shows mean % change of 6.62% in gingival score, whereas Neem shows 9.09% mean % change at 21 day from baseline. Thus, greater mean % change with Neem group as compared to tetracycline group is witnessed.

Hence, it can be inferred that both the groups show statistically significant improvement on 21st day but greater % of improvement is reported by Neem than the control i.e tetracycline fibers.

Lesser change observed in both the groups on 7th day can be attributed to the presence of slough tissue, debris beneath the Coe-pack which was removed on that day.

Results of our study showing reduction in gingival score corroborates with results presented by Lisgarten etal in 1978.[98] They treated 6 human subjects with advanced periodontal disease by tetracycline administration via systemic route, revealed change in plaque microbiota and reduced signs of gingival inflammation thus, markedly reduced gingival index score, gingival fluid flow and probing depth. In a study conducted by lindhe in 1979[69] showed that gingival index score reduced significantly over 37 days with local administration of tetracycline using hollow fibers. It proved that the treatment effects observed were obtained by administration of less than 1/1000 of the amount of tetracycline used in listgarten etal 1978.

Neem leaves are extremely rich in a large variety of polyphenol agents ,which have the capability to bind to both mammalian and microbial surfaces thus, possessing antioxidant properties that can modulate inflammation. The binding of Neem polyphenols to the cell surface

might be further enhanced by RBC and salivary cationic lysozymes, which may act as adhesives. This amplification of antioxidant capacity bears clinical relevance as lysozyme is always present in the oral cavity, while RBC escape injured capillaries, in particular those adjacent to inflamed periodontal tissues. The potent antioxidant activity in Neem extracts might also act as a double-edged sword. On one hand scavenging ROS might lower inflammatory damage to the gum, but on the other hand it may also protect periopathogenic catalase-negative bacteria against oxidants generated by inflammation or cariogenic streptococci.[99]

Antony etal in 2013[100] concluded that a mean change from 2.8±0.223at baseline to 0.737±0.236 at 6 months at experimental site (SRP + neem gel) indicated a higher reduction in gingival index score compared to control (SRP + Placebo gel) showing 2.85±0.22 at baseline to 1.32 ± 0.23 at 6 months .

As stated by Axellson in 1987, of Streptococcus *sanguis* is one of the constituting bacteria of the initial colonizers of plaque neem. Primary colonizers of dental plaque of the free gingival margin are also responsible for the gingivitis initiation.The results of our study were not in accordance with that of the study by Saha S in 2017 [54], who reported that all subjects in neem group showed significant reduction of bacterial count of Streptococcus *sanguis* at 7 days and non significant reduction at 15 days showing that they were not inhibited by neem over along time .

In another study by Botelho etal 2008 [56] assessed the efficacy of neem mouthwash in the treatment of chronic gingivitis. Results supported our observation of statistically significant gingivitis score reduction from 1.52 at baseline to 0.45 at 30 days in the neem group $p<0.001$.

A study by Vennila etal in 2016 [60] showed the similar pattern of gingivitis score reduction using 10% Neem chip when used as an adjunct to SRP, as are the results of our study. Significant reduction of the gingival score from 1.52 ± 0.19 at baseline to 0.329 ±0.87 at 21 day.

Pocket probing depth

The pocket probing depth of tetracycline group is summarized in table 3. On 7thday, PPD reduced with a statistically significant mean improvement of 0.71 and p value <0.0001 which further reduced at 14th day showing statistically significant mean improvement of 1.53 and p value <0.0001.On 21st day PPD reduced and a statistically significant improvement of 1.89 and p value <0.0001 was seen.

The pocket probing depth for Neem group is summarized in table 7. On 7th day, PPD reduced slightly with significant mean improvement of 1.4 and p value 0.0001 and reduced on 14th day showing a mean improvement of 2.19 and and p value <0.0001.On 21st day PPD further reduced and a statistically significant improvement of 2.54 and p value <0.0001was seen .

Tetracycline shows mean pocket depth reduction of 35% , whereas Neem shows mean pocket depth reduction of 47.04 % on 21st day from baseline. Thus, greater mean % pocket depth reduction with Neem group as compared to tetracycline group is witnessed. Newmann etal in 1994 [68] reported the statistically greater improvement in pocket probing depth at 1 month ($p < 0.05$), 3($p < 0.05$) and 6 months ($p < 0.01$) for tetracycline fiber treated sites as compared to SRP treated group.

Lindhe etal 1979 [62] reported that the pocket probing depth measurements showed a significant improvement between the initial examination and examination carried out after 14 and 37 days in SRP+ Tetracycline group.

Various other studies conducted using tetracycline fibers alone or as an adjunct to SRP show significant reduction in pocket probing depth. [66,69,71,97]

Various constituents of Neem either inhibit the growth of microorganism or rupture the cell wall of the microorganism. Extracts from fresh Neem contain quercetin , β sitosterol, polyphenolic flavanoids are know to have potent antibacterial and anti fungal properties. Oil from neem leaves possess wide spectrum of antibacterial action. Neem extract possess potent immune stimulant activity acting by both humoral and cell mediated immune response. Nimbidin show significant antiulcer activity by its tendency to block H_2 receptors thus, acting as anti histaminic agents.[45]

Our result is in accordance with a study by Antony etal in 2013 [100] concluded that a statistically highly significant reduction with p value <0.0001 in pocket probing depth from baseline to 6 months using Neem extract gel is seen.

A study by Vennila etal in 2016[60] showed the similar pattern of pocket probing depth reduction reduction using 10% Neem chip as an adjunct to SRP, as are the results of our study. Significant reduction of the pocket probing depth from 5.10 ± 0.316 at baseline to 2.30 ± 0.483 at 21 day was witnessed.

Clinical Attachment Level

The clinical attachment level of tetracycline group is summarized in table 4. On 7th day, PPD reduced with mean improvement of 0.73 and further reduced on 14th day showing

statistically significant mean improvement of 1.58 and p value <0.0001.On 21st day PPD and a statistically significant improvement of 1.98 and p value <0.0001 .

The clinical attachment level for Neem group is summarized in table 7. On 7th day, CAL reduced slightly with a statistically significant mean improvement of 1.47 and p value <0.0001 and reduced on 14th day showing a mean improvement of 2.37 and and p value <0.0001.On 21st day PPD further reduced to 3.60±1.3 for tetracycline group showing a statistically significant improvement of 2.68 and p value <0.0001 .

Tetracycline shows clinical attachment gain of 32.89% , whereas Neem shows mean clinical attachment gain of 42.68 % on 21st day from baseline. Thus, greater mean % clinical attachment gain with Neem group as compared to tetracycline group is witnessed.

Newmann etal in 1994 [68] reported the statistically greater improvement in clinical attachment level at 1 month (p <0.05), 3(p<0.05) and 6 months (p <0.01) for tetracycline fiber treated sites as compared to SRP treated group.

Radvar etal in 1996 [71] reported that the greatest clinical improvement is seen in SRP+ Tetracycline fibers group than 3 other treatment modalities compared (SRP+ 2% minocycline gel, SRP + metronidazole gel, SRP alone).

Various other studies conducted using tetracycline fibers alone or as an adjunct to SRP show significant gain clinical attachment level.

Neem leaves contain tannins which promote wound healing activity through increased healing response and neovascularization[103, 104]. Neem has alkaloids, glycosides, flaonoids and saponins which exhibit anti bacterial property thereby preventing bacterial colonization and aggregation[103]. Neem regulates pro inflammatory enzyme activity including cyclooxygenase and lipoxygenase enzyme .Nimbidin , a photochemical suppresses the macrophage and neutrophil functions thus reducing inflammation. Extract of neem leaves affect cell mediated and humoral responses affecting immunostimulation further adding to suppress periodontal disease progression[56,102,103]. Neem also shows anti- inflammatory effect by causing reduction in MMP-2 and MMP-9.[39]

A study by Vennila etal in 2016[60] showed the similar pattern of clinical attachment level using 10% Neem chip as an adjunct to SRP, as are the results of our study. Clinical

attachment level improved from 5.20 ± 0.422 at baseline to 2.40 ±0.516 at 21 day showing statistically greater significance than control (placebo gel).

Antony etal in 2013[100] concluded that a statistically highly significant improvement with p value <0.0001 in clinical attachment level from baseline to 6 months using Neem extract gel is seen, with test group (Neem gel) showing greater significance than control (placebo gel).

Tetracycline fibers taken as control in our experiment has improved all the clinical parameters considerably, but neem incorporated collagen fibers proved to be more efficacious in clinical terms.

CONCLUSION

CONCLUSION

In vitro analysis revealed that the 25 mg of collagen fibers contained 255mg of neem extract. Release of Neem constituents from collagen thread and into the tissue at 72 hours was 23.85% and 38.33% respectively which there after remained constant when checked at 5^{th} and 7^{th} day. Thus, therapeutic range of the Neem will be administered before degradation of collagen begins.

Based on the clinical parameters, the following can be concluded from the present study:

Plaque score and gingivitis score reduced significantly(p value 0.002) at 21 days when compared with baseline for tetracycline group.PPD reduced significantly (p value <0.001) from 7 days till 21 days when compared with baseline for tetracycline group. CAL reduced significantly (p value <0.001) from 14 days till 21 days when compared with baseline

Plaque score reduced significantly (p value 0.001) at 14 days when compared with baseline for neem group. Gingivitis score reduced significantly (p value <0.001) at 21 days when compared with baseline for neem group. PPD and CAL reduced significantly (p value <0.001) from 7 days till 21 days when compared with baseline for neem group. Mean % variation observed in plaque score for tetracycline group and neem group was 6.25% and 9.22% respectively. Mean % variation observed in gingivitis score for tetracycline group and neem group was 6.62 % and 9.09% respectively. Mean % improvement observed in Pocket probing depth for tetracycline group and neem group was 35 % and 47.04% respectively. Mean % improvement observed in clinical attachment level for tetracycline group and neem group was 32.89 % and 42.68% respectively.

Neem group proves to be more efficacious when compared with tetracycline fibers on all clinical parameters i.e Neem has higher mean % variation from baseline to 21 days .

From the current study it can be concluded that Neem incorporated tetracycline fibers can be used as an adjunct to SRP proving it to be better than the control i.e tetracycline fibers.

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

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APPENDICES

APPENDICES

ANNEXURE 1

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

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **A Comparative Evaluation of *Azadirachta indica* (NEEM) Fiber with Tetracycline Fiber as a Local Drug Delivery Agent- A Randomized Control Study** submitted by **Dr.Vanchha Sharma** Post graduate student from the Department of **Periodontics** as part of MDS Curriculum for the academic year 2016-2019 with the Accompanying proforma was reviewed by the institutional research committee present on 7th and 8th December 2016 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. (Dr) Vivek Govila
Principal

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
Babu Banarasi Das University
BBD City Faizabad Road, Lucknow-227105

Chairperson Institutional Research Committee

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 V[th] Institutional Ethics Sub-Committee

IEC Code: 08 **BBDCODS/03/2017**

Title of the Project: A Comparative Evaluation of *Azadirachta Indica* (Neem) Fiber with Tetracycline Fiber as a local Drug Delivery Agent- A Randomized Control Study.

Principal Investigator: Dr. Vaanchha Sharma **Department:** Periodontology

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

Type of Submission: New, MDS Project Protocol

Dear Dr. Vaanchha Sharma

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 02[nd] March, 2017.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Neerja Singh Member	Prof. & Head, Department of Pedodontics, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics, BBDCODS, Lucknow
4.	Dr. Manu Narayan Member	Reader, Department of Public Health Dentistry, BBDCODS, Lucknow

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

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

Forwarded by:

(Dr. Lakshmi Bala)
Member-Secretary
IEC

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

(Dr. Shirish Gpvila)
Principal
BBDCODS

ANNEXURE 3

निस्केयर
NISCAIR

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 and Industrial Research)
डॉ. के. एस. कृष्णन मार्ग, (पूसा गेट के पास), नई दिल्ली 110 012
Dr. K. S. KRISHNAN MARG, (Near Pusa Gate), NEW DELHI 110 012
14, सत्संग विहार मार्ग, नई दिल्ली 110 067
14, SATSANG VIHAR MARG, NEW DELHI 110 067

RAW MATERIAL HERBARIUM AND MUSEUM, DELHI (RHMD)

Ref. No.-NISCAIR/RHMD/Consult/2018/3170-19 28/03/2018

CERTIFICATE FOR CRUDE DRUG SAMPLE AUTHENTICATION

This is to certify that leaves sample of *Azadirachta indica*, Neem, received from Dr. Vasudha Sharmavide letter No. Nil Dated 15th February 2018 for authentication has been found **correct as leaves of *Azadirachta indica* A. Juss.** syn. *Melia azadirachta* L. **which is commonly known as Indian Lilac, Margosa Tree, Neem.** 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.

(Dr. Sunita Garg)
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ई-मेल/E-mail: ... वेबसाइट/Website: www.niscair.res.in

ANNEXURE 4

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.....
I received a signed copy of the PID and duly filled consent form
Signature/thumb impression of the subject or legally Acceptable representative Date .....

ANNEXURE 5

Indiatyping

11प्र क्या कर रहे है और संभावित नुकसान का पोलिम के भाग लेनेघ्
कोई खतरा या नुकसान इस अध्ययन में भाग लेने के नहि है।

12प्र कब भाग लेने के संभावित लाभ कर रहे हैघ
इस अध्ययन का विश्लेषण करेंगे और विशिष्ट समय अंतराल पर नीम शामिल लतुओ टेट्रासाइक्लिन फाइबर के साथ तुलना करें। यह आगे
उपचार प्रभावित आबादी के लिए योजना तैयार करने में मदद मिलेगी। थ्

13प्र क्या होगा अगर नई जानकारी उपलब्ध हो जाता हैघ्
अतिरिक्त जानकारी आप इन के बारे में बताया जाएगा अनुसंधान के दौरान उपलब्ध हो जाता है और आप इसे अपने शोधकर्ता के साथ चर्चा
करने के लिए स्वतंत्र हैघ तो अपने शोधकर्ता आपको बताएगा कि क्या आप में अध्ययन जारी रखना चाहते है। यदि आप भाग लेने का
फैसलाए अपने शोधकर्ता अपनी वापसी के लिए उपलब्ध कर देगा। यदि आप में अध्ययन जारी रखने के लिए निर्णयए आपको एक अतिरिक्त
सहमति पत्र पर हस्ताक्षर करने के लिए कहा जा सकता है।

14प्र क्या होता है जब कोई अध्ययन बंद हो जाता हैघ
यदि अध्ययन अनुसूचित समय से पहले समाप्त यह रोगी/अभिभावक को समझाया जाएगा।

15प्र क्या होगा अगर कुछ गलत हो जाता हैघ
किसी भी गंभीर प्रतिकूल घटना होती हैए या कुछ अध्ययन के दौरान गलत हो जाता हैए तो शिकायतों की [illegible] के लिए निर्धारित
द्वारा निर्धारित किया जाएगाए और संस्थागत नैतिक समुदायए और उपचार लागत वाडी प्रधान अन्वेषक द्वारा किया जाएगा।

16प्र मेरे इस अध्ययन में भाग से गोपनीय रखा जाएगाघ्
हाँ यह गोपनीय रखा जाएगा।

17प्र इस अध्ययन के परिणाम के लिए क्या होगाघ्
अध्ययन के परिणाम नीम फाइबर और क्लासिकवर्डस केस में रखा टेट्रासाइक्लिन फाइबर की प्रभावकारिता और समय रिलीज पैटर्न की
तुलना करने के लिए उपयोग किया जाएगा। यह भ्रान्त प्रक्रिया में उनके उपयोग का निर्धारण करने में मदद मिलेगी।

18प्र यो अनुसंधान आयोजित कर रहा हैघ्
इस शोध अध्ययन को शैक्षणिक संस्थान ,ठटवब्ब्द्ध द्वारा आयोजित किया जाता है।

19प्र अध्ययन पूरा होने के बाद अध्ययन के परिणाम उपलब्ध कराया जाएगाघ्
हाँ

20प्र जो अध्ययन की समीक्षा की हैघ्
अध्ययन की समीक्षा की और विभाग के शिरए और आईईसीएचआई आरसी संस्था के द्वारा अनुमोदित किया गया है।

[illegible]

अधिक जानकारी के लिए संपर्क
डॉ. [illegible] शर्मा
विभाग [illegible]
दंत चिकित्सा विज्ञान के बाबू बनारसी कॉलेज
लखनऊ 227105
9450226256

डॉ. [illegible]
प्रोफेसर और प्रमुख
विभाग [illegible]
दंत चिकित्सा विज्ञान के बाबू बनारसी कॉलेज
लखनऊ 227105
9415012444

डॉ.
डॉ. [illegible]
सदस्य [illegible]
दंत चिकित्सा विज्ञान के बाबू बनारसी कॉलेज
[illegible]

मैं इस साक्षात्कार के [illegible] से [illegible] हस्ताक्षर
नाम : [illegible]
दिनांक : [illegible]

ANNEXURE 6

Name of the Patient:

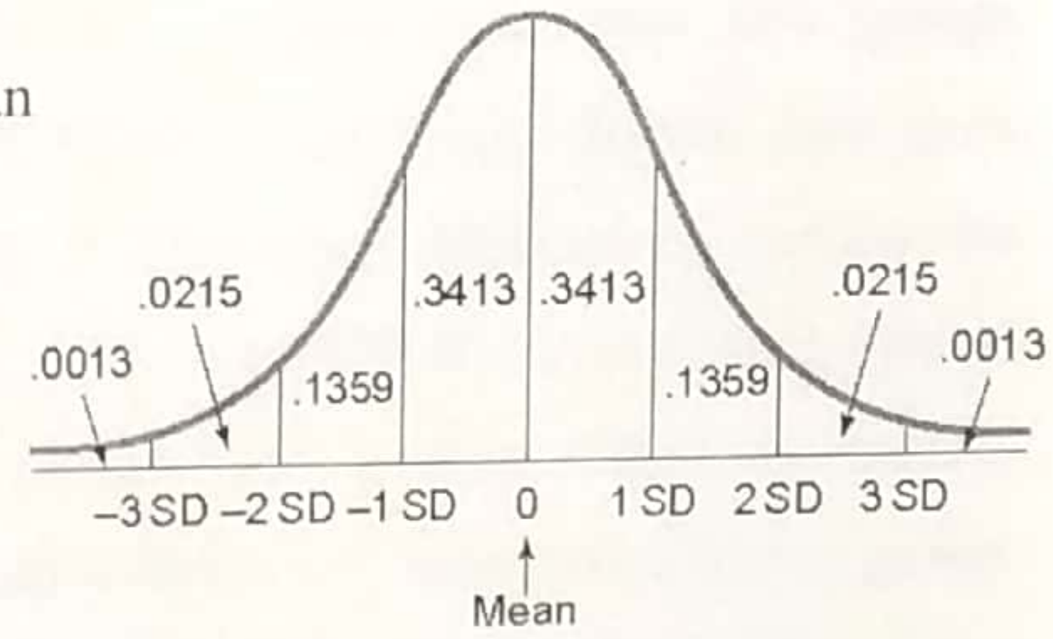
Tetracycline/Neem Incorporated Fibers

Tooth Number	Plaque Score								Gingival Score								Pocket Probing Depth								Clinical Attachment Level							
	0 DAY	M	7 DAY	M	14 DAY	M	21 DAY	M	0 DAY	M	7 DAY	M	14 DAY	M	21 DAY	M	0 DAY	M	7 DAY	M	14 DAY	M	21 DAY	M	0 DAY	M	7 DAY	M	14 DAY	M	21 DAY	M
	X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		X	
	X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		X	
	X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		X	

Finally, you will divide the variance by either the number of items in the data set, which is usually referred to as n, or by one less than the number of items in the set, which is written as n - 1. What you divide by depends on whether you are calculating the variance of the whole population or just a sample. When you are calculating the variance for an entire population, divide by n. For a sample of the entire population, divide by n - 1. Once you have found the variance for the data set, you can then find the standard deviation by taking the square root of the variance.

If the shape of the dot plot or histogram is approximately bellshaped, we would expect

- 68% of the data to be within 1 SD of the mean
- 95% of the data to be within 2 SD of the mean
- 99.7% of the data to be within 3 SD of the mean



$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \overline{x})^2}$$

σ = standard deviation

X_i = each value of dataset

$\bar{x}$ (= the arithmetic mean of the data (This symbol will be indicated as the mean from now)

N = the total number of data points

$\sum_{i=1}^{N}(x_i - \overline{x})^2$ = The sum of $(x_i - \overline{x})^2$ for all datapoints

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

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

Where, n= no. of observations

Minimum and Maximum

Minimum and maximum are the minimum and maximum values respectively in the measure data and range may be dented as below

Range = Min to Max

and also evaluated by subtracting minimum value from maximum value as below

Range = Maximum value-Minimum value

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

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

Where, n= no. of observations

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

P-value

The p-value is a probability that measures the evidence against the null hypothesis. Lower probabilities provide stronger evidence against the null hypothesis.

To determine whether the variables are independent, compare the p-value to the significance level. Usually, a significance level (denoted as α or alpha) of 0.05 works well. A significance level of 0.05 indicates a 5% risk of concluding that an association between the variables exists when there is no actual association.

P-value ≤ α: The variables have a statistically significant association (Reject H0)

If the p-value is less than or equal to the significance level, you reject the null hypothesis and conclude that there is a statistically significant association between the variables.

P-value > α: Cannot conclude that the variables are associated (Fail to reject H0)

If the p-value is larger than the significance level, you fail to reject the null hypothesis because there is not enough evidence to conclude that the variables are associated.

ANNEXURE 8

NEEM

1

pt. no.	tooth	pocket probing depth 0 day (mm)	7 day (mm)	14 day (mm)	21 day (mm)	clinical attachment level 0 day (mm)	7th day (mm)	14 day (mm)	21st day (mm)	plaque index score 0 day	7th day	14th day	21st day	gingival index score 0 day	7th day	14th day	21st day
1 Deepika	42	5	4	3	3	5	4	3	3	1.4	1.38	1.31	1.27	1.33	1.29	1.23	1.2
	43	5	4	3	3	5	4	3	3	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	47	5	4	3	3	5	4	3	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	48	5	4	3	3	5	4	3	3	1.5	1.48	1.41	1.37	1.35	1.33	1.1	1.25
2 Meenu singh	42	5	5	3	2	7	7	5	4	1.2	1.18	1.11	1.07	1.13	1.12	1.06	1.02
	43	5	5	3	2	7	7	5	4	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	45	6	4	3	3	8	6	5	5	1.1	1.08	1.01	0.97	0.9	0.7	0.5	0.2
	46	6	6	5	5	9	9	8	8	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
3 Nisha devi	44	7	5	4	4	7	5	4	4	1.6	1.58	1.51	1.47	1.51	1.48	1.45	1.39
	45	5	3	3	3	5	3	3	3	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
	46	5	4	2	2	5	4	2	2	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	47	5	4	3	2	5	4	3	2	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	16	5	4	2	2	5	4	2	2	1.2	1.18	1.11	1.07	1.13	1.12	1.06	1.02
	17	5	5	3	3	5	4	3	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
4 Neha	35	5	3	3	2	5	3	3	2	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	37	5	3	3	3	5	3	3	3	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	16	5	4	3	3	5	4	3	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	17	7	5	4	4	7	5	5	4	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
5 Mohd Alam	46	5	3	3	3	6	4	3	3	1.6	1.58	1.51	1.47	1.51	1.48	1.45	1.39
	47	6	4	4	3	7	3	3	3	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
	17	5	3	3	3	5	3	3	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
6 Amita	34	5	3	3	2	6	4	4	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	35	5	3	2	2	6	4	2	2	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	36	6	3	2	2	6	3	2	2	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
7 Seema	33	5	4	3	3	5	4	3	3	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	34	5	3	3	2	5	4	3	3	1.2	1.18	1.11	1.07	1.13	1.12	1.06	1.02
	35	5	3	3	3	5	4	3	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
8 Simmi yada	33	6	5	5	3	6	5	5	3	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	34	5	3	3	3	5	3	3	3	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	35	5	5	4	3	5	5	4	3	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
9 Pinki Kanija	46	5	3	3	2	5	3	3	2	1.6	1.58	1.51	1.47	1.51	1.48	1.45	1.39
	47	6	3	3	3	6	3	3	3	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
	16	5	4	4	2	5	4	4	2	1.6	1.58	1.51	1.47	1.52	1.49	1.46	1.4
	17	6	5	4	4	6	5	4	4	1.5	1.48	1.41	1.37	1.41	1.39	1.35	1.3
10 Ghanshaya	43	5	5	4	3	7	7	4	4	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	45	5	4	3	3	7	6	4	4	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
	46	5	3	3	3	7	6	4	4	1.4	1.38	1.31	1.27	1.33	1.31	1.25	1.2
11 Geeta Yada	16	5	3	3	2	6	4	4	3	1.6	1.58	1.51	1.47	1.51	1.48	1.45	1.39
	26	5	4.2	3	3	6	5	4	4	1.5	1.48	1.41	1.37	1.41	1.39	1.35	1.3
	16	6	3	3	3	8	5	5	5	1.6	1.58	1.51	1.47	1.52	1.49	1.46	1.4
	17	5	3	3	3	7	5	4	4	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
	36	5	4	3	3	7	6	5	5	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	37	5	4	4	3	7	6	6	5	1.5	1.48	1.41	1.37	1.41	1.39	1.35	1.3
	25	5	4	3	3	7	6	6	5	1.6	1.58	1.51	1.47	1.51	1.48	1.45	1.39
	26	6	4	3	3	8	6	5	5	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
12 poonam so	43	6	5	4	3	10	9	8	7	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	42	7	7	6	4	8	7	6	4	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	12	5	4	3	3	8	7	6	6	1.4	1.38	1.31	1.27	1.3	1.28	1.22	1.18
	13	6	6	4	4	11	9	7	7	1.4	1.38	1.31	1.27	1.31	1.29	1.23	1.19
13 tarika dutta	36	5	4	3	3	5	4	3	3	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	37	6	5	3	3	6	5	3	3	1.2	1.18	1.11	1.07	1.13	1.12	1.06	1.02
	35	6	5	3	3	6	5	3	3	1.2	1.18	1.11	1.07	1.15	1.14	1.08	1.04
14 Sarla	25	5	3	3	2	5	3	3	3	1.4	1.38	1.31	1.27	1.32	1.3	1.24	1.2
	26	6	4	3	3	6	3	3	3	1.3	1.28	1.21	1.17	1.26	1.24	1.2	1.15
	27	6	4	3	3	6	4	3	3	1.4	1.38	1.31	1.27	1.31	1.29	1.23	1.19
15 RAJPAL SIH	25	5	3	2	2	6	4	3	3	1.4	1.38	1.31	1.27	1.31	1.29	1.23	1.19
	26	6	4	3	3	8	6	5	5	1.5	1.48	1.41	1.37	1.4	1.38	1.34	1.28
	27	6	4	3	3	8	6	5	5	1.3	1.28	1.21	1.17	1.25	1.23	1.19	1.14

APPENDICES

tetracycline

pt. no:	patient name	tooth	pocket probing depth 0 day (mm)	7 day (mm)	14 day (mm)	21 day (mm)	clinical attachment level 0 day (mm)	7th day (mm)	14 day (mm)	21st day (mm)	plaque index score 0 day	7th day	14th day	21st day	gingival index score 0 day	7th day	14th day	21st day
1	TRIBHUVAN	46	6	5	4	4	8	7	6	6	1.4	1.39	1.33	1.3	1.3	1.28	1.23	1.2
		47	5	4	4	3	7	6	6	5	1.5	1.48	1.43	1.4	1.4	1.37	1.32	1.3
2	SHIKHA YDA	42	5	4	4	4	5	4	4	4	1.3	1.27	1.24	1.2	1.28	1.25	1.22	1.2
		41	5	4	4	4	5	4	4	4	1.5	1.48	1.43	1.4	1.45	1.43	1.41	1.39
		32	5	4	3	3	5	4	3	3	1.5	1.49	1.42	1.4	1.45	1.44	1.38	1.37
		33	5	4	3	3	5	4	3	3	1.6	1.58	1.54	1.51	1.5	1.47	1.43	1.4
		16	5	4	3	3	5	4	3	3	1.5	1.49	1.43	1.41	1.4	1.37	1.32	1.29
		17	6	5	4	4	6	5	4	4	1.6	1.59	1.53	1.5	1.3	1.26	1.25	1.22
3	SANJEEV	36	6	6	6	5	8	8	8	7	1.4	1.39	1.33	1.3	1.3	1.28	1.23	1.2
		37	6	5	5	5	8	7	7	6	1.5	1.48	1.43	1.4	1.45	1.43	1.41	1.39
		34	5	5	3	3	7	7	5	5	1.4	1.38	1.34	1.31	1.3	1.28	1.23	1.21
4	Neha	35	5	3	3	3	5	3	3	3	1.5	1.48	1.43	1.4	1.45	1.43	1.41	1.39
		37	5	3	3	3	5	3	3	3	1.5	1.49	1.42	1.4	1.45	1.44	1.38	1.37
		16	5	4	3	3	5	4	3	3	1.6	1.58	1.54	1.51	1.5	1.47	1.43	1.4
		17	6	5	5	4	6	5	5	4	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
5	POONAM	36	5	5	4	4	5	5	4	4	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
		37	6	5	4	4	6	5	4	4	1.6	1.58	1.54	1.51	1.45	1.43	1.41	1.39
		46	6	5	4	3	6	5	4	3	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
		47	5	4	4	3	5	4	4	3	1.5	1.48	1.43	1.4	1.45	1.43	1.41	1.39
6	ALAM KHAN	24	5	4	4	4	8	7	7	7	1.5	1.48	1.43	1.4	1.45	1.43	1.41	1.39
		25	6	5	4	4	10	9	7	7	1.5	1.49	1.42	1.4	1.4	1.37	1.32	1.29
		26	5	5	4	4	10	10	9	9	1.6	1.58	1.54	1.51	1.5	1.47	1.43	1.4
7	NISHA KHAT	36	6	5	4	3	6	5	4	3	1.3	1.27	1.24	1.2	1.2	1.18	1.15	1.14
		37	6	6	5	4	6	6	5	4	1.2	1.18	1.13	1.1	1.18	1.15	1.13	1.12
		38	6	5	4	4	6	5	4	4	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
8	SIMRAN	26	5	5	4	4	7	7	6	6	1.5	1.47	1.45	1.42	1.45	1.43	1.41	1.39
		27	6	6	5	4	8	8	7	6	1.5	1.49	1.42	1.4	1.4	1.37	1.32	1.29
9	GUDDI MIS	16	6	5	4	4	6	5	4	4	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
		17	6	5	4	4	6	5	4	4	1.3	1.27	1.24	1.2	1.2	1.18	1.15	1.14
10	CHAMPAK S	35	5	5	4	3	5	5	4	3	1.2	1.18	1.13	1.1	1.18	1.16	1.14	1.13
		36	6	5	5	3	6	5	5	3	1.3	1.27	1.24	1.2	1.2	1.18	1.15	1.14
11	SHWETA KU	44	5	5	3	3	5	5	3	3	1.5	1.48	1.43	1.4	1.4	1.37	1.32	1.29
		45	5	5	3	3	5	5	3	3	1.5	1.49	1.42	1.4	1.4	1.37	1.32	1.29
		46	6	5	4	4	6	5	4	4	1.5	1.47	1.45	1.42				
12	RICHA DIW	34	5	4	3	3	6	5	4	3	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
		35	5	4	3	3	6	4	3	3	1.5	1.48	1.43	1.4	1.4	1.37	1.32	1.29
		36	5	4	4	3	5	4	4	3	1.6	1.58	1.54	1.51	1.5	1.47	1.43	1.4
		37	5	4	3	3	5	4	3	3	1.5	1.47	1.45	1.42	1.4	1.37	1.32	1.3
13	SATISH JHA	46	6	5	4	4	6	5	4	4	1.4	1.39	1.33	1.3	1.3	1.26	1.25	1.22
		47	5	5	4	4	5	5	4	4	1.3	1.27	1.24	1.2	1.2	1.18	1.15	1.14
14	REKHA CHO	44	5	5	4	3	5	5	4	3	1.2	1.18	1.13	1.1	1.18	1.16	1.14	1.13
		45	5	5	4	3	5	5	4	3	1.3	1.27	1.24	1.2	1.2	1.18	1.15	1.14
		46	6	5	4	3	6	5	4	3	1.5	1.48	1.43	1.4	1.4	1.37	1.32	1.3
15	Jaiveer sing	35	5	5	4	3	5	5	4	3	1.4	1.39	1.33	1.3	1.4	1.37	1.32	1.3
		36	5	5	3	3	5	5	3	3	1.6	1.58	1.54	1.51	1.4	1.37	1.32	1.3